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Journal of Himalayan Life Sciences (JHLS)

Aim and Scopes

The Journal of Himalayan Life Sciences (JHLS) is the leading forum for perspectives that give insight into biological processes. The aim of the Journal of Himalayan Life Sciences is to become the primary source of high quality research from all over the world and to disseminate scientific knowledge and principle. JHLS focuses on specific research discipline, contributing resourceful and impactful platform where research joins together with technology. It scope is global and covers a very wide range of topics which is of interest to biologists in many areas of research.

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"ब्रह्म सत्यम जगत मिथ्या..."

The upnishadas mention “Knowledge is the only truth”. The prosperity of a nation depends upon the knowledge creation and efficiency in its application. India has always been a land of truth and thereby knowledge, seekers. The faculty of School of Life Sciences have created “Himalayan Life Science Society” with the aim to train the future minds in various aspects of life sciences and to inculcate the understanding of righteousness and strengthen their yearning for perfection.

It is the duty of “those who know” to transcend the knowledge to “those who want to know” and the medium of written form is one of the most effective medium for knowledge management and transfer. It is with great sense of appreciation; I invite you to participate in this path setting event of the launch of the inaugural issue of the “Journal of Himalayan Life Sciences” (JHLS) under the auspices of The Himalayan Life Science Society. JHLS aims to discover, catalogue and spread the novel concepts and the emerging applications in various disciplines of life sciences. This journal shall prove to be a platform for the researchers from academic circles and industries to present their research and perspectives on a plethora of scientific issues. Though the path to achieve academic excellence is tortuous and difficult but the human spirit is known for its indomitability.

I have full trust that the journal will be an invaluable source for the researchers, academicians and students who wish to do further research and contribute to the arena of “knowledge based wisdom”. I hope that the articles published in JHLS will boost the researchers to undertake new research ventures.

It is imperative that all of us strive to learn, discover and apply knowledge to propel Indian economy in this century so that we may transform India into a developed nation.

(Prof. Sat Prakash Bansal)
Patron-in-Chief
Himalayan Life Science Society

Welcome message by President

It is with profound joy and anticipation that we celebrate the launch of inaugural issue of the new journal "**Journal of Himalayan Life Sciences" (JHLS)**, published by Himalayan Life Science Society under the aegis of School of Life Sciences, Central University of Himachal Pradesh. On behalf of the JHLS editorial team, I would like to take this opportunity to thank to all individuals who have stepped forward to contribute to the launch of the journal a success.

The Journal of Himalayan Life Sciences is primarily focused on the rapid publication of basic research from all areas of the life sciences, including the latest and scientific one. It also provides great opportunity for researchers to improve and disseminate their research data across the spectrum of biology. The publication aims to facilitate the exchange of ideas between researchers from different countries in different formats: full and extension research articles, survey articles, best practice articles from experts from all sections of the scientific community. We are committed to publishing all discoveries, methods, resources, and reviews that significantly advance the field of Life Sciences and its applications.

Finally, I wish to encourage more contributions from the scientific community to ensure a continued success of the journal. Authors, reviewers and guest editors are always welcome and with their support, I see very bright prospects for JHLS to serve science even better in the future.

Please enjoy the issue and we look forward to your future submissions. We also welcome comments and suggestions that could improve the quality of the journal.

Thank you. I hope you will find JHLS informative.

(Prof. Pardeep Kumar)
Patron
Himalayan Life Science Society

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Review Paper

Bhavna et al

Journal of Himalayan Life Sciences
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Morphometric studies of fish and its relationship with Length- weight

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Abstract: Freshwater fish morphometric characteristics and length-weight relationships (LWRs) could be employed as markers of environmental changes and fish health. As fish industry is the fastest growing industry and major contributors towards the national economy, assessment of fish health and biology are mandatory for monitoring the proper output from the industry. This Paper emphasizes on morphometric characteristics of fish and role of length-weight relationship.

Keywords: Morphometry, condition factor, length weight relationship**.**

Introduction

Fisheries and aquaculture are one of the world's fastest-growing businesses and contributes towards the socioeconomic development, good sources of foreign exchange and source of employment for weaker section of society of any country. Fish is a cheap, affordable, highly nutritious and significant dietary source of protein, lipids and other essential micronutrients accounting for over a fifth of all animal protein consumed worldwide [1]. It has been considered as healthiest and most nutritious food [2] and fisheries sector contributes 15 % of the animal protein in the human diet worldwide [3]. Degradation of aquatic life and their habitat due to pollution and anthropogenic activities can cause in low fish composition, resulting in insufficient fish product availability in the commercial market, directly affecting economy of any country. Therefor assessment of fish

Morphometry

Fish morphometric features are the measurable characteristics that all fishes share (depicted in Figure 1). Landmarks are randomly picked spots on a fish's body that aid in the analysis of the particular fish morphology. A landmark is an object that

population dynamics, fish health, proper growth and suitable habitat becomes essential for the conservation of fishery sector [4]. Morphometry is an important tool for determining the proper health, wellbeing and growth of fish. The length-weight relationship (LWR), according to Pauly [5], provides crucial knowledge about the fish's surroundings environment. Fish length-weight ratio characteristics are indications of fatness, well-being, and gonadal development in relation to the environment and help to identify variations from expected weight for known groups of fishes [6, 7]. These metrices also play important role in investigating the different life stages including gonadal maturity and to identify changes between different unit stocks of the same species [8]. The role of length-weight relationships in fisheries for growth comparisons and management studies has also been described [9].

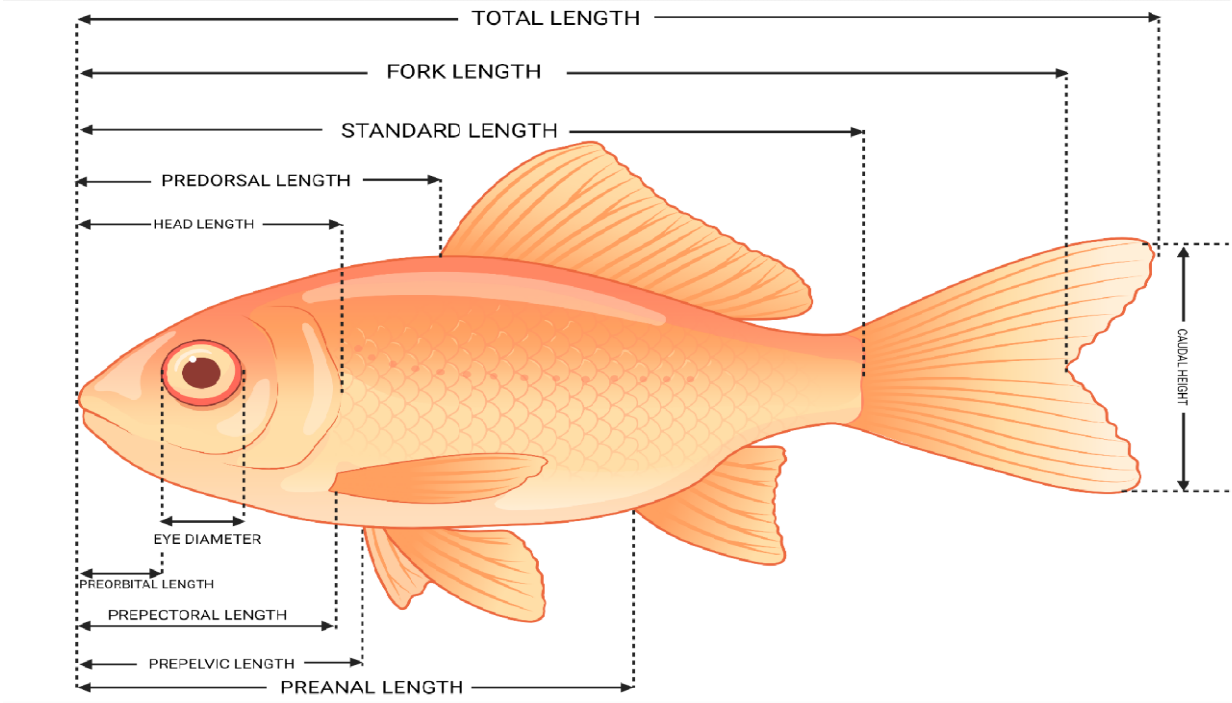

Figure 1. The general diagram of a fish showing various morphometric parameters.

has a point of identification and correspondence, matching between and among populations. Although morphological, physiological, behavioural, and biochemical traits have all been used to identify and categories fish species. Nonetheless, metric (body length, body depth, head length, eye diameter, jaw length) and meristic (fin ray, scale, tooth, gill raker, and lateral line pore counts) measures have been more common [10]. Morphometric analysis long has been used in fish identification and classification and a useful method for determining the discreteness of a particular single species [11] and fish stock assessment. Costa et al. (2003) emphasized the role of morphometric variations in stock identification among groups of fish [12].

Among all other morphometric parameters body length is the most frequently used trait in sampling programmes as it is very easiest and quickest tool to be measured at the spot [13], [14] moreover the weight can easily be predicted from length [5] .Various studies have been done on the morphometric traits of fishes by various researchers. Onsoy *et al*. (2011) conducted a comparative study on Standard, fork length, total lengths (TL) of three popular fish species, and concluded standard length to be more reliable if the museum or preserved specimens are to be assessed as the caudal fin gets brittle with time and increases the chances of break of [15]. Fish preservation procedures, which can induce significant shrinking of specimen, are likely to have the significant impact on alteration of morphometric characters. Not only preservation methods there are number of factors altering the morphometric characters like life stage, quality and abundance of food, size range, health and overall fish condition, additionally sampling strategy, such as size and length range of sample [16, 17].

Length Weight relationship

During development, organisms typically grow in size in all aspects that is length, girth and weight. There are number of parameters like size and age, the quantity and quality of feed, the percentage of fish using the similar food resource, temperature, dissolved oxygen, physicochemical properties of water and reproductive age of fish all influencing fish growth. Throughout their lives, all creatures increase in all dimensions, and their length weight ratio follows a basic pattern. The length-weight relation, which is critical in fishery evaluations, is one of the most popular methods for obtaining accurate biological data. To determine the relative condition or robustness of a population of fish, parameter estimates for the relationship can be compared to overall mean parameters for particular location, parameter estimates from preceding years, or parameter estimates within the different fish groups. The length weight relations are necessary for constructing yield equation; additionally, it can also be used as a trait to distinguish "small taxonomic groups." It also helps with the conversion of one variable to another [18]. Hence morphometric assessment of fishes is useful in the study of ecology and population dynamics.Length weight relationship is expressed as:

$W=aL^b$[19]

Or

Log W = Log a + b Log L

Where W= body weight

L=total length

a=coefficient related with body form

b=exponent

Here a and b, the length and weight parameters are calculated from the given length weight data. The exponent b denotes the type of fish growth. If the fish keeps its shape and its specific gravity constant, it is said to be isometric. The value of exponent 'b' will be 3 if fish maintains its constant shape and its specific gravity [20], means weight proportional to cube of length (cube law). The value of b <3 indicates negative allometric growth while b>3 indicates positive allometric growth. The ideal value of b to be lying within the range 2.5 and 3.5 [21].

Condition factor

Condition factor, also known for fish wellbeing, is a valuable metric for keeping track of feeding intensity, aging, and development phases and growth of fish [22] additionally acting as environmental sustainability indicator [23]. This parameter is also used to measure deviations from the cube law according to which weight of a fish equals to cube of its length. It is considered as an index of physiological state to evaluate fish health. Earlier, formula $K=W/L^3$ was used to calculate.Which was later on replaced by expression $K=W/L^b$ because the exponent 3, is not relevant for vast majority of fish species. It is evident from above equation that K or condition factor is directly proportional to the body weight. So, if the individual lengths from a population are statistically almost same, the condition factor is decided by the weight of fishes [24].

Relative condition factor

Kn or Relative condition factor, assesses a fish's divergence from its expected length or weight and is stated as: observed or actual weight/calculated weight.

Length weight relationship study of fishes in India

A study of LWRs for a species can reveal crucial information and insight about the species' ecology [21], health of that ecosystem and in addition also useful for

comparative growth research of species [9]. In this review we compiled LWRs of some common freshwater fishes based on data from various investigation conducted in India during 2012 to 2020 (depicted in Table 1).

Families	**Species**	**Sampling area**	**Value of a**	**Value of b**	**References**
Scombridae	*Scomberomorus commerson*	Kerala	0.0092	2.883	[26]
Carangidae	*Alepes vari*	Kerala	0.0236	2.722	[26]
	Alepes kleinii	Kerala	0.0085	3.021	[26]
Cyprinidae	*Schizothorax niger*	Dal lake, Jammu and Kashmir	0.028619	2.572044	[27]
	Schizothorax richardsonii	Kumau, Uttarakhand	0.006861	3.027319	[27]
	Schizothorax labiatus	Jhelum Kashmeer	-3.849	2.578	[28]
	Labeo bata	River Ganga Uttrakhand	1.154	1.0116	[29]
	Puntius sophore,	Gomti Uttar Pradesh	-4.28	1.94	[30]
	Labeo bata	Varanasi	0.31	1.84	[31]
	Puntius ticto	Rapti Uttar Pradesh	-2.34	1.94	[30]
	Amblypharyngodon mola	Ganga, Uttar Pradesh	2.21	1.92	[30
	Rasbora daniconius	Ganga U.P.	2.33	1.99	[30]
Siluridae	*Ompok pabda*	River Ganga Uttrakhand	1.488	0.935	[29]
	Ompak pabda	Varanasi	0.37	1.8	[31]
	Wallago attu	Ganga, Uttar Pradesh	1.92	2.02	[30]
Channidae	*Channa punctate*	River Ganga Uttrakhand	0.009	1.205	[29]
	Channa punctatus	Varanasi	0.67	1.53	[31]

Clupeidae	*Tenulosa ilisha*	Narmada River, Gujrat	.0000006monsoon 0.025 winters	3.07 2.76	[32]
Mastacembelidae	*Mastacembelusarmatus*	River Ganga Uttrakhand	2.620	0.993	[29]
	Mastacembalus armatus	Ganga Uttar Pradesh	1.61	2.65	[30]
	Macrognathus puncalus	Rapti Uttar Pradesh	2.33	2.34	[30]
Clariidae	*Clarius batarachus*	Varanasi	0.13	2.09	[31]
Ailiidae	*Ailia coila*	Varanasi	0.0009	3.65	[31]
Clupidae	*Gudusia chapra*	Ganga Uttar Pradesh	2.32	2.06	[30]
Bagridae	*Rita rita*	Varanasi	0.05	2.54	[31]
	Mystis vittatus	Kangsabati River, west Bengal	-4.983 Summers -0.2325Monsoon	1.207 1.110	[33]
	Rita rita	Gomti Uttar Pradesh	1.65	1.32	[30]
	Sperata seenghala	Rapti Uttar Pradesh	-3.43	1.30	[30]
	Sperata oar	Ganga, Uttar Pradesh	2.37	2.30	[30]
Schilibidae	*Clupisoma garua*	Ganga, Uttar Pradesh	-3.32	2.54	[30]
Ambassidae	*Chanda nama*	Ganga, Uttar Pradesh	2.12	1.54	[30]
Osphronmide	*Colisa faciatus*	Ganga Uttar Pradesh	2.21	1.77	[30]
	Colisa sota	Rapti Uttar Pradesh	2.54	1.77	[30]

The length-weight correlation and the condition factor can be used to make comparisons among populations of a particular species from various locations. The LWR equation establishes a statistical relationship between the length and weight variables, allowing to make out unknown variable directly from the known value. According to Pauly, these morphometric relationships and ratios can be used to determine weight based on length measured during yield evaluation [5]. It is also used to calculate expected weight based on length and weight, and as a fatness indicator. The relationship also reveals information about the maturity and reproduction status of fish. The length-weight relationship (LWR) gives crucial information about fish's surroundings, making it useful for proper fish utilization and management and exploitation [5, 33]. The relationship also reveals information about the maturity and reproduction status of fish and also provides crucial knowledge about a fish's surroundings, making it useful for proper fish utilization, management and control.

Conclusion

Morphometric characters, Length weight relationship and Condition Factors are commonly used metrices or indicator in fish biology research because they provide vital information about a fish's physiological condition based on common perception that for individual of a certain length, those that have a larger weight are having better condition and for condition factor the idea is larger fish of same length are in better physiological shape[7]. The value of b can be used to check whether the growth is isometric (b=3) or allometric (b>, < 3). According to this, weight of fish should increase as a cube of length for isometric ideal growth. All fish, however, do not obey the cube law, which could be due to many other factors like availability of food resources, feeding intensity, spawning stress, physiological condition of the fish or physicochemical properties of water. But knowledge of all these factors can be used for assessment of habitat health and physiological stress and pollution level of surrounding water, which can be further used for Conservation and management.

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Assessment of microbial water quality of river Beas and its tributaries: Present scenario and future perspectives

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Abstract: River Beas rises from the Himalayas of Himachal Pradesh. It joins the River Sutlej at Harike inPunjab. River water pollution is a major concern because the river is the primary water source for human consumption. This review evaluates the microbial status of the Beas River and looks at waterborne disease incidence in India and around the world, as well as its limitations. Few studies have been conducted on river Beas. Fecal coliform and total coliform are reported elevated in the river in all seasons. To precisely assess the risks posed by waterborne disease, it is essential to understand microbial distribution and strategies within water distribution networks, as well as to employ methodologies capable of detecting not only the presence but also the viability and infectivity of the microbe. Improper dumping of industrial and municipal wastes and a lack of water filtering and disinfection facilities are major reasons behind water contamination. There is an immediate need for emergency measures to prevent future degradation of water quality and to enhance existing water quality to safeguard the population from widespread waterborne illnesses.

Key words: Beas, coliform, microbial contamination, *E. coli*.

Introduction

The river water quality is considered an important parameter for sustaining industrialization, urbanization, agriculture, and transportation purposes but a continuous deterioration of the river water quality has been noticed due to anthropogenic activities. Chenab, Jhelum, Ravi, Sutlej, and Beas river come under the Indus river system[1]. The River Beas is a significant contributor to the Indus river system, and it is the only tributary of the Indus system that is entirely contained within India [2]. River Beas is also called Vipasa in Vedas and Hyphasis in Greek. It originates from Beas Kund which lies in the Rohtang Pass of Dhauladhar Range at an altitude of about 4060 meters.The Beas River rises in the Himalayas of central Himachal Pradesh, India, at 31.51° N latitude, 77° 05′ E longitude, at an elevation of 2050 meters above sea level [3]. River Beas covers 923 km, which includes 297 Kms of the main river and its tributaries cover a stretch of 623 km [4]. Parvati, Sainj, Hurla, Tirthan, Suketi, Uhl, Looni, Binwa, Bakkar, Baner, Neugal, Banganga, Chakki, and Mankhad rivers are important tributaries of Beas in Himachal Pradesh [2]. Beas river is a perennial river that receives its water from snow and rainfall and is surrounded by lush flora [5]. The Beas River and tributaries of Beas are the main sources of drinking waterfor the people of Himachal Pradesh's Hamirpur, Mandi, Kangra,and Kulludistricts [6]. Because the Beas is the primary supply

of water for both human use and aquatic species inhabitation in the river, any pollution of water of the river poses a huge environmental threat [7]. The water quality indicators in the river Beas are significantly affected by domestic, agricultural, and industrial activities [3]. They proved that as river water runs down from Himachal Pradesh to Punjab, the river's water quality starts to deteriorate.

In India, approximately half of total fecal matter is released into rivers water, and other bodies of water without appropriate treatment [8]. When fecal contaminants like *Escherichia coli*, *Salmonella*, *Shigella*, *Vibrio cholerae* enter thewater supply system, the most dangerous form of water contaminationoccurs, andingesting contaminants from water sources can cause many illnesses [9].

E.coli is associated with multiple health problems including diarrhea [10]. According to the WHO, diarrhea accounts for approximately 4.1 percent of global daily disease burden and kills 1.8 million people every year[11]. The waterborne outbreak of *E. coli* and *Campylobacter jejuni* in 2000 in Walkerton caused seven deaths and over 2,300 illnesses after which more stringent waste management and risk assessment laws have been implemented in several municipal systems all over Canada [12].

Role of microbes in water-born diseases

Water-borne disease is considered to be a significant health concern in both the developing and developed worlds[13]. Fecal contamination of water by humans transmits microorganisms that result in both diarrheal diseases, like cholera, and extremely deadly non-diarrheal diseases, like Hepatitis A, when adequate sanitation is lacking[14]. Many outbreaks have already been caused by water-borne diseases (gastrointestinal distress,diarrhea) caused by numerous viruses,bacteria, and protozoans [15]. According to WHO (2010), over 2 billion people around the world have no access to safe and contamination-free water for drinking purposes, which causes approximately 2 million deaths each year, out of which 1.4 million reported deaths are of children. Continuing to enhance the water quality could indeed decrease the risk of disease by approximately 4% (WHO 2010). Indicator organisms, *i.e*., water-borne pathogen marks of water supplies, are commonly used to evaluate pathogen concentrations in water supplies. In general, such indicator bacteria are non-pathogenic andcan be used to signify if the water has been contaminated with faeces and presence of pathogens in water [16]. Observing the concentrations of biological indicators (such as fecal coliforms and *E. coli*) is a very commonstrategy for estimating the potential pathogen loads in natural water bodies[17]. Virulent *Escherichia coli* strains, including enteroinvasive, enteropathogenic, enteroaggregative as well as Shiga toxin-producing *E. coli* (STEC) are indeed a significant and powerful class of water-borne pathogenic organisms [18].

Microbial contamination in groundwater

Groundwater has been widely used as the major source of household drinking water all across the globe, but also water pollutants greatly increase the hazard to human health. Numerous research studies have found that groundwater contains a fairly stable concentration of pathogenic microorganisms including enteroviruses, *Salmonella*, *E.coli* [19]. Pathogen transport from surface water to groundwater makes groundwater more vulnerable [20]. Total coliforms are usually monitored as a pollution indicator and under normal environmental conditions, non-fecal coliforms have the potential for proliferation among total coliforms, total coliforms, on

the other hand, might well indicate the presence of fecal coliforms, which seem to be common indicators of fecal contamination (WHO 2004). *E. coli*, which commonlyresides in the digestive tracts of many animals is the most suitable coliform bacteria to demonstrate warm-blooded fecal pollution [22].

Microbial contamination in surface water

Surface water is especially susceptible to pollution both from point sources (described as distinct inconveniences such as pipes or man-made ditches used to discharge pollutants into river systems) and non-point sources including run-offs from urban and suburban streets, farming land, microbes from atmospheric water precipitation, and so on [23]. Human pathogens that contaminate water include *Escherichia coli*, *Salmonella typhi*, *Campylobacter*, *Shigella*, *Aeromonas*, *Vibrio cholera*,*Yersinia enterocolitica*, *Pseudomonas aeruginosa*, etc. Most of these pathogens cause serious health risks. As long as they exist in any water supply source, they can cause disease [24]. These infectious diseases include viral hepatitis, polio, typhoid and paratyphoid fever, amoebic and bacillary dysentery, botulism, cholera, schistosomiasis, salmonellosis, primary amoebic meningoencephalitis, and Giardiasis. The pathogens of these diseases exist in the urine and faeces of infected people, and can enter any body of water that caneventuallybeusedfor drinking and other household purposes after being discharged (WHO, 2004). These organisms may also be present in the environment.

Water quality parameters of beas during summer, winter, and monsoon seasons

Sharma and Walia studied water quality parameter status in selected sites of Beas in Himachal Pradesh during the summer season. Physio-chemical properties including total dissolved solids (TDS), pH, alkalinity, conductivity, temperature, turbidity, etc., Metals such assodium, calcium, magnesium, etc. and Non-Metals such asfluoride, chloride, nitrate, biochemical oxygen demand (BOD), chemical oxygen demand (COD), Coliformand *E. coli* were determined and all physio-chemical parameters were within the limits prescribed by WHO, 2011 and BIS, 2012 except Iron,Cadmium,Total Coliform, and*E. coli* in all sampling stations[2]. The water quality parameters (different physio-chemical, and biological parameters) of Beas in the state during the winter were observed. Except for 2 stations, coliform and *E. coli* were found in all the selected sampling stations. All other physicochemical parameters, except for pH at 1 station, were within the permissible limits prescribed by the WHO 2011 and BIS 2012 for drinking water in India [25]. During the Monsoon Season, Sharma and Walia (2016) monitored the water quality parameters of the Beas River in Himachal Pradesh. Turbidity, lead, and iron levels were reported to be significantly higher than the BIS 2012 acceptable limit for drinking water in India. Also, *E. coli* and coliform were found in all of the selected sampling stations. The rest of the other physiochemical parameters were found within theWHO 2011 and BIS 2012 limits [5]. The mean values of Total coliform and Fecal coliform of Beas in Himachal Pradesh were reported as 1451 MPN/100 ml and 479.16 MPN/100 ml [26].

Discussion

Water pollution is a serious environmental issue, particularly in underdeveloped and developing countries. Most dangerous type of water contamination takes place when fecal contaminants like *Escherichia coli* encounter the supply of water. Because the majority of waterborne disease is caused by

fecalcontamination of water bodies, the microbiology of water is strongly determined by the necessity to define coliforms and *E. coli* as markers of fecal pollution [27]. Pollutants ingested into the water system cause a variety of problems[9]. During the summer, winter, and monsoon seasons,Sharma and Walia 2016bmonitored the status of quality of water of Beas in the region of Himachal Pradesh and measured Alkalinity, conductivity, and other parameters such as pH, total coliform and fecal coliform. The status of pollution in the River Beas in India was studied by Kumar et al. 2016. They created a water quality index utilizing nine factors, which were discovered to be effective. COD, BOD, and Total Coliform were over BIS allowed limits. River Beas Water Quality Index has been regarded as being of medium quality [3]. The mean fecal coliform values of river Beas from the year 2002 to 2015 were reported between 42 to 375 MPN/100 ml which is alarming as there is no permissible limit for fecal coliform because it can cause serious gastrointestinal diseases [28]. The mean value of Total coliform of Beas in Himachal Pradesh was reported as 1451 MPN/100 ml and that of Fecal coliform of the riverwas reported as 479.16 MPN/100 ml [26].

The primary sources of microbiological pollution of the Beas River include improper industrial and municipal waste disposal, inadequate sanitary and drinking water supply systems, and a lack of water filtering and disinfection techniques. Weak enforcement of environmental regulations and a lack of public knowledge exacerbate the situation. Waterborne infections are common in the area, but a lack of appropriate diagnosis and record-keeping at hospitals makes it difficult to determine the actual burden of water-related disorders. There is an urgent need for emergency measures to prevent future degradation of water quality and to enhance current quality to safeguard the population from prevalent waterborne illnesses. To treat industrial and domestic wastewater, necessary steps must be taken, before discharging it into the water. These are required to protect the aquatic biodiversity of the Beas River.

Conclusion

This review looks at finding from many disciplines to improve understanding of microbial contamination in the Beas River. The global prevalence of pathogen contamination is a severe problem, and it is critical to improving awareness ofmajor sources of pathogen and their impact on water sources. On the river Beas, only a little research on contamination caused bypathogens has been done at laboratory or field level; more focus should be placed on such studies to advance our understanding of pathogen-environment interactions. Monitoring of bacteriological quality of water should be carried out more. Creating new models and improving widely used modelling techniques to forecast pathogen levels in water would most likely aid in analyzing pathogen contamination.Despite the constraints of conventional models for predicting pathogen contamination, advancements and the establishment of innovative models have always been required to more precisely forecast pathogen levels. Trying to integrate knowledge from various fields such as microbiology, hydrology, and ecology would enable a better knowledge of levels of pollution and potential causative factors of pollution, andas a tool for developing long-term plans for improving water quality.

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A review on chromosome study on Aphid species

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Abstract: Crops are destroyed by different number of insect-pests. Aphids are agriculturally important pest feeds on plant sap most of the species of aphids are found in the temperate climate. Rate of multiplication of these insect are very high. Different aphid's species contain different number of chromosomes. To study the chromosome number karyotype of aphids species were detected. Aphid chromosomes are devoid of a central centromere, and kinetic activity is dispersed across the whole length of the chromosomes. Banding techniques allow enough chromatin differentiation to produce patterns that allow homologous chromosomes to be reliably identified.

Keywords: aphids, karyotype, chromosomes, chromatin.

Introduction

Aphids are economically significant insect pests. Aphids are microscopic insects that belong to the Hemiptera order and have a global distribution, with the majority of species found in temperate regions of the planet, where at least one species of aphid colonises one out of every four plant species [1]. There are about 5000 described aphid species worldwide in about 510 presently accepted genera infesting about 300 plant families [2,3,4,5].

Aphids have a very high rate of multiplication and build up large populations in a very short time [1]. Most of the aphids are heteroecious, exploiting both primary and secondary host plants. Holocyclic aphids have a series of parthenogenetic cycles consisting of a single sexual generation, whereas holocyclic aphids have deleted the sexual portion of their life span [6].

Review of Literature

Life cycle and occurence of several morphs in the life cycle of aphids pose many taxonomical problems. Studies on the chromosomes of aphids started in the beginning of the twentieth century. Stevens [7,8] was the first to investigate chromosomes in *Aphis rosae* and *Aphis oenothera* germ cells to better understand sex chromosomal activity and the occurrence of parthenogenesis in aphids. However, rather than scientific names, she named the aphid species after their host plants; the data she gathered could not be trusted. Later, several workers [9,10] studied the cell division, sex chromosomes, parthenogenesis and sex determination in aphids.

Makino (1951) reported the number of chromosomes in 93 different aphid species. However, according to Fox (1956), out of the 93 species of aphids whose chromosome numbers are stated by Makino, at least 31 of

them are meaningless due to a lack of accurate identification. Basilova *et al*, studied the karyotypes of seven species of *Cryptomyzus* [11].

Because aphid chromosomes have experienced significant evolutionary modifications, there is still debate about whether aphids with a high or low chromosomal number are primitive [12]. Gut (1976) looked studied the number of chromosomes in parthenogenetic females of 55 different aphid species. He stated that because the taxonomy of aphids is not stable at the genetic and species level, taxonomic conclusions should not be drawn from the current chromosomal data.

Aphids are an intriguing cytogenetics model because their chromosomes are holocentric, with centromeric activity distributed throughout the entire chromosomal axis [13,14,15,16]. Because they operate as if the spindle attachment is not localised during mitotic anaphase, these chromosomes are also known as holokinetic. The chromatids migrate apart in parallel, rather than forming the iconic V-shaped patterns seen when monocentric chromosomes move [14]. Aphids may be especially useful for investigating holocentric chromosomal architecture since mitotic chromosomes may be found to be very effective from early embryonic tissues [14,15].

Many chromosomal rearrangements in aphids have already been noted, mostly involving autosomal translocations and fission [17,18,19] and they may have played a significant role in aphid evolution because they can influence host choice, as shown in the corn leaf aphid, *Rhopalsiphodium maidis* (Fitch), which feeds on barley and sorghum [18].

According to Blackman [20] certain dominant genera in a group contain disproportionately more species. These were the oldest genera, which had undergone substantial diversification and were therefore unlikely to retain primitive characteristics. As a result, in order to determine which chromosomal sequence was the original, cytological data must be carefully selected from a systematic standpoint.

Sethi and Nagaich [21] found 2n=12 and 14 in *Myzus persicae* from India. The chromosomes of 27 aphid species were published by Khuda-Bukhsh and Kar [22]. The chromosomes of seven aphid species from the Northwestern Himalayas were published by Gautam and Sharma [23]. Panigrahi and Patnaik [24] looked at how many chromosomes each aphid species had.

Various researchers from Himachal Pradesh have been studying aphid chromosomes [25,23,26]. Gautam and Dhatwalia [27] described the karyotypes of 21 different aphid species. 2n ranged from 6 to 16 in these species. For the first time, Gautam and Kumari [28] reported the karyotype of the green apple aphid, Aphis pomi De Geer, from India. This species has eight diploid chromosomes (2n=8), according to research. Aphid karyotypes infesting Quercus and Rhododendron were described by Gautam and Kumar [29].

In the study of aphid cytogenetics, kyotype banding is quite valuable. Aphid chromosomes are devoid of a central centromere, and kinetic activity is dispersed across the whole length of the chromosomes (Smith, 1960). Banding techniques allow enough chromatin differentiation to produce patterns that allow homologous chromosomes to be reliably identified. C banding chromosomal preparations in Eucerphis species were obtained by Blackman (1976). Lauritzen (1982) employed Q and G banding patterns to identify the chromosomal rearrangements in Myzus persicae.

There have been reports of chromosomal diversity in a number of aphid species. Myzus persicae (Blackman, 1971) is a well-known example of chromosomal variants, with 2n recorded as 12, 13, and 14. Changes in chromosomal number and organisation have been seen in this species. This species' normal female has 2n=12. This species from India was likewise found to have the same diploid chromosomal number [21].

Conclusion and Future prospective

Aphids are insect species with a wide range of chromosomal number variations. Aphids have a complex life cycle and chromosomal arrangement is examined. More research is needed to determine why various species of aphids have such big variances in chromosomal counts. Characterization of Karyotypes, as well as chromosomal investigations combined with enzyme polymorphism, will undoubtedly shed light on Aphids species' evolutionary patterns in the future.

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Functional foods from plants: a new perspective for healthy life

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Abstract: Food is the basic requirement of all living beings. Food is not just a source of energy and basic nutritionbut alsohas health promoting role. Functional food is the food that provides health benefit beyond basic nutrition due to the presence of bioactive compounds. Phytochemicals are the most potent sources that can be exploited for the commercial production of functional food products. Polyphenols, carotenoids, phytosterols dietary, fibres, glucosinolatesetc are some examples of phytochemicals with functional value. Scientific validation and verification of this claim of health benefit from various plants is required for their approval as functional food ingredient as well as industrial exploitation. The present paper reviews the phytochemicals that are potential candidates to be used as functional food ingredients. It also provides an overview of development of concept of functional food development and various definitions proposed by different organizations and authors. Future of the functional food industry depends upon their approval by regulatory organizations of each country for which scientific validation through clinical trials is necessary. Keeping in view this some clinical studies on the health promoting role of bioactive components of plants have also been mentioned.

Keywords: Functional foods, nutraceuticals, bioactive compounds, phytochemicals, antioxidant, anti-inflammatory.

Introduction

Food is the basic requirement of all living beings that sustain life. It has been believed for generations, that food can provide additional benefits beyond merely providing energy. Healthy lifestyle is often correlated with the balanced nutritional intake. Medicinal properties of the food are well known to mankind since ages, evident from the famous old saying of Hippocrates, “Let food be the medicine and medicine be the food. Whosoever gives these things no consideration and is ignorant of them, how can he understand the disease of man” The concept of functional food was first introduced in Japan in 1980s with the goals to improve quality of life, control healthcare cost and increase life expectancy. A related term ‘nutraceuticals’ was coined by DeFelice for any substance food or part of a food that provide medical or health benefits, including prevention and treatment of disease [1].

Several age related chronic disorders and other degenerative diseases have been found to be connected with our daily dietary habits by various authors[2,3,4].Large expenditure due to very high cost of medication and

health care facilities decrease the standard of living and thus the quality of life. In addition to this chemically synthesized drugs mostly have several side effects thus negatively affect our health. Development of functional foods from natural sources is an active area of research due to continuously increasing demand of natural products with least side effects. Market of functional food is rapidly growing but scientific validation of claimed food products with functional value through in vitro studies and clinical trials is still scarce.

There is a long history of utilization of plant and plant products for curing various ailment and diseases. Plants are suitable candidates for the functional food development as they contain certain metabolites/chemicals which affect cellular process and thus our health positively. These metabolites could be successfully exploited for the commercial production of nutraceutical products. Developing countries like India that are rich in biodiversity and have great wealth of traditional knowledge regarding use of local natural resources for medicinal purposes can be the central points of such research activities.

Loss of traditional crop cultivations and food culture, increased awareness on link between nutrition and health, deteriorating health due to busy lifestyle, urbanization and side effects of the allopathic drugs are some of the reasons that strengthens the need of scientific investigation in area of functional food development. These can also help in improving the economic conditions of poor countries with rich tribal culture along with the preservation of their traditional knowledge and biodiversity. Developed countries like USA, Europe, Japan, Germany, France, Russia etc are very much interested in the natural food products with heath benefits which can be formulated and supplied by the developing countries.

Current review presents the information available on the concept of functional food with special focus on the phytochemicals that could be exploited for their functional value. To highlight the importance of clinical trials few clinical placebo studies are also summarized. It is expected that the provided information will encourage the scientific community of the developing nations to do the further research in this area and will also increase the interest of companies in commercialization of the developed functional food products.

Concept of functional food

'Physiologically functional food' term was first used in Japan (1984) by the Japanese Ministry of Education Science and Culture with the further introduction of category 'Foods for Specified Health Uses' [FOSHU] in 1991 [5]. Since then many definition of functional foods has been proposed by various institutes, organizations and authors but so far there is no universally accepted definition for this category of food. After reviewing several definitions some selected definitions are listed in table 1.

Bioactive compounds from plants as functional food ingredients:

Bioactive compounds are any compounds present in food either from plant or animal source which affect the body of organism that consumes it. In several studies intake of these bioactive compounds has been linked to the lower incidence of chronic diseases [8,9,10]. Plants produce many such compounds which can help in improvement of health, thus can be effectively utilized as functional food ingredient [11]. These compounds preserve their characteristics

even after extraction with various solvents. The frequently used methods for extraction of bioactive phytochemicals from plants areSoxhlet extraction,hydrodistillation and maceration [12].

Clinical studies

Despite of the presence of enormous literature claiming health benefits of plant bioactive compounds, the numbers of clinical trials and studies to prove this claim are still insufficient. There aresome clinical studies available on the health promoting role of functionl foods that are listed in table 3.

Table1: Definitions of functional food

Definition	Reference
"Foods which are, based on the knowledge between foods or food components and health, expected to have certain health benefits, and have been licensed to bear a label claiming that a person using them for specified health use may expect to obtain the health use through the consumption thereof"	FOSHU, Japan 1991
"Foods or food component that may have health benefits that reduce the risk of specific diseases or other health concerns"	National institute of Nutrition, 2000
"A functional food is a conventional food or a food similar in appearance to a conventional food, it is part of a regular diet and has proven health-related benefits and (or) reduce the risk of specific chronic diseases above its basic nutritional functions"	Health Canada 2006
"Foods that encompass potentially healthful products, including any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains"	National academy of Sciences, USA
"Functional foods serve naturally primarily the supply of nutrients, but they offer a special advantage for the health"	European food information council
"Foods that with their specific health effects could, in the future, indicate a new mode of thinking about the relationships between food and health in everyday life"	Ballali and Lanciai 2012
"Industrial processed or natural foods that when regularly consumed within a diverse diet at efficacious levels have potentially positive effects on health beyond basic nutrition"	Granato et al., 2017

Source- [6,7]

Fig1. Functional food effects on human health

Table2: Physiological effects of the plant bioactive compounds

Phytochemical	**Plant source**	**Physiological effect**	**Reference**
β-Carotene	Fruits and yellow, orange ,green leafy vegetables	Antimutagenic, antioxidant, vitamin A precursor	[13][14][15][16]
Lutein	Green leafy vegetables	Improvement of cognitive functions, anti-inflammatory, antioxidant, antidiabetic, anticancer, prevent age related eye diseases	[17][18] [19][20][21]
Lycopene	Tomato, melon, peach	Anticancer, antixidant, cardiovasular protective	[22][23][24]
Zeaxanthin	Spinach, Kale	Antioxidant, anti-inflammatory, chemopreventive, prevents ocular diseases, cognitive function	[25][26][27]
Curcumin	Curcuma	Antioxidant, antidiabetic, nuroprotective and cardioprotective	[28][29][30]
Limonoids	Citrus fruits	Cardioprotective, anticancer and pulmonary tissue protection	[31]
Saponins	Legumes(Soyabean)	Immunostimulatory, cytotoxic anticancer, antioxidant and cholesterol lowering activity	[32][33]

Chromanols	Whole gain germ /bran and Palm oil	Cardiovasular protection, inhibit breast cancer cell growth	[34]
Betalain	Beetroot, red dragon fruit, pricky pear (Opuntia spp), Amaranthus.	Antioxidant, anticancer, antilipidemic, antimicrobial	[35]
Phenolic acids	Tomatoes, carrots, garlic,peanuts, plums, cherries, apples, kiwi	Antioxidant,anticancer, anti-inflammatory, antiproliferative	[36][37][38][39] [40]
Flavonoids	Apples,onions, broccoli,whole grains,nuts,berries, citrus fruits	Reduce the risk of cardiovascular diseases, cancer, antioxidant activity	[41][42][43]
Stilbenes(resveratrol)	Grapes, Peanuts	Cardioprotective and anticancer	[44][45]
Catechins	Green and black tea	Reduces the risk of stroke and cancer	[46][47]
Lignans	Flaxseeds, lentils, soybeans, cereals, carrot, garlic and carrots, pumpkin and sesame seeds	Antiestrogenic, antioxidant, reduces risk of cardiovascular diseases	[48][49]
Dietary fibres: (B-glucan, resistant starch and oligosaccharides)	Cereals-Wheat, Barley, Oat	Reduce LDL, cholesterol level and risk of coronary heart diseases, stimulate growth of gut bacteria	[50][51]
Omega-3 fatty acid (Linolenic acid)	Flax, walnuts, canola oil	Antiobese, increase immunity and bone density	[52]
Phytosterol	Cereals, fruits, vegetables, peanuts,	Cholesterol lowering, antioxidant, anticancer, antiathergenicity, anti-inflammatory, anti- Alzheimer	[53][54]
Sulfur compounds	Cruciferous vegetables, onion, garlic	Anticancer, antiviral, antioxidant, antiatherosclerotic, antibacterial and antifungal	[55][56]

Table3: Some clinical studies on plant bioactive compounds

Reference	**Study design**	**Number of subjects**	**Health condition of participants**	**Treatment**	**Outcome**
[57]	Placebo control	14(Male)	Hypertriacylglycerolemic	High fat meal (72% fat)+Extra virgin olive oil	↓Inflammatory markers
[58]	Placeb	50	Hypercholesterole	Daily oral dose of	↓Total

	o control	26-Male 24-Female	mic	Nutraceutical combination (500mg berberine, 200mg red yeast rice and 100mg policosanol)	cholesterol level, LDL-Cholesterol and Triglycerides
[59]	Placebo control	11(Male)	Overweight atherosclerosis prone	High fat meal(30% fat)+ 250g black current based juice	No effect on inflammatory markers
[60]	Placebo control	45 (>18Y)	Distal ulcerative colitis (UC)	NCB-02 enema plus oral 5-ASA (Standardized curcumin preparation)	↓UC disease activity index
[61]	Placebo control	36 boys	Duchene Muscular Dystrophy (DMD)	ω-3 long chain poly unsaturated fatty acid	↓Pro-inflammatory markers ↑ Anti-inflammatory markers
[62]	Parallel placebo control	138 79-male 59-female	With Type-II diabetes mellitus(44) At risk (94)	Phytosterol added low fat spread (2g/d)	↓ Total cholesterol level, LDL-Cholesterol and Triglycerides (Fasting serum level)
[63]	Placebo control	51 29-Male 22-Female	Healthy subjects	Active supplement containing 10mg Lutein+2mg Zeaxanthin to active supplement group	↑ CNS Xanthophyll level, Improved cognitive function

Future prospects

There is a growing trend in consumers' desire for food products that are naturally functional. The establishment of industries dedicated to the research and production of functional foods may be extremely beneficial to the market's favorable growth. Multidisciplinary research is needed for the success of functional food industry. The consumers should be well informed with the health benefits of the functional food products. The best way to achieve this is the establishment of strong alliance between science, technology and health. Complex clinical trials are required to provide evidence in support of the claimed health benefit. This will further increase the interest of peoples in functional value of natural food products. Functional food utilization could improve the quality of life by lowering the incidence of diseases. In addition to this, increased value of functional food could also help in protecting

several neglected traditional underutilized crop plants with health benefits from extinction. Considering the significance of functional foods, this area should be explored by planning multidisciplinary research activities.

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Review Article

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Valeriana jatamansi: Its Traditional Uses, Phytochemistry and Pharmacology

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Abstract: *Valeriana jatamansi* a subtropical Himalayan herb that grows as a perennial herb. "Valerian" is the most commonly used name for the plant. The plant is a member of the Valerianaceae family. Because of the various organic components such as sesquiterpenes, lignanoids, alkaloids, as well as flavonoids are present in the rhizomes and roots of the plants and used traditionally for the treatments of ulcers, hyperbilirubinemia, chronic cough, seminal weakness, body infections, leprosy, and insomnia improvement. It is very important for antioxidant, antibacterial, anxiolyticand antibacterial activities.

Keywords: *Valeriana jatamansi*, traditional uses, pharmacological properties, phytochemistry

Introduction

Plants are well known for their significance. The plant kingdom is a great source of potential medicines, and there has been a growing recognition of the value of medicinal plants in recent years. *Valeriana jatamansi* is one of the medicinal plants which have various medicinal properties. The genus Valeriana is member of a family Valerianceae (Caprifoliaceae), which contains of approximately 250 sp. that are found all over the world's temperate regions. In India, 16 species were discovered, with two sub-species and five species of the genus found at high altitudes in the Central Himalayan region. “Valeriana” is the commonly used name for this plant. It is also referred to as India Valerian, Muskbala, Sugandhbala, and Tagar [1]. The species can be found throughout the temperate areas of the Himalayan, from the Kashmir to Bhutan and the Khasi hills. Naturally it grows at elevations ranging from 1800 to 3000 meters in the Northwestern Himalayan, and within 1200 and 1800 meters among Assam and North-East India.

V. jatamansi a small perennial herbaceous plant that grows approximately 50 cm tall with creamy rhizome bundles covered in linear descending fibres, pubescent stem as well as radical leave 1–3 cm in length. Flower seems to be white and pink-tinged, and fruit have always been crowned by something like persistent pappus calyx. Flowering and fruiting occur between March and April. The Seeds ripen between April and May. *V. jatamansi* a plant that is used in the Ayurvedic and Unani medical systems. Anxiety, blood and circulatory ailments, breathing problems, chronic cough, liver disease, seminal weakness, cardiovascular debilitation, and body infections are treated with the roots and rhizomes. Its herbal oil is popularly used in fragrances and insect repellent formulations. The root of *V. jatamansi* usually contains 0.8 percent essential oils which are used at the pharmaceutical

companies as well as hair treatments [2, 3]. Their root has been used in the form of powder in doses ranging from 1-3 g [4].

The species has been observed to be a psychoactive agent and a natural source of valepotriates [5]. *V. jatamansi* contains valerenic acid and valerinone, which are sources of the drug valerian. The drug valerian is ranked eighth among the highest herbal remedies[6].

Classification

Kingdom	Plantae
Class	Asterids
Order	Dipsacales
Family	Caprifoliaceae
Genus	*Valeriana*
Species	*Jatamansi*

Synonyms

Valerianawallichii, V. mairei, V. harmsii, and V. hygrobia

Vernacular names

India Valerian, Nihani, Tagar, Muskbala, Sugandhbala (Hindi), Jatamansi, Natah, Tagarh (Sanskrit), Shadamangie, Takaram (Tamil), Takaram (Malayalam).

Fig: *Valeriana jatamansi*

Phytochemical constituents

The main chemical components from rhizomes and roots of *V. jatamansi* arevalepotriates [7], flavonoids and flavones glycosides [8,9], lignans [10], sesquiterpenoids [11], phenolics [12], essential oils [13] and other phytochemicals. "Chatinine, norphoebine, thaliperphine, nantenine, phenanthrene, phoebine, dehydroaphine, valerine, valeriane, and oxoaporphine are all alkaloids. Valeriana flavonoids include acacetin, hesperidin, and methylapigenin, as well as diosmetin, luteolin, quercetin, kaempferol, linarin, and luteolin. Volatile oils, essential oils, sugar, bitter extractive matter, starch, gum, resin, and ketones" are among the phytochemical constituents [14, 15].

Traditional uses

Root of this plant are used in traditional medicine for the treatments of ulcers, convulsions, asthma, severe wheeze, jaundice, seminal weakness, vascular debilitation, and body infections, leprosy, general weakness, as well as sleep improvement. *V.jatamansi* roots or rhizomes are suggested for the treatment of insomnia

as well as body fluid, cardiovascular, and mental problems. It activates the central nervous system, behaves as a nerve tonic, and utilised to treat nervousness and muscle spasms. The plant has been used as a stimulant, hypoglycemic, and tranquillizer. The oil is used in the production of perfumes as well as the formulation of insect repellents [16].

Pharmacological activities

V. jatamansi has been observed a variety of uses and pharmacological activities.

Neuroprotective and stress effects

Various substances are obtained from *V. jatamansi* have also shown different degrees of efficacy in reducing tension and nervous abnormalities. This herb's extract has been used to reduce the tension, nervousness, and depression [17]. The genus has also been found useful for cerebro-spinal system, hypochondriasis, sleeplessness, headaches, and neurosthemia [18]. The effects of the chlorophyll and water extraction observed on ischemia and reperfusion- induced brain damage was significantly reduced in terms of reduced ischemic size, increased short term memory, motor control as well as lateral drive reactions [19]. A study was performed on 121 adult people with sleep disruption of at least four weeks, and participants were given 600 mg of valerian or a placebo for four weeks. There was no improvement at first, but after two weeks of treatment, there was a significant global improvement in the valerian group, but no meaningful changes in other measures. After four weeks, all measures of sleep and mood improved significantly in favor of valerian [20].

Antioxidant activity

The polyphenol and flavonoid content of dried roots of *V. jatamansi* was determined using methanolic, chloroform, and aqueous extracts. Three solvent extractions of *V. jatamansi* root as well as essential oils (100 g/ml) were evaluated, using the 2, 2-diphenyl-1-picrylhydrazylhydrate (DPPH) radical-scavenging and ferrous chelation power method for their antioxidant activities [21]. When compared to essential oils, methanolic extract was found to be superior [22]. In another study, hydroalcoholic extracts of *V. jatamansi* were evaluated for antioxidant and anti-inflammatory activity by DPPH free radical scavenging method as well as percentage inhibition of denatured proteins, with diclofenac sodium used a standard reference. Presence of bio-active compounds like flavonoids, polyphenols and tannins, resulted in a significant reduction of various mediators of inflammation [23].

Anxiolytic activities

The anxiolytic activity was tested with an iridoid fraction extracted from *V. jatamansi* rhizomes and radix using D-101 resin. The significant components were preliminary analysed using TLC, UV spectrophotometry as well as HPLC, and its anti-anxiety impacts on 6 mg/kg, 9 mg/kg, as well as 12 mg/kg had been assessed by using the Elevated plus maze technique, Vogel's drinking conflict technique, Open field drink technique, as well as LDB technique. Using ELISA techniques, mode of actions was demonstrated by regulating GABA levels [24]. The anxiolytic characterizations of *V. jatamansi* were also examined in mice [25].

Anti-inflammatory activity

The anti-inflammatory activities of water and methanolic extracts of *V. jatamansi* rhizomes were demonstrated. It reduces inflammation in rats using the carrageenan-induced paw edoema method at doses 100, 150, as well as 200 mg/kg for

appearance of saline which is used a control and aspirins as standard drugs. Extraction markedly reduced inflammation by inhibiting the synthesis of inflammatory mediator's histamine, prostaglandin, and serotonin at 200 mg/kg dose [26]. Essential oils are extracted from the entire plant of *V. jatamansi* suppressed xylene as well as induced topical anti-inflammation. This was discovered that informative applications of essential oils to mouse ears occurred in effective inhibition of intense edemia stimulated by xylene, implying that this herb's topical anti-inflammatory effect is significant compared to the standard drug diclofenac [27].

Antibacterial activity

V. jatamansi has been shown to various antibacterial and antifungal properties against a diverse range of pathogenic bacteria and fungi [28, 29]. Antimicrobial activity in several solvent systems (methanol, chloroform, hexane, and water) was observed to be quite efficient than positive controls [30]. Hexane, chloroform, or methanolic extracts of *V. jatamansi* rhizomes were tested for antibacterial activity. *Escherichia coli, Enterobacter aerogenes, Klebsiellapneumoniae*, and *Hafnia alvei* generate increased spectrum B-lactamase, which was evaluate using a double disc diffusion assay with three solvent extracts [31]. Hexane extract inhibited urinary tract infection significantly and are used in conjunction with other antibiotics as a new experimental therapy [32].

Hepatoprotective response

Cirrhosis of the liver develops as a result of hepatocellular injury induced by chronic hepatitis, alcoholism, non-alcoholic hepatic steatosis, innate metabolic error, and a diverse range of organic and toxic compounds. This is really the terminal phase of hepatic cirrhosis that has resulted in liver shrinkage, portal hypertension, and liver failure [33, 34]. The effect of a *V. jatamansi* dried rhizome extract on cell proliferation in an animal study of cirrhosis of the liver, cell growth. In thioacetamide-induced liver cirrhosis, oral administration of this herb extracts partially reversed the high amount of alkaline phosphatise, γ-glutamyl transferase, as well as other biomarkers of hepatotoxicity, and drugs metabolising enzyme [35].

Conclusion

This review provides a comprehensive overview of phytochemical components, medicinal values, and therapeutic activities. This species are commonly used in both traditional and innovative drug. *V. jatamansi* is an herb that grows in high altitudinal range of the Himalayas and used in Traditional medicinal system due to its various medicinal properties. It is also an important Ayurvedic plant with a wide range of uses. The plant's roots and rhizomes contained a significant amount of organic components like iridoids, liganoids, valeriandoids, and valepotriates that are used for treatment of a variety of diseases. Recent researchers suggested that it exhibits various activities like anxiolytic, anti-inflammatory, antioxidant and anti-stress activities. These observations validate the ancient assertions of Ayurveda about the therapeutic potential of *V. jatamansi*.

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Review Article

Devi et al.

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A review on the ethno-botanical, phytochemistry and pharmacological activities of *Arnebia euchroma* (Royle) Johnston: A critically endangered medicinal herb

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Abstract: *Arnebia euchroma* (Royle) is an important critically endangered medicinal herb distributed in the Himalayan subalpine and alpine zones. It is a high-value herbaceous perennial plant, commonly known as 'Ratanjot', belonging to the Boraginaceae family. Morphologically, this herb has hairy stems, infundibular corolla, and sub-globular and in terminal inflorescence with purplish rootstock. *A. euchroma* has broadly been utilized in the traditional system of the Yunani, Chinese and Ayurvedic medicines formulations because of its anti-microbial and anti-fungal effects. In the Lahaul valley, India, the locals use the roots of the herb as a hair tonic, an antiseptic, and for various complications such as hypertension, headache, and back pain. It is also used in the treatment of measles, constipation, burns, mild frostbite, eczema, and wound healing disorders. Roots of *A. euchroma* contain important chemical compounds such as Naphthoquinone, alkannin, and by-products of iso-hexenyl naphthazarin esters that have many pharmacological properties. These natural pigments, which are obtained from the wild, are in high demand. Overexploitation of natural habitat has resulted in a population decline, making this plant species severely endangered. Plant cell and tissue culture technologies might be a feasible alternative for producing such pigments in this case.

Key words: *Arnebia euchroma*, ethno-botanical uses, phytochemicals, naphthoquinone, endangered.

Introduction

One of the unique biodiversity hotspots 'The Indian Himalayan Region' provides essential ecosystem services to living beings. So far, 1,788 medicinal plant species have been discovered in this hotspot [1]. Due to increased anthropogenic (human beings) activity and strong demand for homeopathy medicine production in the pharmaceutical industry, the Himalayan area is currently dealing with a significant risk of biodiversity loss [2]. *Arnebia euchroma* (Royle) is a typical example of a severely endangered herb located in Himachal Pradesh's trans-Himalayan area. It is a high-value herbaceous perennial plant that belongs to the Boraginaceae family. Ratanjot (Hindi), Demok (Spiti Zanskar) and Khummed (Bhoti) are the names given to it in India. It is well known traditional, hairy, and upright herb with numerous stems appearing from the axil of its lowest leaflets, and found in arid parts of Northern Africa and Asia [3]. *A. euchroma* can grow in sandy and nutritionally deficient soil but cannot flourish in shade. It has a sweet and bitter taste, as well as a cooling effect and it

acts on the liver and heart channels. It regulates blood circulation and protects against toxicity caused by measles, sores, and inflammation. It is traditionally used in the cure of jaundice, eczema, dyschezia, burn, frostbite (coldness), dermatitis, and other skin ailments in India and China's traditional medicinal system [4]. *A. euchroma* is a key component of the Tzu-Yun-Kao ointment, which is used in Asia to treat burns and wounds.

Roots of *A. euchroma* contain different types of bioactive compounds including arnebinols, naphthoquinone pigments, and meroterpenoids [5] that are employed as a colorant and have a variety of pharmaceutical activities like anti-microbial, anti-bacterial, anti-cancer, anti-oxidant, antipyretic, and anti-inflammatory. Only three species of *Arnebia* are reported from Lahaul and Spiti area [6]. *A. euchroma* is the only one that species is used economically on a broad scale. In 1998, *euchroma* species was put under the threatened plants list [7]. Plants of *A. euchroma,* however, have been overexploited due to their therapeutic value, and have now been added to the list of Himachal Pradesh's critically endangered plants according to the current International Union for Conservation of Nature (IUCN) categorization [8]. In this respect, plant cell and tissue culture methods could be a feasible alternative to fulfill the increasing demand of *A. euchroma* natural metabolites [9].

This review is mainly based on ethno-botanical uses, phytochemistry, and pharmacological activities of *Arnebia euchroma*.

Taxonomic Description

Kingdom- Plantae
Phylum- Tracheophyta
Class- Magnoliopsida
Order- Boraginales
Family- Boraginaceae
Genus- *Arnebia*
Species- *euchroma*

Distribution

A. euchroma (Ratanjot) is found mostly on open slopes and shrubberies in the alpine and subalpine zones of Kashmir, Kumaon, Garhwal, and Nepal, at elevations ranging from 3000 to 4300 meters. Some of the common localities are Har-ki-Doon, Himtoli, Kedarnath, Mana, Valley of flowers, Gorson, Dronagir Malari in Uttarakhand; Great Himalayan National park, Pin valley, Chamba, Lahaul and Spiti, Rohtang, Kinnaur valley in Himachal Pradesh and Kurram valley, Deosai, Kakra, Kagan valley, Bedori, Aliabad, Pir Panjal in Kashmir. In the sub-alpine zone, *A. euchroma* occurs mostly in shady areas, but it is found in open sunlit areas in the alpine zone. It can also be found in North Africa, Turkey, and the Northern provinces of Iran especially in the mountain territories [10].

Botanical Description

Arnebia euchroma is a 15-40 cm tall hairy perennial herb. The roots of this herb are thick, stout, and purplish, and stems are usually single or many, branching, erect and hirsute. The leaves are thorny and linear, with long bristly hair that defends the herb from animals. Flowers are purplish and borne on terminal and sub-globular inflorescence. The flowers of *A. euchroma* are hermaphrodite, heterostylous (the stigma and anther grow at different heights which do not favor self-pollinated), and pollinated by insect [11]. There is less fruit production in them because the plants of *A. euchroma* are self-incompatible (unable to be fertilized by their pollen).

Fig: A plant of *Arnebia euchroma* [12].

Ethno-botanical uses

A. euhcroma is a popular component in the traditional systems of the Yunani, Chinese, and Ayurvedic medicine formulations. Previously, *A. euchroma* liquid extract was assorted with beeswax to make slurry known as "Ghuriti", which was used as a hair tonic, antiseptic as well as cure of cough, cold and chronic ailments [13,14]. The color isolated from the roots of this plant and assorted with mustard oil is utilized in the Lahaul and Spiti area for hair strengthening, the coloration of Chog, preparation of achar, chutney, and other dishes [15]. In the Spiti valley, the roots powder of *A. euchroma* is utilized to cure cough and different disorders of lungs [16]. Instead, the locals of the Spiti valley in Himachal Pradesh used it like a toothache, earache, cuts, eye sickness, healing of the skin, burns, and hair tonic [17]. This herb is also used to treat backaches, colds, hair difficulties, pulmonary problems, and blood vomiting.

The decoction created from *A. euchroma*, also called Arnebiae Radix (Zicao), is utilized for the treatment of skin, cardiovascular, post-herpetic neuralgia, and dermatitis in Chinese traditional medicine [18,19]. Different therapeutic herbs are blended with the roots of *A. euchroma* and its allied species to make an extraction known as Chog, Ghuritu, Khari, and Sharbeth, which is utilized to treat a variety of ailments. Different ailments like chronic constipation, cough, cold, fever, jaundice, and blood purification are all treated with 'Sharbeth' decoction.

Phytochemistry

Phytochemical analysis of roots extract of *A. euchroma* reported that the plant contained various phytochemical constituents which are Naphthoquinone (shikonin), phenolic compounds and its by-products have significant biological effects in the treatment of various diseases. Shikonin, acetyl-shikonin, β,β-dimethylacryl-shikonin, iso-butyryi-shikonin, isovalery-shikonin, arnebinone, deoxy-shikonin, B-hydroxy-isovaleryl-shikonin, stigma sterol, isobutyl-shikonin, and arnebin-7 are all the constituents of *A. euchroma*, which are mainly known for their different medicinal effects such as anti-cancer, anti-microbial, and anti-immunodeficiency [13]. Shikonin aids in the early stages of diabetic retinopathy by preventing LDC cholesterol from being

oxidized. Furthermore, it inhibits carcinogenic chemicals that have a negative effect on the system of action in cells, which speed up the carcinogenicity procedure in the body [20].

Alkaloids, anthraquinones, flavonoids, and phenolic substances are extracted from the upper parts and roots of *Arnebia* spp. [21]. Some important phenolic compounds such as alkaloids, O9-angeloylretronecine, O7-angeloylretronecine, 2a-hydroxyursoic acid, tormentic acid, pyrrolizidine, flavonoids, and triterpene derivatives are extracted from *A. euchroma* and having certain therapeutic properties [22]. Some previous research has suggested that phytochemical elements like acetyl-shikonin, β,β-dimethylacryl-shikonin, tetraacyl-shikonin can be utilized to cure carcinogenic disease, but additional research is needed.

Shikonin

Shikonin is a natural colorant biological active constituent in the roots of *A. euchroma*. It has a pleasant scent and a bright red color. It is reported to have anti-fungal, anti-tumour, anti-bacterial, anti-pyretic, anti-diabetic, wound healing, analgesic and chemo-preventive anti-inflammatory properties [23]. Its components, such as deoxy-shikonin, benzoquinone, naphthoquinone, isovaleryl-alkannin, arnebin-5, arnebin-6, and alkannin, are employed in therapeutic formulations for antimicrobial, anti-cancer, anti-HIV, and anti-inflammatory effects.

Pharmacological Activities

Roots extract of *A. euchroma* plant have been described to produce a variety of phytochemical constituents which possess different therapeutic effects.

Anti-inflammatory activity

Anti-inflammatory activity of *A. euchroma* roots extract is most noteworthy. There are very few evidences about the anti-inflammatory effects of this herb. Bioactive compounds isolated from the roots of this herb were discovered to possess the constituents such as glycosides, steroidal, sterols, and triterpenes which are exhibiting anti-inflammatory and anti-oxidant effects [24]. Naphthoquinone molecules have been found to have anti-inflammatory and anti-oxidant effects. It inhibits the production of NF-kappa B, Leukotriene, TNF-α, and Prostaglandin, all of which are involved in the response of inflammatory [25]. It also inhibits the generation of LPS-induced interleukin (IL)-6 in the brain which is liable for the inflammatory reaction [26,27].

Anti-cancer activity

In HT29 and DLD-1 cells, the bioactive compound deoxyshikonin extracted from *A. euchroma* substantially downregulated Pl3K and p-Pl3K/Akt/mTOR pathway proteins. Acetylshikonin, a compound isolated from *A. euchroma*, has been shown to inhibit the growth of tumor in the A549 human lung adenocarcinoma cell line [28]. Shikonin has additive and synergetic interconnection with possible pharmaceutical medicines used in cancer treatment, according to preliminary clinical research.

Anti-obesity activity

Obesity is a worldwide health issue linked to a variety of metabolic difficulties. Metabolic syndrome is a co morbidity that is linked to body waist size and stomach fat thickness. Liposuction and other methods to remove fat in particular areas are readily accessible to decrease fat thickness around the belly. The external experiment of an ointment containing *A. euchroma* extracts

has been shown to lower body weight (2.96 kg), stomach circumference in obese women (11.3 cm), and stomach fat thickness (2.3 cm) [29].

Cytotoxic activity

Using MIT assay, the alkaloids, pyrrolizidine, geranylquinol, and meroterpenoids by-product constituents extracted from the roots of *A. euchroma* may have a strong cytotoxic effect against an epithelial cancer that originates from hepatocytes and human lung carcinoma [30]. Some important phytochemical constituents extracted from the roots of this herb were experimented in opposition to cytotoxicity in different cancer cells; the propionyl alkannin had strong cytotoxic effect with less IC50 values [31].

Conclusion

The Indian Himalayan Region (IHR) is equipped with different varieties of therapeutic plants. Traditional medical practitioners in this hotspot utilize *A. euchroma* for the cure of different disorders (hair issues, remitting, cough, cold, fever, chronic condition, etc.,) as well as a vegetable colorant, textile dyeing, and other uses. It is a highly important medicinal herb, and demand for it is steadily rising across the world. Three species of the *Arnebia* genus are establish in the frigid desert of Spiti and are used by local populations for medical and religious purposes. Phytochemicals like acetyl-shikonin and shikonin are used to cure a variety of ailments including wound healing, skin and hair problems, and so on. In addition, knowledge is scarce on its clinical and pharmacokinetics applications. As a result, further study is needed to better explain the species' bioavailability and pharmacology. The Himalayan perspective also lacks in-situ and ex-situ conservation strategies for the maintenance of this threatened species. There is immediate requirement to investigate the prospects for its protection and the creation of agro-technological methods.

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A review on phytochemical profile, traditional uses, and therapeutic activities of *Bauhinia variegata*

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Abstract: *Bauhinia variegata* a deciduous flowering tree, member of Fabaceae family. This plant is known for its therapeutic potential as described in ancient system of medicine. It is commonly distributed along temperate and tropical Indian continent, South east Asia and China. It is grown as an ornamental for its showy flowers and buds are eaten as staple food in some parts of the country. Kachnar possess various phytochemicals that exhibit pharmacological activities. This review summaries the overall information of the plant including its traditional uses and phytopharmacological activities.

Key words: *Bauhinia variegata*, rakta kanchan, Himachal Pradesh, Bauhinione, anti-carcinogenic.

Introduction

Since ancient times, mother nature has been a major source of medicines and human have been reliable on plant for his requirements. The significance of plant products in the treatment of human disorders cannot be ignored. According to the report of World Health Organization (WHO) about 80% of world population depends on indigenous system of medicines [1, 2]. In British National Formulary approximately 56% of the active compounds for medicines are natural products. Phytomedicines used to treat various disorders is well acknowledge in Ayurveda, Unani and Charak Samhita system of medicines.

Bauhiniavariegata L. a flowering plant, member of family Fabaceae and sub-family Cesealpinioideae. Commonly it is known in Hindi as Kachnar, in Sanskrit as Rakta Kanchan, in English as Mountain Ebony and in Bangla as Kanchan. In Veda, it is referred as Kanchan (gold) since this plant was well known among various indigenous groups in India for many years because of its curative properties for number of ailments. *B. variegata* is well mentioned in Ayurveda as it is used as medicines in various formulations. In ancient system of medicine various plant parts are used to cure ailments. Kachnar is indigenous to temperate and tropical Indian Sub-continent (India, Nepal and Pakistan), South-east Asia (Vietnam, Laos, Myanmar and Thailand) and China [3]. It is generally planted as ornamental plant in park, garden, and roadside areas. Because of its ability to fix nitrogen it is well known to restore fertility of soil [4]. In Himachal Pradesh (India), *Bauhinia variegata* is well known tree species. In the month of March and April, the buds are eaten as food called 'Karalenkisabji'. Buds of Kachnar (locally known as karalen) are consumed seasonally

when available. Buds as a vegetable are also eaten in the North-eastern parts of India [5].

Distribution

B.variegata is commonly found in tropics and distributed all over the India mainly in Punjab, Central and South parts of the country. Kachnar is extensively present in Sub-Himalaya and outer Himalayas up to altitude of 1300m. In Himachal Pradesh, most commonly 3 species of *Bauhinia* are found i.e. *B*. *variegata* commonly known as Mountain Ebony, distributed in Bilaspur, Chamba, Hamirpur, Mandi (Joginder Nagar, Sundar Nagar, Nachaan), Sirmaur, Solan, Una districts. *B*. *Purpurea* commonly known as Butterfly tree, Khairwal,distributed in Bilaspur, Hamirpur, Kangra, Sirmaur, Solan, Una districts. *B*. *racemosa* commonly known as Purple Bauhinia, Kachnal, Ashta, distributed in Bilaspur, Hamirpur, Kangra (Nurpur), Kinnaur, Mandi (Nachan), Sirmaur (Nahan), Solan, Una districts [6].

Ecology

Bauhinia variegata is a deciduous tree which thrives well in tropical and sub-tropical climate. It grows in warm and dry summers with light winters, requires full sun or limited shade. Kachnar is susceptible to fire but drought resistance. Though frost destroy the leaves of sapling and seedlings, but they recover during summers. *B*. *variegata* can be found up to altitude of 1800m, with annual temperature ranges from 0-47° C and rainfall 500-2500mm. It can thrive in any type of soil, but mostly requires sandy and rocky, loamy soil with proper irrigation.

Botanical Description

Bauhiniavariegata tree attain height of about 10-15cm and girth of 50cm. The bark is pinkish, fibrous and bitter internally and light greyish-brown, smooth to slightly fissured and scaly externally. At young stage twigs are slender and zigzag, light greenish, fairly hairy, and angled, turns greyish brown. Leaves are with small stipules; petiole is puberulous to glabrous; lamina is ovate to circular, mostly wide than longer; 11-13 nerved; lobes tip are round; base is cordate; upper and lower surface is glabrous when grown fully. At the end of twigs the unbranched flower clusters are present. Few flowers are with short, stout stalks and green hypanthium. The light greenish, slightly hairy calyx forms a pointed five-angled bud and; five unequal petals with wavy margins, narrow to the base; five curved stamens; pistil with 1-celled ovary, style and dot like stigma. Fruit is pod, hard, flat, dehiscent and 10-15 seeded. Seeds are brown, flat, almost circular with coriaceous testa [7]. Since Kachnar is a deciduous tree, its leaf starts falling down in the month of November or December and remain leaf-less from January to April. Mostly propagation of *B*. *variegata* is done by seed and air layering [8].

Fig. (a) *Bauhinia* Bud (b) *Bauhinia* Flower

Traditional uses

Ayurvedic literature states that Rukshaguna, Shitavirya, Kasaya rasa and Katuvipaka are present in plant. In Ayurveda, *B. variegata* is known by the name Kachnar, Gandhari, Yugampatra and Karbudara. Its stem bark is used to cure Krinnrog, gandmala, apaci and vrna. It is used in ayuvedic formulations for dysentery, goitre, diarrhoea, lymphadenitis, rectal prolapse and worm infestation. In Unani, the bark of *B. variegata* is consumed as tonic to the liverand astringent to bowels. It is also used in case of asthma, leukoderma, leprosy, menorrhagia, ulcersand wounds [9].

The plant possesses antitumor properties[10]. Insulin like protein is present in *Bauhinia variegata* leaves so it is widely used as an antidiabetic agent [11]. Its root is antidote to snake poison and carminative. The flower juice is consumed in case of stomach disorders. Dried buds are practiced in case of diarrhea, dysentery, worm piles and tumours [8].

Phytochemical constituents

In Stem bark, quercitroside, lupeol, glycoside, β-sitosterol isoquercitroside, rutosidemyricetol and kaempferol glycoside have been reported. 2,7-dimethoxy-3-methyl-9,10 dihydro phenanthraquinone, Phenanthraquinone, dihydro phenantrene-1-4, dione (Bauhinione) have been extracted from stem extract [12,13]

In leaves alkaloids, fats, lupeol, oil, terpenoids, phenols, kaempferol-3-glucoside lignin, rutin, quercitin, saponins, vitamin C, β-sitosterol, apigenin, apigenin-7-o-glucoside amides, protein, fibre, carbohydrates, reducing sugar, calcium, phosphorous are present [14].

In seeds, protein, palmitic acid oleic acid, linoleic acid, steric acid is present (Yadav and Reddy, 2001). The root extract of Kachnar a flavone (2S)-5,7-dimethoxy-3-4'-methyleneflavone has been reported [15].

In buds, glycine, aspartic acid, serine, glutamic acid, alanine, oxaloacetic acid, and ketoglutaric acid and phosphoenolpyruvic acid are found[5].

Pharmacological studies

Anticarcinogenic Activity

While investigating anticarcinogenic activity of *B. variegata*, in methanol extract of stem bark anticarcinogenic activity was reported in case of 7, 12- dimethylbenz anthracene and croton oil-induced skin oncogenesis in skin papilloma model of Swiss albino mice using two-stage protocol [16].

Anti-diabetic Action

A recent experimental study suggests that Kachnar performs anti-diabetic activity. In induced diabetic model rats the glucose level in blood is found to be lowered on treatment with alcohol and hydro alcohol extract of Kachnar leaves administered orally. This is because Kachnar has a domain structure that contain same sequence of amino acid as Insulin hence reduction in elevated blood glucose level was seen [17].

Anti-inflammatory & Analgesic Activity

While identifying analgesic and antiinflammatory activity of *B. variegata*, a triterpene saponins (Compound 9) in leaves has been reported which reduces edema along with notable lowered level of Prostaglandin E2 in serum, granuloma and liver homogenate. After treatment with compound 9, significant reduction in diameter of hepatic and pulmonary granuloma was observed. The results were attributed to its antiinflammatory function [18].

Anti-Microbial Activity

B. variegata shows antimicrobial property against Gram-negative bacteria [19]. Bacterial growth inhibition was observed with leaf extracts. Antimicrobial spectrum of *B. variegata* is relatively narrow. Antimicrobial activity was prominent against *E. coli, Klebsiella pneumoniae* and *Pseudomonas* species. Administration of *B. variegata* bark powder on *Staphylococcusaureus* resulted in antibacterial, bio enhancing, and anti-inflammatory effects [20].

Antioxidant Effects

Antioxidant potential of the crude extracts was examined. With use of DPPH radical scavenging assay antioxidant potential was evaluated. Moderate scavenging activity was noted with ethyl acetate, methanol, and n-hexane fractions as compared to the standard Quercetin. Methanol extract of bark and its fractions shows notable antioxidant activity, resist H_2O_2-induced oxidative damage to pBR322 DNA. The ability to prevent DNA and antioxidative activity property may be because of presence of phenolic/flavonoid compounds in abundance [21].

Anti-tumor Activity

Kachnar also reported to perform anti-tumor activity. The ethanol and aqueous extract of stem showed anti-tumor effect against Dalton's ascetic lymphoma on swiss albino mice [22].

Antiulcer Activity

A study reported antiulcer activity of *B. variegata* in case of induced gastric ulcer in model rats. Ethanol extract of stem decreased the amount of gastric secretion, ulcer index, and total free acidity with respect to control, which increases during ulcer [23].

Chelation Action

In an experimental study the chelation action of *B. variegata* was examined. The plant extracts exhibited dose-dependent effect in metal ion chelating ability at different concentrations. Aqueous extract showed more chelation property as compared to the non-polar extract. This activity for different extracts was also determined [24].

Haemagglutinating Activity

Crude seed of *B. variegata* contains certain protein that has haemagglutinating activity [25]. This activity is widely used to detect the presence of viral particles.

Haematinic Activity

Kachnarstem bark ethanol and aqueous extracts reportedly increased the amount of hemoglobin in hemolytic anemic rats thus suggesting its haematinic activity [26].

Hypolipidaemic Activity

*B*auhinia *variegata* ethanol and aqueous extracts of stem and root bark shows hypolipidaemic activity. On administration of both the extracts, plasma lipids and lipoprotein level lowers effectively and the high-density lipid level increases in test albino rats induced with Triton WR-1339 (iso-octyl poly-oxyethylene phenol) [21].

Nephroprotective Activity

On investigation, nephroprotective activity of Kachnar extracts was seen in *invivo* cisplatin-induced nephropathy model in rats. Kachnar stems ethanol extract was administered for fourteen days and it resulted in reduction of different biochemical and histological symptoms of nephrotoxicity. Body weight and urine output were found to be increased meanwhile Creatinine serum level and urea were decreased[27].

Neural Activity

Specific neural activity was identified in Kachnar. Acetylcholinesterase inhibition activity was found by Chromatography. The enzyme inhibition activity was noticed specifically in flowers. Lower inhibition or no inhibition was observed in branches and leaves[28].

Conclusion

Since ancient times plants have been known as reliable therapeutic agents as explained in various indigenous system of medicine. These plants have been tested and shown potential to be used as source of modern drugs in pharmacological world. *Bauhiniavariegata* is one such medicinal plant with a potential to cure number of human ailments. Various biological activities have been proved by scientific research for the treatment of various disorders. More clinical and pharmacological screening should be done to probe unexploited the potential of *Bauhiniavariegata*.

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Multimodal ORMOSIL nanoparticles for biomedical application: A Review

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Abstract: Nanomedicine is a novel approach to conventional medicine in which problems are tackeled from bottom up rather than the top down, medical actions are carried out at the single cell level, tailored therapeutic prescriptions are carried out, and theronosis is promised. The synergistic effect of nanotechnology and biotechnology allow to develop multifunctional nanoprobe for combined therapt and diagnosis of diseases. This talk will highlight the use of multifunctional ORMOSIL NPs with combined imaging, diagnostic, and therapeutic functions for nanomedicine. ORMOSIL NPs serve as a new generation drug carrier for PDT of cancer, as well as for an efficient non viral gene delivery. The concern to use these multifunctional nanoprobes for clinical application lies within toxicity effects which need more investigation to carry out efficient clinical trial.

Keywords: Multifunctional, drug carrier, PDT, gene therapy.

Introduction

The biomedical applications of nanomaterials are covered by an interdisciplinary research area nanomedicine, that brings together nanotechnology, biology, chemistry, physics, material science, molecular biology and biotechnology for the enhancement of medical care. Quantitative superiority, multifunctionality, nano-size effect, additive/multiplier effect are just a few of the characteristics that make nanoparticles a desirable alternative for biomedical applications over conventional medications and imaging agents [1]. Nanotechnology provides a comprehensive technological platform for bioprocessing, molecular medicine, environmental and agricultural systems, as well as novel solutions for the transformation of biosystem. The size-dependent properties of nanoparticles contributes to their potential role in biomedical nanotechnology. Through the introduction of new generation of a multimodal nanoprobe, nanomaterials have the potential to solve the current constraints of sensitivity and specificity in medical diagnostics, as well as leads to betterment of existing and developing therapies. However, certain aspects which need consideration while designing these nanoprobes for

diagnostic and therapeutic applications are desired composition, size, and surface functionalities, biocompatibility and biodegradability, and in vitro and in vivo testing safety [2].

In biomedical applications, such as integrated imaging, diagnostics, drug delivery and therapy, multifunctional designed nanoparticles with improved magneto-optical characteristics have been extensively researched. Combining magnetic and fluorescent nanopatrilces into a single entity allows for the creation of innovative multifunctional nanocomposites that can be employed as multimodal contrats agents, in bioseperation, for controlled drug delivery, biosensors and catalysis [3].

In the past few decades, silica-based nanoparticles exhibiting the potential of target-specific and sustainable delivery of therapeutics have become a topic of great interest to researchers. Excellent biocompatibility and tailorable physiochemical properties of these classic nanomaterials contribute to their tremendous attention in biomedical applications. Furthermore, the presence of silanol groups on the surface adds to the improvement of therapeutic efficacy of drugs and reduced side effects by providing suitable conditions for conjugation of targeting ligands or stimuli-responsive "gate keepers" [4]. Porous silica (SiO_2) nanoparticles possess unique qualities, such as large specific surface area, superior biocompatibility, huge pore volume and controlled particle size which contributes to its increasing applications in biomedical field. It has been studied for use in drug delivery and biosensors. Silica has been widely employed as an organically modified silica (ORMOSIL) (shell) coating on different metal/ metal oxide nanoparticles including gold, silver, and ferrous for antibacterial, tumor targeting along with imaging purposes [5]. As a result it is possible to conclude that biocompatible and stable multifunction advanced system is formed by encapsulating metal core in polymeric structure. Several studies have shown that hemolysis is caused by –OH group of silica surfaces, however this could be resolved by substitution of –OH group with an amine group. Cornell dots (C dots) is the first fluorescent core-shell silica nanoparticles that has received approval for a first-in-human trial by FDA. The particles are safe for human usage and leave no trace after renal excretion, according to the findings of the first-in-human clinical investigation [6]. Since the single imaging modalities now in clinical use have either very high sensitivity and reactivity or vice-versa, the value of combining imaging modalities has been recognized and gained favour in recent years. However, multimodal system comprises blending of potential of different imaging modalities. The biocompatibility and flexibility of silica contributes to its efficacy in clinical nanotheronostics and diagnostic probes. The nanoparticles used for drug delivery or gene delivery can simultaneously be used for providing diagnostic information by a variety of in vivo imaging method. Unlike single imaging modality, multimodality imaging helps in analyzing in vivo system providing substantial information about anatomy, physiology and molecular structure of system.

Hybrid organic/inorganic substance ormosil is a type of organically modified sol-gel silica that is made by adding functional groups to inorganic alkoxides. When compared to inorganic sol-gel, such

materials have a number of appealing characteristics. By altering the amount and type of organic moieties in the silicon monomer, a variety of pore sizes can be generated in the ormosil network. The physical size of the organic substituents determines the pore size. ORMOSIL nanoparticles(NPs) are mesoporous with big pores in the matrix, which can facitilate encapsulation of both hydrophilic and hydrophobic drug/dyes. The tendency of ORMOSIL NPs to undergo surface functionalization with various chemical groups (e.g. carboxyl thiol/ amino/ hydroxyl) and its ability to conjugate with different targeting biomolecules such as proteins and antibodies leads to its mushrooming in biomedical field [7]. Despite of promising applications of ORMOSIL NPs in biomedical field, cytotoxicity effect of these nanoparticles is a matter of concern. Though, they are considered biocompatible several studies reveal their toxic effects. Various factors such as surface properties, aggregation, size and shape have been considered in relation to toxicity of silica nanopatricles, but general conclusions lack strong evidence against them. It has been investigated that PEGylation of ORMOSIL NPs minimizes the toxicity of these nanoparticles in certain lung cancer cells [8]. Various healthcare complications arises due to infections caused by medical devices, which may cause risk of life to patients. It has been reported that doping of phosphotungstate ormosil with core –shell SiO_2 @TiO_2 resulted in antimicrobial activity against various strains of bacteria. Thus it provides a protean and reproducible strategy for preparing self sterilizing film which adhere to innate material without significantly affecting the vascular cells [9]. Several attributes of silica and ORMOSIL nanoaparticles make them attractive platform for the development of a perfect nanoprobe. Multimodal ORMOSIL NPs conjugated with a near-infrared (NIR) flourophore as optical probe and with positron emission (PET) imaging probe Iodine-124 showed very reliable clearance result from body without causing any toxic effects. This shows the potential of these multimodal nanoparticles to be used as safe and efficient probes for in vivo imaging [10]. The overall aim of this review is to address basic routes of fabrication of ormosil nanoparticles , their characterization techniques and their biomedical applicatons.

Review of Literature

Few applications of multifunctional ORMOSIL NPs as therapeutic and diagnostic agent are as follows:

Multifunctional ormosil nanoparticles for photodynamic therapy (PDT) and diagnostics

In past few years, PDT has experienced a surge in popularity for cancer treatment replacing the conventional chemotherapy and radiation treatment. In cancer treatment, photo sensitizers (PS) are used to create singlet oxygen which is then combined with photo irradiation. The dosimetry of activation and PS, which modulates the photodynamic reaction at depth in dead tissue, determines the efficacy of PDT to a large extent. The biocompatibility and accessibility for multifunctionalization of nanoformulated PS lead to its promising applications in cancer treatment. However, due to hydrophobic nature PS are poorly soluble in blood plasma which lead to alteration in the photophysical properties of PS. Moreover, conjugation of

tumor targeting moiety with chemical PS is difficult leading to damage of healthy cells as well. To address these issues, researchers are showing their interest in nanoparticles encapsulating PS. Both organic (e.g. PLGA) and inorganic (e.g. inorganic oxides, metals and ceramics) materials are used as nanodelivery systems for PS. The efficacy of ORMOSIL NPs to encapsulate both hydrophobic and hydrophillic drugs, make it an attractive candidate for delivery of PS drugs. ORMOSIL NPs synthesized by polycondensation and alkaline hydrolysis of triodobenzyl-pyrosilane were found effective for PDT. The PS incorporated was iodobenzylpyropheophorbide. Cellular uptake of nanoparticles by carcinogenic cells and phytotoxicity of these nanoparticles towards cancerous cells were confirmed by in vitro studies [11]. A comparative study was carried out by Tang et al. on PS loading and PDT efficiency of hydrophilic methyl blue(MB) in three different nanoparticles (a) hydrophilic polyacrylamide having dimension between 20-30nm, (b)190 nm sol-gel silica nanoparticles and (c)ORMOSIL nanoparticles of 160 nm size. Lowest rate of MB loading was recorded in polyacrylamide nanoparticles due to their small size. Among ORMOSIL and sol-gel silica nanoparticles, although the delivery of $^1O_2$ per milligram of nanoparticles was found more in later due to higher loading, the former showed a higher kinetic rate of reaction of the generated $^1O_2$ with ADPA after irradiation at 650nm [12]. F.Selvestrel and co workers in 2013 studied PEGylated and non-PEGylated ORMOSIL nanoparticles for their microstructure and ability to deliver photoactive agents. One pot synthesis was applied for the preparation of dye doped, PEGylated and targeted nanoparticles. A comparission was made between Stöber silica nanoparticles and VTES –ORMOSIL nanoparticles doped with the alkoxy silane porphyrin derivative 3 to investigate surface properties. Substantial changes in the microstructure and surface properties was observed with organic modifications. The targeting efficiency of ORMOSIL NPs was improved by functionalization with various targeting agents such as antibody Cetuximab, cyclic RGD peptide, folic acid, and biotin. Results record the interference of PEG with small targeting agent but not with bulky antibodies. Though enhanced uptake was investigated from efficient targeting, but it is not sufficient to ensure high therapeutic efficiency. The final fate of the nanoparticles following administration was determined by interaction with proteins. The results supported the prevention of such interaction in case of PEG coated nanoparticles. Based on information provided by these nanoformulations a new nanosystem for tumor therapy could be developed [13]. L Mmoritlas-Becerril et al. also investigated PEGylated ORMOSIL NPs loaded with anticancer drug 3N-cyclopropylmethyl -7- prhenyl-pyrrole-quinolinone (MG2477) as nanovectors for cancer therapy. Synthesis of PEGylated ORMOSIL NPs was done by microemulsion condensation of vinyl triethoxysilane. PEG-Si and NH_2-PEG-Si were used as PEG derivatives to coat the NPs. These nanoparticles were also conjugated by anti-CD44V6 antibody and hyaluronic acid (HA) using thiol-maleimide and amide coupling chemistries respectively. Selective cytotoxicity of drug loaded nanocarriers was demonstrated by using an anti-CD44V6 antibody or HA as targeting agents for a receptor over expressed in cancer stem cells. The enhanced activity drug in the presence

of targeted nanovectors due to the much more efficient uptake pathway provided by these nanovectors as well as encapsulation of the drug, prevention of its aggregation and precipitation and rapid delivery in the cells by receptor mediated uptake . Cytoxicity was found more in NPs conjugated with lower amount of antibody. However it was investigated that cytotoxicity of unconjugated NPs was even lower than the free drug. In case of HA conjugated NPs reverse effect was observed i.e. NPs conjugated with higher amount of HA induced higher toxicity compared to that with lower amount of HA conjugated NPs. These studies not only describes the attractive potential of ORMOSIL NPs for cancer therapy but also highlight the general potential of NPs in cancer therapy [14].

ORMOSIL NPS for gene therapy

Development of a model which could analyze the uncovered genetic/molecular abnormalities is necessary for development of corrective gene therapies and to investigate changes in gene structure and activities. The synergy of nanotechnology into biomedical research is believed to change scenario of diagnosis and therapy. For tremendous and safe delivery of genes nanobiotechnology aims to develop non-viral, nanoparticles based vectors. Single gene defects as well as factors causing changes in function of several genes could be traced with these vectors. C Rejeeth reported the significant expression of P53 gene in cancer cell line MCF-7 cells using ORMOSIL NPs. ORMOSIL NPs were prepared in oil-in-water microemulsion and treatred along with pCMV-Myc (3.8kb) plasmid vector construct carrying P53 gene before being transfected into the breast cancer cell line MCF-7 cells. The uptake of P53 genes into MCF-7 by NPs was evaluated via Western Blot Analysis. Results shows expression of P53 gene in MCF-7 by ORMOSIL NPs thus, confirming potential of these NPs to be used as superior gene delivery vehicle. In vitro growth assay was performed to study the effect of transfection of P53-pCMV/ORMOSIL on the rate of MCF-7 cell growth. Compared to non-transfected cancer cells ORMOSIL/P53/ pCMV –Myc transfected cells grew at a much slower rate. Gene transfer efficiency of ORMOSIL NPs was demonstrated using a Transmission Electron Microscopy at cellular level. This revealed the accumulation of NPs in the cytoplasm and the nucleus of the cancer cell transfected with P53 gene. The results of this strategy, shows bright future of ORMOSIL NPs as transfected agent for therapeutic manipulation of cancer cells and in vivo applications [15]. In order to study the gene delivery efficacy and ecotoxicity of amino-functionalized silica NPs (NH_2-ORMOSIL NPs), *in vitro* and *in vivo* assays were carried out by J.C. Matos and coworkers. NH_2-ORMOSIL NPs were synthesized by sol-gel method and were bioconjugated with pVAX.1-GFP (pDNA) resulting in an ORMOPLEXEs (for short). Direct incubation of pDNA and NH_2-ORMOSIL NPs in a mass ratio of 1:20-1:350 (pDNA: NPs) formed ORMOPLEXEs and its formation was confirmed by agrose gel electrophoresis. The test results showed total complexation between NPs and pDNA. Further, labeling of these ORMOPLEXEs with Rhodamine fluorescence marker lead to formation of rhodamine labeled ORMOPLEXEs which helped in monitoring ORMOPLEXEs during in *vitro* studies. *In vitro* studies using cultured CHO cells revealed the gene delivery performance of

ORMOPLEXEs, which showed an incipient level of transfection. In vitro studies on Zebra fish embryo was done to assess the transfection efficiency and biodistribution. The results revealed that transfection did not occur at tested condition. In vivo ecotoxicity assay showed no significant effects on mortality, development delay, hatching, and malformations of Zebra fish embryo thus, confirming the non toxic nature of ORMOSIL NPs. Hence, these reproducible, monosized NH_2-ORMOSIL NPs, appear to be a potential gene delivery nanocarrier [15, 16].

Ormosil nanoparticles as Drug/dye delivery vehicle

(INTRO) .A. Nagesetti et al. studued the effect of NIR laser on the release pattern of doxorubicin hydrochloride (Dox) and its cytoxicity towards ovarian cancer cells (Skov-3). Microemulsion mediated multifunctional ORMOSIL NPs(PEGCDSIR820) incorporating both anticancer drug and Near Infrared(NIR) dye (NIR,IR820) were developed and surface modified with polyethylene glycol (PEG) which improve its aqueous stability via the enhanced permeability effect. Skov-3 cells were cultured and treated with PEGCDSIR820. It was inferred that these NPs showed no toxicity in Skov-3 untill exposed to 808nm laser. The enhanced cytoxicity was due to combination of adjuvant hyperthermia(43^0C) and enhanced Dox release. The release of Dox was found to increase on exposure to laser. Cell necrosis caused due to hyperthermia was observed with the help of confocal imaging. Furthermore, cell toxicity studies reveled that upto a concentration of 360µg/ml PEGylated particles without NIR exposure did not show any toxicity, whereas non-PEGylated particles showed toxicity in a dose dependent manner. Exposure of PEGCDSIR820 to laser resulted in cell death. Along with PEG these NPs could further go modification with cancer specific antibodies (HER2) and small molecule (folates) for selective targeting of cancer cells as well as other cells (Nagesetti, Srinivasan, McGoron, & Biology, 2017). To study the effect of surfactant on conjugation of dye/drug to nanoparticle surface V.Shirshahi and co-workers in 2013 demonstrated the effect of surfactant on dye doped, Herceptin-functionalized ORMOSIL NPs by carrying out the active targeting of HER2-positive breast cancer cells. These NPs were separately synthesized in the non-polar core of two different micellar system consisting of AOT/1- butanol/DMSO and Tween 80/ 1-butanol/DMSO in deionized water. Herceptin was conjugated to these NPs using a water soluble carbodimide. The morphology and size of these NPs were determined using TEM. TEM micrographs revealed larger diameter of Aerosol OT (AOTORM) compared to that of Tween 80(TWNORM) NPs. DLS measurements showed larger hydrodynamic diameter in case of both NPs after entrapment of fluorophore NR within ORMOSIL NPs, thus confirming successful encapsulation of dye. The results of fluorescence spectrometry was same for both doped and free NR, thus determining the fluorescent properties of NR after encapsulation within silica matrix. Effect of surfactant on the surface of ORMOSIL NPs was demonstrated by calculating Zeta Potential of these NPs. The results confirmed the huge impact of surfactants on the surface charge of NPs. In vitro experiments performed to study active targeting of Herceptin conjugated dye doped ORMOSIL NPs to

SKBR3 cells, as a model of HER2 overexpressing breat cancer. They concluded from these studies that efficient bioconjugation is affected by the presence of surfactant molecule on the surface of ORMOSIL NPs. Better conjugation resulted in differentiating within breast cancer in HER2 positive or HER2 negative, thus providing a platform for drug/gene delivery to breast cancer cells in future works [17].

Bioimaging

The creation of multimodal diagnostic probe that will allow for combination diagnostics, ideally addressing both anatomical and physiological features of diseases can facitilate a comprehensive diagnostic visualization about a diseases cell/tissue/organ. A dual modality nanoprobe consisting of ORMOSIL NPs co-encapsulating fluorophore(Nile Red) and iron oxide (IO) nanoparticles were prepared in *in vitro* bioimaging. These NPs were prepared by microwave assisted method in micellar media and were characterized for their shape and size using TEM. The images obtained from TEM showed diameter of nanoparticles around 120nm. XRD pattern revealed crystalline nature of these NPs. Vibrating Sample Magnetometry(VSM) was used to investigate magnetic properties of these NPs which resembles to that of superparamagnetic material. This helped in finding that co-encapsulating with a fluorophore within ORMOSIL NPs did not significantly alter the magnetic behavior of IO NPs. U.V. visible and fluorescence spectroscopies were used to study the optical properties of these NPs. The substantial decrease in the absorption peak of NR (around 560nm) with IO within ORMOSIL NPs was attributed to optical quenching by IO. Fluorophore emisision spectra ascertained the presence of NR in nanoparticles smaples by showing characteristic emissioin peak of NR although with decreased intensity due to nanoencapsulation. Cell viability assay demonstrated the biocompatibility and nontoxicity of these NPs. It was found that IO NPs exerted a negligible toxic effect on the cells whether in free form or co-encapsulated with the NR within ORMOSIL NPs [18,19]. Hong Whung Tran et al. demonstrated the synthesis and bioapplications of dye doped water soluble ORMOSIL NPs. The nanoparticles were synthesized by modified Stöber method using methyltriethoxysilane(MTEOS) as precursor and surface was bifunctionalized by bovin serum albumin(BSA) and also with different biocompatible chemical groups like amino.hydroxyl and thiol. Rhodamine(6G)(Rh6G) and Rhodamine(B) were used for doping. The size and shape of NPs were determined using TEM, showing spherical shape of particles. It was observed that encapsulation of these dyes within NPs enhances its photostability, brightness, life time and bioenvironmental stability compared to that of bare dyes in water. Immune labeling of bacteria E.coli 0157:H7 and breast cancer cells was carried out using these NPs. The results showed amplified fluorescence microscope signal of bacteria, thus identifying bacteria through antigen-antibody interaction and recognition. They treated breast cancer cell with anti-HER monoclonal antibody nanoparticle complex and reported efficient optical imaging and detection than those treated by antibody-free dyes. They concluded that these dye doped silica based NPs have potential to work as markers for different bioapplications [19,20]. Cellular uptake and photodynamic toxicity of photosensitizer entrapped

ORMOSIL NPs induced by surface attached gold NPs were investigated. Three ORMOSIL NPs with different diameter (30/55/80 nm) entrapping photosenstizer MB were synthesized. Using micellar approach TEM and DLS data were obtained to measure the shape and size of these NPs. The assembly of NPs on the surface of ORMOSIL NPs was confirmed with UV-Visible absorption spectra and FTIR spectra. Time-dependent release pattern of MB from these ORM NPs showed sustainable release for all 3 pattern smallest particles showing least release. Effect of Au NP aggregation over the ormosil surface was observed using ABMDMA photobleaching studies showing faster rate of photobleaching in case of aggregated AuNPs over ORMOSIL NPs. Maximum rate was noticed in case of MB-Au-ORM nanoparticles with smallest size out of three formulations. Invitro studies using pancreatic cancer (Panc-1) cells were carried out to demonstrate the cellular uptake and photodynamic cytotoxicity. These studies also favoured substantial results in case of smaller NPs. Surface transfer energy was considered the main cause for this enhanced PDT. Based on these studies, authors suggested possibility of dual therapeutic modes as well as real-time PDT monitoring with photoacoustic imaging [21, 22]. The use of ORMOSIL NPs in bioanalytical chemistry for the development of ultrasensitive and simple bioassays is of great significance in clinical diagnostics. Massimo Di Fusco and co-workers reported the synthesis of homogenous ORMOSIL NPs incorporating thermochemiluminiscent (TCL) 1,2-dioxetane derivatives and demonstrated their suitability as highly detectable nanoprobes for biospecific assay. They further functionalized NPs with biotin to develop a general purpose label having potential to send any streptavidin-conjugated biospecific probe. Anti-streptavidin antibody was immobilized to capture streptavidin, and the antibody-bound streptavidin was detected by biotinylated TCL ORMOSIL NPs in a quantitative non-competitive immunoassay to streptavidin. Similarity in performance was observed when compared to that of obtained by chemiluminiscent detection using horseradish peroxidase as label, thus allowing reagentless detection with amplified luminescence signals. The accessibility of organic groups of ORM NPs towards bio-specific probes(such as antibodies, gene probes) makes it a suitable candidate to detect a broad range of analytes or even to produce multi-functional nanoprobes [22,23].

Biocompatibility

Several nanoprobes such as Quantum dots, liposomes, polymers, ceramic or polymeric NPs are used as a theronostic tool for in vivo imaging also possessing therapeutic applications. However, long term use of these nanoprobes in invivo applications may cause dilution of their efficiency. Silica-based nanomaterials have emerged as a significant candidates owing to their property of inertness, porosity, optical transperacy, ability to conjugate desired fluorophore. Several investigations have been made by researchers on the use of ORMOSIL NPs encapsulating fluorophore and targeting ligands for optical imaging of tumor cells invitro. However very less data is available on biocompatibility, biodistribution and toxicity of ORMOSIL NPs in living organisms. Considering this issue, F.Barandeh and co-workers made very first thorough investigation on the potential of ORMOSIL NPs in living neuron with

Drosophila melanogaster by targeting them to neurons. For their investigation, they prepared (receptor and peptide) conjugated and un conjugated Rhodamine-ORM NPs(RORM). DLS and TEM techniques were used to study the morphology of synthesized NPs. Effects of ORMOSIL throughout the life cycle of fly was evaluated. Their findings showed that ORMOSIL NPs did not affect the fly in any stage of their life cycle i.e. larval, pupal, and adulthood. The therapeutic efficiency of ORMOSIL NPs in neuronal diseases was confirmed when they found that ORMOSIL NPs did not cause aberrant neuronal cell death pathways or interference with normal neuronal process. Thus, they concluded from their observations that ORMOSIL NPs are biocompatible and non-toxic and can be safely used in living neuronal tissues without causing any change in normal cellular functions [24,25]. Hydrophobic nature of various dyes, PS or drugs restricts their ability to be used as an effective agent for various biomedical applications. Vitamin-D plays a crucial role in cell proliferation and differentiation, and immune regulation. Being a hydrophobic entity, their higher doses leads to side effects such as hyper calcemia, hypercalciuria and bone decalcifice. To overcome this limitation a dye doped multifunctional ORMOSIL NPs conjugated with several Vitamin-D_3 deraivatives was fabricated using one pot synthesis. The conjugation of Vitamin-D with NPs was done through carbonate function forming carbamate derivatives of Vitamin-D NPs. Using this method nanoparticles formation occurred in the hydrophobic core of surfactant stabilized microemulsion. PEGylation of these NPs was done to improve its biocompatibility and reduce pro-cogulant properties. The Vitamin-D_3 conjugated NPs were characterized by DLS, Zeta potential, UV-Viisble absorbance, TGA,NMR, and TEM. DLS and TEM data confirmed average diameter of particle around 90mm. ^{1}H-NMR was recorded confirming successful purification. Vitamin-D_3 cojugation was confirmed by UV-Viisble spectroscopy. Surface Plasmon resonance investigated the interaction between NPs and Human serum albumin used as a mode protein for transporting insoluble Vitamin-D_3. The results obtained demonstrated that PEGylation of D_3 conjugated ORMOSIL NPs resulted in formation of a suitable biological receptor for hydrophobic molecule Vitamin D_3. Also the affinity of albumin for Vitamin D_3 was enhanced [26].

Conclusion and Future prospective

The development of multifunctional nanoparticles allow integration of various therapeutic and diagnostic strategy such as imaging, drug delivery, or active targeting of genes within a single entity. ORMOSIL NPs owing to its ability to undergo rapid surface functionalization possess great significance for various biomedical applications. In this review, we have discussed the effectiveness of ORMOSIL NPs for a number of biomedical applications such as PDT, gene therapy, targeted optical imaging, and active drug delivery vehicle. Different synthetic routes and characterization techniques have been presented in brief. Bioconjugated ORMOSIL NPs can be utilized as a sensitive biomarker to label malignant cells, allowing surgeons to better visualize and safely resect the tumor while minimizing damage to healthy tissue. Use of multifunctional ORMOSIL NPs for corrective gene therapy provides a great platform to detect various gene defects.

However, future clinical applications of multifunctional ORMOSIL NPs need few issues to be addressed including biocompatibility, toxicity, in vivo targeting efficacy, and long term stability. Furthermore, investigation of characteristics of multifunctional ORMOSIL NPs in a living organism will open the way to more efficient diagnosis and therapy of diseases in future.

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Genomics in drought stress tolerance for crop improvement

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Abstract: Drought stress is the leading cause of crop loss worldwide, accounting for the majority of abiotic stresses. Consequently, the only environment friendly solution available to breeders to combat this problem is to raise stress tolerant cultivars. Crop plant's genetic improvements could be brought by combining conventional plant breeding methods with cutting-edge molecular biology techniques. These both have made significant contribution to global agricultural production. Genomics is exploring many new study areas and playing a distinct role in improving standard of global crop produce in terms of quality and quantity. Three technologies namely genomics, DNA markers, and genetic engineering will undoubtedly speed up the crop improvement efforts around the world. Plant stress response involves activation of a series of stress-related genes and their regulation including interaction of several molecular networks. Several dehydration tolerant cultivars have been raised through combination of r-DNA technology, genomics and traditional breeding approaches. The main objective is to genetically engineer transgenic plants depending upon the different stress-responsive mechanisms. Therefore, genomics is a new advancement in the field of science crucial for solving the future food scarcity situations.

Key words: Genomics, Signal transduction, Drought tolerant transgenics.

Introduction

Non-biotic stresses namely water, temperature severity, salinity or heavy metal stress all have several negative impacts on the productivity and growth of plant eventuating activation of a cascade of external, functional, metabolic and genetic changes in plants and are the major reason behind crop loss worldwide. Among these water scarcity or drought stress is the most prominent and extensively studied abiotic stress affecting any plant. In routine, crop plant faces range of drought episodes in a variety of ways to varying degrees. Unfortunately, the global variations in climate are continuously shooting up the frequency and extremity of drought episodes [1]. Therefore, it would possibly result into a catastrophic reduction in food crop yield or fertile land over the next 50 years [2]. It is assumed that by 2025, approximately 1800 million populations will be residing in countries having absolute water deficiency while rest of the world's people could be under drought stress situation [3]. Hence, food availability in this era is greatly dependent upon the delivery of drought resistance and high yield, stable cultivars [4]. To aim this goal, in plant biotechnology programs, breeding for abiotic stress tolerant food crops must be of supreme importance. In crop plants, the genetic improvements achieved via classical plant breeding

methods have made enormous contributions to global agriculture production later supplemented by tools and techniques of molecular biology. Consequently, genes functioning in protection and structure or function preservation of the cellular components can improve stress tolerance in plants are engineered. As our understanding of stress-related metabolism is still limited so complete listing of stress-linked metabolites is most appropriate procedure for guaranteed genetic breeding for stress-resistant crops. Further stress-related gene assets recovered from crop plants and extremely dehydration-tolerant model individuals will be unlocked in the future, allowing molecular inspection of non-biotic stress-tolerance procedures in prime crop plants [5].

In comparison to biotic stress tolerance, determined mainly by monogenic traits, abiotic stress responses are polygenic and thus more complicated to manipulate and balance. Hence, genomics as a new technology is unfolding several new fields of studies.

Genomics

It is the computer-assisted study of the structure and function of a living organism's entire genome (*i.e.* the structure of a chromosome and activity of all genes). Genomics has now become the forefront of biology and its techniques are extremely powerful, productive, profitable and reproducible in order to tackle complicated genetic problems. The application of genomic techniques in plant breeding and genetics has now become all important.

Types of Genomics

Structural genomics

It means study of an organism entire genome structure, attempting to identify all of its genes, (also known as gene discovery) and determining their chromosomal locations. It determines the genome size in megabase (Mb) and number of genes in the entire genome of a species. Thus, it is concerned with the determination of the entire genome sequence or the complete protein set produced by an organism (proteome). One of the most well-known applications of this type is the analysis of quantitative trait loci (QTL) via genome mapping. This type brings out the construction of high resolution genetic and physical maps**,** DNA sequencing and proteome determination in an organism.

Functional genomics

It refers to the study of an organism all genes function constituting its entire genome and determining its phenotype. It is concerned with transcriptome and proteome, the determination of later is called proteomics. It involves techniques such as molecular markers, automatic gene sequencing, microarray/biochip *etc*.

Comparative genomics

The understanding of the association between genomic structure and its activity over diverse range of species or strains, as well as the study of differences and similarities in genome structure and organization referred to as the comparative genomics. It has aim to recognize natural selection process and to transform the DNA sequence information into polypeptides of known activities so to understand gene organization and expression, as well as evolutionary differences. In terms of both methodologies and outcomes, genomics approaches are highly intertwined to each other [6].

Genomics weapons for crop improvement

It involves polymorphic DNA markers (microsatellites, SNPs *i.e.* Single Nucleotide Polymorphism), expression approaches (microarray), and bioinformatics (databases). SNPs are the dominating molecular markers used in genetic breeding programs as reviewed by the prevalence of softwares associated with their discovery [7]. They are frequently found markers in the genome therefore, gaining popularity in crop species like rice whose genetic data has been sequenced. SNP is defined as a single nucleotide DNA sequence variation — A, T, C, G —among the individuals of a species or their homologous chromosomes. A number of SNPs have been recognized between *Oryza sativa* var. *indica* and var. *japonica* of rice and data associated with it is freely available online [8]. A candidate gene refers to a gene linked with the desired character. The prominent method of recognizing this type of gene is through DNA array technique**.**

A target specific first-generation tool for genome editing known as Clustered Regularly Interspaced Palindromic Repeats (CRISPR/Cas9) has been in use to combat major non-biotic stresses including water stress and salinity [9]. This technology use was demonstrated in different crops being rice, wheat, maize [10], tomato [11], soybean, carrot, lettuce [12] *etc*.

Genomics allow gene mapping for almost all types of traits including morphological characteristics, gene regulating quality, adaptation to various agro-climatic conditions, photo/thermo insensitivity, male sterility, self incompatibility, fertility restoration, toxicants, non-biotic and biotic stress resistance in crop plants *etc.* The preliminary necessities of mapping whole genome are extremely specialized technical expertise, modern laboratory facilities, expensive equipment, chemicals, glass wares, instruments *etc.*

Stress-associated genes, signaling and control

The molecular control mechanisms are dependent on the activation and regulation of unique stress-related genes, engrossed in the entire stress response sequence starting from signaling, transcriptional control, membrane and protein protection, specific osmolyte biosynthesis, anti-oxidant and anti-toxicant activities. Moreover, various researches at this level have begun to pay off and genetic alterations for abiotic stress tolerance have yielded outstanding results which may eventually implemented to agro-economically important crop plants. The central point of attention is on engineering transgenic plants, manipulated by using different stress-response mechanisms. On exposure of a plant to abiotic stresses several genes get activated, resulting in an increased level of several metabolites and proteins, some of which may be responsible for conferring some level of resistance to respondent plant against these stresses (Fig.1) [5]. The knowledge of types of cellular, biochemical and molecular level changes occurring in response to any abiotic stress is crucial for progress towards breeding for stress-tolerant plant crops or better crops under stress. Recent molecular technologies entail the discovery and utilization of molecular markers that can improve breeding programs. However, the introgression of stress tolerant related genomic portions (QTLs) frequently introduces undesirable agronomic characters from the contributor species. This is due to unavailability of a precise understanding of the important genes carrying QTLs [13].

Mitogen-activated protein (MAP); transcription factors such as heat shock factor [HSF]; C-repeat-binding factor or dehydration-responsive element binding protein [CBF/DREB] and ABA-responsive element binding factor/ABA-responsive element [ABF/ABRE] families) and phospholipases are the highly studied genes participating in signal communication and in gene expression regulations [14] (Fig. 2.). However, majority of studies included short-term experiments, provided no conclusion about crop real stress tolerance conditions. This is determined by long-term monitoring of plant performance in terms of harvest/yield, biomass, and extent of stress retrieval. Recent practices to upgrade stress resistance in plant through genetic transformation have yielded a number of significant results; however, the complex genetic pathways of non-biotic stress tolerance made the procedure critically impossible [5]. As a result, biotechnology must be completely integrated with traditional breeding and physiology. However, a lot of studies have shown that stress-associated proteins regulation took place during and after protein synthesis in response to external stress [15].

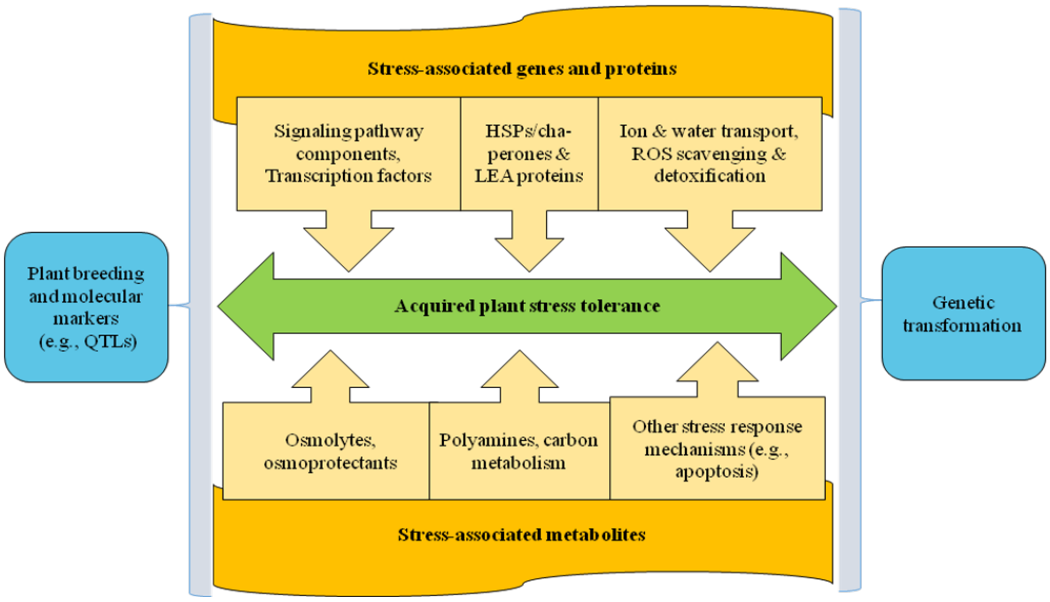

Fig. 1: Stress tolerance acquired by plant could be increased by altering stress-related genes and polypeptides, as well as by over-expression of stress-related metabolites. Plant non-biotic stress resistance is based on the integrity of numerous genes, polypeptides and metabolic routes all working in combination. It can be targeted through genetic engineering as well as by traditional plant breeding approaches in association with genetic markers and QTLs [5].

HSP- Heat Shock Proteins; **LEA**- Late Embryogenesis Abundant; **ROS**- Reactive Oxygen Species.

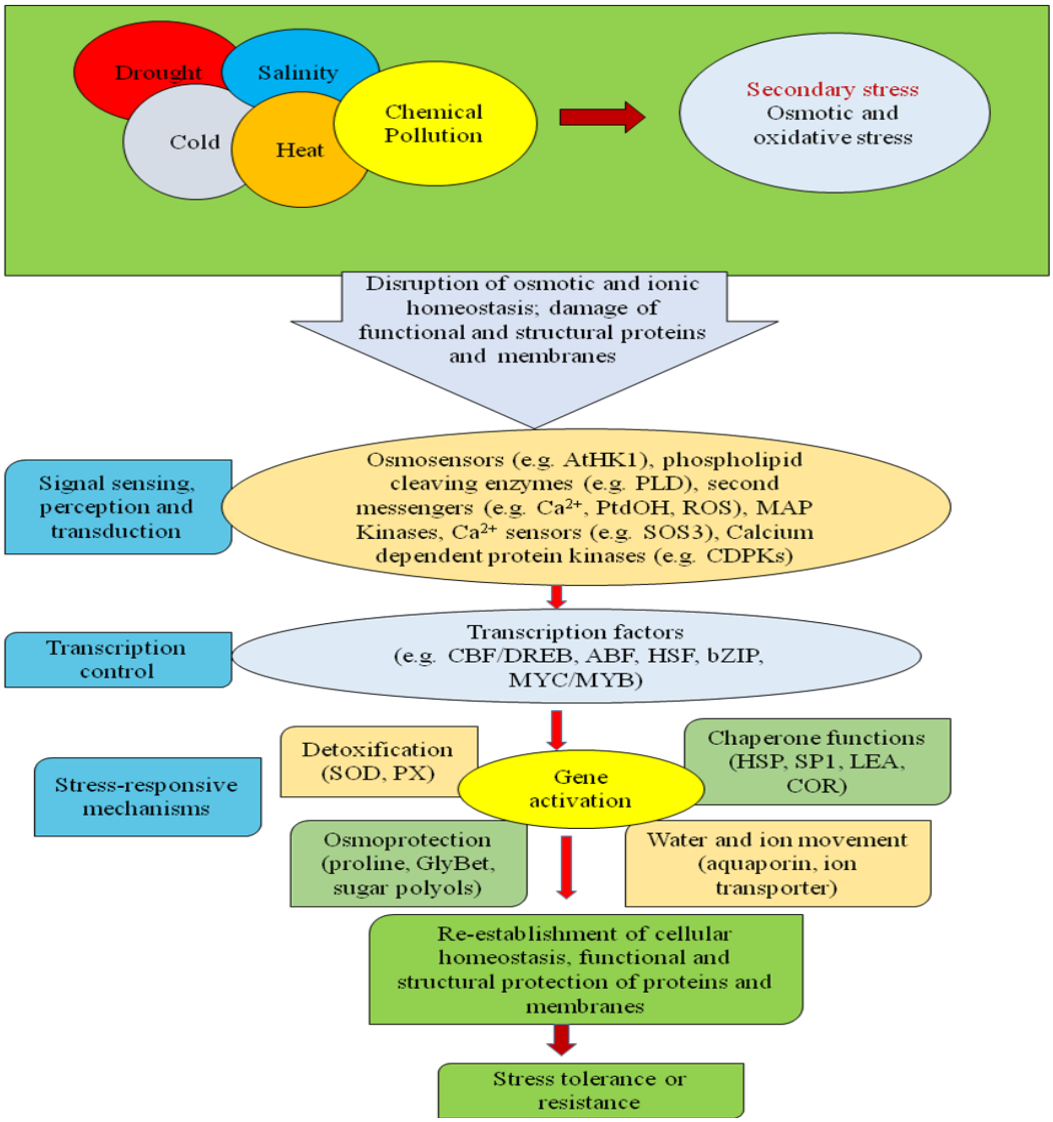

Fig. 2: Plant responds to non-biotic stress in a very complicated way. Common stresses like drought, salinity, temperature extremities (low or high), and chemical pollution are frequently inter-related causing cell damage and second level stress (*i.e.* oxidative and osmotic stress). In the beginning, stress signals (*i.e.* ionic and osmotic effects or variations in temperature or membrane fluidity) activate the downward signaling process and gene expression regulation, triggering stress-responsive system to evoke equilibrium and thus shield and restore destructed membranes and proteins. Insufficient response at one or more signal transduction stages and during gene triggering process could lead to irreversible alterations in cell equilibrium, additionally causing dismantling of functional and structural proteins and membranes, ultimately cell apoptosis.

Abbreviations: ABF, ABRE binding factor; AtHK1, *Arabidopsis thaliana* histidine kinase-1; bZIP, basic leucine zipper transcription factor; CBF/DREB, C-repeat-binding factor/dehydration-responsive binding protein; CDPK, calcium-dependent protein kinase; COR, cold-responsive protein; MAP, mitogen-activated protein; PLD, phospholipaseD; PtdOH, phosphatidic acid; PX, peroxidase; SOD, superoxide dismutase; SP1, stable protein1 [5].

Metabolic listing

The complete profiling of stressed plant metabolites aids in studying stress-induced changes in metabolites. Plants subjected to a combination of drought and heat stress accumulate sucrose and other sugars such as maltose and glucose, according to *Arabidopsis* metabolic profiling. It has been reported that on exposure of a plant to drought stress, proline got concentrated in that plant whereas the same osmolyte has been substituted by sucrose as the primary osmoprotectant under extreme stress conditions of combined heat and drought [16].

Among the common types of plant abiotic stress responses, avoidance mechanisms are less flexible to practical manipulation because they are primarily the outcome of external form and physiological alterations at the whole-plant level and based on the traditional breeding methods and QTL analysis. Tolerance mechanisms, on the other hand, are result of cellular, molecular and biochemical changes and are amenable to biotechnological manipulation and correspond to genetic engineering for a specific gene. All abiotic stresses elicit a chain of physiological or molecular events and few of which lead to similar response level as physiological water scarcity. A comprehensive explanation of non-biotic stress confronting system and smart breeding approach for stress resistance, need logical and exact solution to many queries like type of proteins and genes upregulated or downregulated under stress conditions, exploration of their activities, selection of a candidate gene and molecular marker for breeding transgenic plants, eventually choosing stress-tolerant genotypes/cultivars. Therefore, several plant genetic engineering policies for stress tolerance are based on the transcription and translation of genes involved in signaling and regulatory pathways, encoding enzymes of functional and structural metabolites biosynthesis. QTLs analogous to drought stress tolerance in cotton have been recognized [17]. Recent advances in genomics, metabolomics, transcriptomics and proteomics have provided numerous options for the isolation and depiction of novel abiotic stress-responsive genes and mechanisms [18].

Marker-assisted breeding (MAB) is a better and well-organized technique that identifies the importance of thousands of genomic regions of a crop under stress conditions, which was earlier impossible [19]. QTLs associated with drought resistance have been reported for different kind of traits in number of crops [20]. But due to the high genetic and environmental interdependence, enormous genes encoding yield and incorrect mapping population use, QTL mapping process for higher produce under water shortage situations got affected adversely. A transgenic amendment to this hurdle appears more satisfying and reasonable.

Genetic manipulation of important plants and release of drought tolerant transgenics

The system of transgenic amendment permits only the insertion of unique genes copies into an organism so limiting the transmission of unwanted genes from contributor to recipient species than conventional breeding programmes. This strategy also leads to concentration of genes having similar impacts. Rapid advances in recombinant-DNA technology, moreover, expansion of accurate and structured gene-transfer methods have made transgenics production successful in variety of crop species [21].

Tolerance to drought is a measurable quantity with heritable, epigenetic and ecological elements adjusted by a group of distinguished and non-distinguished genes frequently influenced by site specific natural factors. Acclimatization, regulation at all developmental stages and root morphology, penetration or flexibility in various soils are all crucial in this operation.

Table 1: Inventory of some drought associated genes and drought tolerant transgenic crops:

Engineered gene	**Source organism**	**Transgeni host**	**Improved traits in transgenic plant**
TPS1	*Yeast*	*Nicotiana tabacum* L.	Trehalose accumulation, lance-architecture of leaves, dwarfism, low sucrose concentration and better dehydration tolerance [22]
Zm-ASR1	*Sorghum bicolor*	*Zea mays* L.	Enhanced natural water use efficiency, simultaneously increased dry weight on exposure to drought [23]
MTLD	*Escherichia coli*	*Triticum aestivum L.*	Plant height, flag leaf length, fresh and dry weights improved [24]
LEA (HVA1)	*Hordeum vulgare L.*	*Oryza sativa* L.	Water volume relative to leaf become higher, small decrease in plant growth, enhanced cell membrane protection [25]
MNSOD	*Pisum sativum L.*	*Oryza sativa L.*	Decreased oxidative damage, electrolyte leakage, injury whereas SOD activity & photosynthetic rate improved [26]
CAXTH3	*Capsicum. annuum L. cv. Pukang*	*Arabidopsis thaliana L.*	Abnormal leaf architecture with several twist and bend along their edges produced severe wrinkled leaves so improved severe water scarcity tolerance [27]
P5CS	*Vigna aconitifolia*	*Triticum aestivum L.*	Primarily conferred oxidative stress protection, high proline concentration [28]
LEA (HVA1)	*Hordeum vulgare L.*	*Morus Indica*	Improved stability of cell membrane, higher photosynthetic rate, least light induced

			oxidative losses and efficient water utilization under water stress conditions [29]
SINAGS1	*Arabidopsis thaliana L.*	*Lycopersicon esculentum L.*	Better germination rate and higher ornithine collection imparted water scarcity resistance [30]
MH1	*Medicago sativa L.*	*Arabidopsis thaliana*	Improved plant growth, seed germination, osmolytic adjustments, 'superoxide dismutase (SOD)' and 'ascorbate peroxidase (APX)' performance [31]
GsGST over-expression	*Glycine soja*	*Nicotiana tabacum*	Raised tolerance against stresses like drought and salt [32]
MdVHA-B, a V-ATPase over-expression	*Malus domestica*	*Solanum lycopersicum*	Significantly reduced water loss and malondialdehyde amount and enhanced free proline and activity of H+-ATPase [33]
SiMYB56 over-expression	*Setaria italica* (Foxtail millet)	*Oryza sativa L.*	Considerably elevated drought tolerance (both at intial and later developmental stages) by adjusting biosynthesis of lignin and ABA transduction route without having any deleterious impact on plant normal growth [34]

Abbreviation used: Trehalose-6-phosphate synthase(TPS1); *Zea mays* ABA/osmotic stress/ripening activated polypeptide (Zm-ASR1); (LEA; HVA1); Mannitol-1-phosphate dehydrogenase (MTLD); Manganese superoxide dismutase (MNSOD); *Capsicum annuum* xyloglucan endo-transglucosylase / hydrolase *(*CAXTH3); Δ^1-Pyrroline-5-carboxylate synthetase (P5CS); N-acetyl-L-glutamate synthase (SINAGS1); *Medicago sativa* helicase 1(MH1); Glutathione S-transferase gene (GsGST); a drought-activated R2R3-MYB transcription factor (SiMYB56).

Conclusion

Thus, unification of current technologies like genomics, tools of molecular biology, proteomics, metabolomics, transcriptomics along with traditional breeding practices for food crops will undoubtedly speed up the crop improvement programs worldwide. Physiologists and scientists have been utilizing this genomic data in their research to determine association among different abiotic stress genes and crop harvest potential. This amalgamation of various branches has potentially improved both quality and quantity of agricultural areas, which is critical for averting future food crisis issues. Other techniques, particularly gene knockouts and RNA interference (RNAi) studies, are assumed to be helpful in future to add more knowledge to our understanding of the diverse functions of the unknown genes conferring abiotic stress tolerance.

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Review Article

Guleria et al.

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A review on ethnomedicinal properties and biological activities of *Zanthoxylum armatum*

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Abstract: *Zanthoxylum armatum* is an aromatic sub deciduous, branched shrub and it belongs to family Rutaceae. Its maximum height is approximately 5-6 meters with alternate leaves,the flowers are small, light yellow in color. It is widely spread in Himalayas region from Jammu to Bhutan, Pakistan and Nepal. In Indian subcontinent, most part to the Andhra Pradesh and Orissa is covered by *Zanthoxylum armatum*.This plant is significantly used to heal stomachic, tooth pain, antiseptic, and treatingchest infection. A large number of phytochemical compounds such as alkaloids, tannins, amino acids, terpenoids, glycosides are present in the leaves of *Zanthoxylum armatum*. This review has collected information about its botanical position, morphology, pharmacological properties of different parts like seed, bark, stem, leaves, and roots of *Z. armatum*.

Keywords: *Zanthoxylum armatum*, ethno-medical, biological activities, anti-bacterial, leech repellant.

Introduction

The medicinal plants are untapped reservoirs of essential medicines. Plants containing active ingredients used to treat diseases and relieve pain are known as medicinal plants. The Indian subcontinent has the wealthy traditional healing systems on the earth. In India and other developing countries, the plants are major national resources of health protection system [1]. Indian Himalayas has been identified as 34 biodiversity hotspots in the world. It has been found that around 1,748 distinct species of medicinal plants are present [2]. *Zanthoxylum* Linn. belongs to family Rutaceae is an aromatic, prickly, dioecious, or monoecious shrub or tree distributed in tropical region [3]. *Zanthoxylum armatum* is the most important medicinal plant in Indian medicinal literature. In India, about 10 species of *Zanthoxylum* are grown and almost all parts of this plant are used to treat various diseases. Its important medicinal properties have been further reported through scientific research. *Zanthoxylum* is very valuable as it has medicinal, pharmacological and phytochemical properties and is also used in various conventional medicinal applications such as appetizer, carminative, dyspepsia, stomachic, antipyretic, and toothache [4-5]. All most of all portions of this plant contain essential oil called as *Zanthoxylum* oil. Major essential oil reported in seeds, leaves, and fruits of *Z. armatum* are limonene and linalool [6-7]. Many more compounds also have been reported from volatile oil i.e., oleic acid, myrcene, palmitic acid, trans-beta-ocimene, methyl ester alpha and beta-pinene [8]. Several monoterpenes have also been recognized from leaves. The main

monoterpenes are cymene, geraniol, carvone limonene, linalool, tridecanone, terpinolene, *trans*-caryophyllene and ocimene. Another research reported 2-undecaone, the main compound that occurs in leaf oil of *Zanthoxylum armatum* [9]. *Zanthoxylum armatum* DC. (Synonym. *Zanthoxylum alatum* Roxb.) is a major plant which is also known as toothache tree, Nepal pepper, Timur and Indian prickle ash. This plant is widely distributed in India. *Zanthoxylum armatum* is a small shrub sometimes a tree with spiny leaves that are specifically trifoliolate associated with leaf stalk winged [10].

Distribution

The plant of *Zanthoxylum* is widespread scattering in India from Kashmir to Bhutan at 2500 meters altitude. In addition to this, it occurs throughout North East India, Taiwan, Nepal, China, Pakistan, Malaysia, Japan, and the Philippines with varying altitudes from 1300 to 1500 meters [11].

Nomenclature of *Zanthoxylum armatum*

Kingdom:	Plantae
Class:	Magnoliopsida
Order:	Sapindales
Family:	Rutaceae
Genus:	*Zanthoxylum*
Species:	Z. *armatum*

Common Names of *Zanthoxylum aramatum*

English name: Prickly Ash, Toothache tree
Sanskrit name: Tejovati
Urdu name: Tamu
Nepali name: Timur, Nepali peeper
Oriya name: Tundopoda
Bengali name: Gaira, Tambul
Hindi name: Tejphal, Nepali dhaniya

Morphological characteristics

Zanthoxylum armatum is evergreen spiny shrub sometimes a tiny tree, it reaches height of six meters. The leaves of *Zanthoxylum armatum* are 4-20 cm long, spiny, fragment, petioles are glabrous, petioles are long, narrow, and there are two stipules at the base. The leaflets of the plant are glabrous below and appear in 2 to 6 pairs. This plant can be identified by its shrub habits, dense foliage, spicy aroma, thorny trunks and branches, nearly spherical fruits. The flowers are small yellow in color and arise in leaf axils. Flowers appear in a dense terminal and are green to yellow in colour. In the flowers, the petals are absent and six to eight acute sepals are present. The male flowers possess larger anthers due to this the flowers appear yellow in colour and possess 6-8 stamens. Female flowers have 1-3 celled ovary, diameter is 3mm, pale red when it ripe it is splitting into two. Seeds are round, 3mm in diameter, shinning black in colour. Mature carpels are generally solitary, tumorous and reddish. The seeds are spherical, shiny, dark in colour. Blooming takes place from March last to May and the fruiting arises from July – August[12].

Figure: ***Zanthoxylum armatum***

Zanthoxylum armatum **constituents and parts used:** Stem bark, fruits, and seeds are used.

Root: Contains xanthoplaninemagnoflorine, dictamine and gamma-fagarine.
Seeds: The seed contains tambulin, flavonoids and tambulol.
Oil: The oil acquired from dried fruit contains limonene, linalool, sabinene linalyl acetate, citral and geraniol methyl cinnamate.
Bark: The bark contains lignans-sesamin, eudesmin, fargesin, a lactone pulviatide, dictamine, 8- hydroxydictamine and magnoflorine.
Leaves: Contain linalyl acetate, methyl-n-nonylketone, tricosane, and terpene [13].

Ethnomedicinal Uses

The branches, fruit, and thorns of *zanthoxylum armatum* are used to heal tooth diseases and to cure toothache. By mixing the bark powder with honey the bleeding of the gums can be cured. Seeds and fruits of the plant are used to prepare tonic for dyspepsia, for expelling nematodes and fever [14,15]. It is also considered as a condiment, stomachic tonic, abortifacient, insecticidal, antifertility agent [16]. The volatile oil is used as an antidiarrheal agent, preservative, deodorant, and anti-catarrhal medicine. The oil has great toughness and values for fixative quality [17,18]. Timur fruits are generally used by pharmaceutical companies for preparing toothbrushes. In North-East India and Southeastern Asia, it is most commonly used in conventional ways. Anthelmintic and hypoglycemic activities have been reported in the various parts of a plant. It is also used in the therapy of tonic for headache, cholera, remedy for skin diseases, mouth and teeth diseases [19].

Biological Activities

Antibacterial Activity

The highest content of flavonoids has been identified in ethanol extract of *Z. armatum* fruit as compared to ethanol extract of its bark [20]. The main flavonoid present in *Z. aramtum* is 3,5-diacetyltambulin reported. The plant contains certain phytochemicals that were found active against both gram-positive and gram-negative bacteria. By using Agar well diffusion technique, the plant antibacterial activity was reported [10].
The leaf oil of *Zanthoxylum armatum* has been tested against different bacteria that are gram-positive and gram-negative. These bacteria are *Escherichia coli*, *Micrococcus*,

Staphylococcus aureus, *Bacillus subtilis*, and *Streptococcus viridans*. Ciprofloxacin is used as a standard drug [21]. *Streptococcus viridans* and *Bacillus subtilis* were found to be more susceptible. The methanol fraction showed stronger antibacterial activity than the ethyl acetate, while the hexane was not found to be active against bacteria. Oil extraction from fresh leaves of *Zanthoxylum armatum* was analyzed by gas chromatography/ mass spectrometry[22]. The *Zanthoxylum armatum* essential oil showed antibacterial properties for *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli,* and *Micrococcus luteus.*

Antifungal Activity

The leaves of *Zanthoxylum armatum* contains essential oil was evaluated for antimycotic capability against different fungal stains like *Microsporumcanis, Fusarium solani, Aspergillus flavus, Candida albicans, Trichophyton longifusis*and *Candida glabrata*.
With increasing the concentration of the tested sample, the effect become more prominent. The best antifungal effect was noticed in the case of *C. albicans*, *A. flavus* and *F. solani*. The other fungal stain was also affected. It is found in the result that inhibition of total fungal stains was done by the oil obtained from *Z. armatum* leaves [20,21].

AnthelminticActivity

Extracts of the seed of *Z. armatum* such as methanol and Petroleum ether, aqueous were tested against earthworm (*Pheretima posthuma*). The standard used was Piperazine citrate. The minute concentration of the extract cause of paralysis and even the death of earthworm *(Pheretima posthuma)*. Out of these three extracts the methanol extract was found to be most potent[21].

Insecticidal Activity

Zanthoxylum armatum may find applications in the pesticide and food industry due to its use as a source of insecticidal compounds as shown by results[23]. In mustard aphid (*Lipaphiserysimi*), the insecticidal activity of the extract was observed. To determine the insecticidal activity of the extract, the methods of food poisoning were used. In a 6-inch Petri plate with brassica leaf the insect was put in then, a spray of plant extract was done with a chromatographic sprayer. The observation was recorded with insect mortality numbers after every 6, 12, and 24 hours of spraying. The fruit oil of the *Zanthoxylum piperitum* and the seed oil of *Zanthoxylum armatum* together increase repellent function in case of yellow fever mosquito. A comparison of reactions was done with the N, N diethyl-3-methylbenzamide repellent. It has been observed that the plant shows insecticidal properties against *Culex pipiens, Aedes alpepictus, Anophales stephensi, Aedes aegypti, Culex quinquefasciatus,* and *Pieris brassica*[24]**.**

Antidiabetic Activity

The bark extract of *Zanthoxylum sp.* in another study examined for antidiabetic property in diabetic rat induce by streptozotocin. To induce diabetes in the rat the Streptozotocin was used. Overnight fasted rats blood samples were collected on 7, 14 and 21 days of analyzed and treatment for lipid profile and for blood glucose. After 21 day the rats were sacrificed and kidney and liver tissues were excised. It was established that oral management of the *Zanthoxylum* extract for 21 days result outstanding reduction in total cholesterol, blood glucose and very low-density lipoproteins. The streptozotocin diabetic rat there was a significant increase in body

weight and high-density lipoproteins. This study result that *Zanthoxylum* plants show medicinal value in diabetes and other related difficulties[25].

Leech Repellant

The leech repellent action has occurred in the volatile oil of *Z. armatum*. Investigation on perseverance of anti-agent's activity of DEET (N, N diethyl phenyl m-toluamide), DEPA that is N, N diethyl phenyl acetamide, N-benzoyl piperidine, Dimethyl phthalate and oxazolidine on material tried against leeches in deciduous back woods and evergreen rain of Assam. The result obtained were contrasted with unpredictable oil of it to assess its adequacy as parasite repellant[10,19,28].

Anti-inflammatory Activity

Anti- inflammatory property *invivo* of ethanol extract of bark and stem of the plants was estimated in wistar species of the rats by using carrageenan paw edema model. Edema in rats to be biphasic system. First stage is because the reveal of serotonin and the second stage is because of the reveal of prostaglandins, bradykinin, proteases, and lysosomes. Its effects may be due to the inhibition of cyto-oxygenase, thereby inhibiting the formation of prostaglandin[22,29]**.**

Hepatoprotective Activity

A study was done for the evaluation of hepatoprotective properties extract of ethanol the bark of *Z. armatum* in carbon tetrachloride generated hepatotoxicity in the rats. The damage produced by CCl_4 is histologically similar to viral hepatitis. Due to variations in endoplasmic reticulum (ER), the toxicity starts that results in the dropping of metabolic enzymes present in the intracellular structures. The different biochemical parameters were used to assess the process such as alkaline phosphatase, aminotransferase, total protein, serum bilirubin, and serum enzymes (antioxidant) with histopathological studies in liver cell.

The hepato-proactive effect on liver injuries by CCl_4 was observed in the extract and results suggest that berberine present in the bark of *Zanthoxylum armatum.* Berberine has curative and preventive roles in chemically induced hepatotoxicity in rodents, and it is an isoquinaline alkaloids[26].

Antioxidant Activity

In the methanol extract of leaves, antioxidant property was assessed by DPPH that is 1,1-Diphenyl-2-picrylhydrazyl free radical scavenging, reducing power and phosphomolybdate. The standard used was Ascorbic acid. The antioxidant capability assessed by the 3 assays enlarges in a concentration-dependent manner. The study shows that fungus has a significant scavenging effect upon DPPH, and has significant total antioxidant property and reducing. The phytochemical analysis of *Zanthoxylumarmatum* showed the existence of different flavonoids, glycosides, flavonol, terpenoids, alkaloids, lignans, phenols, amino acid, and fatty. Many authors report that the presence of phenols, flavonoids, carotenoids, and ascorbic acid in the *Zanthoxylumarmatum* is the main reason for the efficient anti-oxidant activity[11,27].

Conclusion

It is concluded that medicinal plants are the center of drug discovery and are source of traditional and western medicines. The above review provides the updated information regarding the *Z. armatum*. Essential oils obtained from *Z. armatum* show good antibacterial, antioxidant, anti-inflammatory, antifungal, and anthelmintics activities. Every part of this plant has a significant medicinal use. All essential oil

present in seeds of *Zanthoxylum armatum* has antimicrobial potential, drugs used for the treatment of microbial diseases.

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Production of Plant Derived Immune Checkpoint Inhibitors: A Comprehensive Review

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Abstract: Cancer has become a serious threat to the human population. Numerous therapies are available to reduce or eliminate cancer,however these treatments have demerits too that somehow hinders the complete eradication of the problem. Immunotherapy is blooming as a new pathway to help fight cancer by directly attacking the cancerous cells. The immune checkpoint inhibitors act as blockade system and help to bring out the immune response. These monoclonal antibodies are generally produced from mammalian cells which are highly expensive and difficult to maintain. Plants can be used as a transient expression system to produce complex recombinant proteins such as monoclonal antibodies at a low cost. This review focuses on recent advancements in using these cost effective, versatile and safer sources for the production of immune checkpoint inhibitors and to check their effectiveness to combat cancer.

Keywords: Cancer, immune checkpoint inhibitors, immunotherapy, *Nicotiana*,transient expression host.

Introduction

Recombinant proteins are class of complex proteins which are constructed exogenously in expression hosts. They have immense importance as they are used for diagnostic purposes as well as in human therapies as vaccines, drugs or monoclonal antibodies [1]. Because of being this important and the increasing market demand, recombinant proteins create an opportunity for various expression hosts to be developed in a way that they can be used to manufacture these proteins following the complex standards for human health and veterinary applications. Industrially, the recombinant proteins are produced in already present well established platforms using bacterial or eukaryotic expression systems because of their structural advantages [2]. These systems include *Escherichia coli*, yeast, insect, mammalian cell cultures etc. and have strict regulatory approval which seems to be problematic to convince industries to accept new or advanced technology in this area. Although these production systems are currently in use but they do possess demerits such as high operating costs, longer production time period, limited protein yield, high chances of pathogenic contamination and limited post-translational modifications. It might seem difficult but to challenge the existing platforms, alternative system must possess unique beneficial characteristics that can be useful to overcome the problems associated with existing ones.

Cancer is one of the most dreadful diseases since it has the ability to metastasize and our lack of total understanding of development mechanism of disease can be the reason behind failure in management. The abnormal cellular proliferation in a manner that can't be controlled and failure to maintain normal

cell cycle to death of cells can be termed as cancer. The cancer cells are known to enter in healthy tissues and organs and eventually move to blood and lymphatic systems. Although significant improvement have been made in cancer treatment including chemotherapy, radiation therapy, surgery and targeted therapy that are either combined or given in sequence to reduce or eradicate tumors. These strategies are found to be effective against non metastatic and early cancers making enduring relief pains but they are unsuccessful in dispensing long term benefits in patients suffering from late stage disease. Few exceptions are there such as certain lymphomas, germ cell tumors and testicular carcinomas, leukemia's etc. These multi-dimensional therapies face problems such as no long lasting effect and presence of severe side effects [3]. These treatments are highly expensive and impose great burden on cancer patients. Therefore, there is an urgent need for exploring an alternative for this.

Therapeutic approaches can be manipulated to alter innate immune system which can cause cell death and under suitable environment, adaptive and innate immunity together can lead to oncolysis with long term memory responses [3]. Immunotherapy is an effective alternative to suppress the progression of cancer as it directly influences the malignant cells with efficiency to only target and attack the diseased cells [4]. Immunotherapy can be considered as an effective and promising tool in combating cancer and the most frequent immunotherapeutic agents are ICIs. According to National Cancer Institute (NCI, U.S.), Immune Checkpoint Inhibitors (ICIs) are part of the immune system. They are specifically formed to prevent an immune response which can be strong enough to kill healthy cells in the body. This type of therapy is concerned with the release of brake like mechanism on the immune system responses that actually prevents the overly strong immune response which not only damages the abnormal cells but also normal cells. In this brake mechanism T-cell surface proteins called immune checkpoint proteins are involved. These proteins recognize certain unique partner proteins on other cells, bind together and an off signal is sent to stop T-cells to not express an immune response against those cells [5].

Thus, it can be concluded that this immune response can be stopped from killing the abnormal cells. The immunotherapy drugs or ICIs play key role by blocking the checkpoint proteins so that they unable to bind partner proteins. This blockage mechanism avert the off signal from being sent hence allowing the T-cells to complete their job of killing the cancer cells. With recent advancements in technology, various ICIs have been made and approved for usage. The target checkpoint proteins which are inhibited by ICIs are CTLA - 4 (Cytotoxic T-lymphocyte antigen-4), PD-1 and its partner protein PD-L 1. There are some tumors that are capable of turning down the T-cell response by increasing the production rate of PD-L1which cause T-cells to switch off and thus help cancer cells to escape immune destruction. Thus immune checkpoint inhibitors act as a great asset to overcome this problem enabling strong immune response. There are various ICIs available for the curing various types of cancer such as bladder cancer, breast cancer, cervical cancer, colon cancer and neck cancer, Hodgkin lymphoma, liver cancer, lung cancer, skin cancer etc. Moreover, these ICIs also play an important role for the treatment of solid tumor which is unable to repair the DNA errors that occurs while DNA has been copied [5].

Among all, the very first ICI which was approved for treating advanced

melanoma was Ipilimumab, known to target CTLA-4 [6-8]. This antibody prevents the inhibition of T-cells thus promoting the activation of the effector T-cells. Current scenario shows that varieties of clinical trials are going on to evaluate the efficiency of multiple ICIs as monotherapy or in combination. Two ICIs i.e., Pembrolizumab and Nivolumab which target PD-1 have shown promising results in treatment of melanoma and carcinoma [9-11]. Urethral bladder cancer treated with PD-1/PD-L1 ICIs resulted in an overall responsive rate within 13 and 24% [12]. Currently seven ICIs are approved for therapeutic purposes by U.S. Food and Drug Administration (USFDA) including ipilimumab, nivolumab, pembrolizumab, durvalumab, atezolizumab, cemiplimab, and avelumab [13]. However, the production cost of these drugs make them expensive and affects the accessibility to common people suffering from cancer.

For recombinant protein production, *Nicotiana* genus is often used among plants because it has fast growth rate and easy to manipulate. Tobacco plant is mostly used for the development of complex proteins such as vaccines, cytokines, hormones, growth regulators etc., hence it is known as workhouse of plant world in field of molecular biology. *N. benthamiana* and *N. tabacum* are commonly used species for transient stable production and expression of complex proteins. Apart from *Nicotiana*, there are number of other plants such as lettuce, tomato, rice, maize, alfalfa, potato also tested for their potential to be used in plant molecular farming [1].

Experimental Studies

Establishment of plant based platform is necessary to be used to rapidly express and assemble immune checkpoint inhibitors. From manufacturing perspective, in comparison to traditional production protocols, plants have remarkable simpler physiology and low raw material cost as compared to existing transient systems[14,15]. Recombinant proteins produced from plants have to meet the construction criteria such as stability, appearance, half-life, performance etc. same as the proteins produced in mammalian cells [16]. A recent experimental study reveals plant as potent transient expression system to produce monoclonal antibody Pembrolizumab [17]. Plant of interest here was *N.benthamiana.*Transient expression of this antibody was seen after 4 days of the agroinfiltration in wild type *N.benthamiana*showed accumulationup to 344.12 ± 98.23 μg/g of fresh leaf weight. Comparative analysis of plant produced Pembrolizumab with commercially produced Pembrolizumab (Keytruda®) showed that both are physicochemically similar. SDS-PAGE and western blot analysis revealed that Pembrolizumab produced by plant has molecular weight comparable to Keytruda®. Both antibodies had similar protein aggregation with same secondary and tertiary protein structures. It was confirmed that the inhibitory performance of plant produced Pembrolizumab between PD-1 and PDL-1 interaction was comparative to Keytruda®. Further, *invitro* efficacy for the activation of T-cell, the plant-produced Pembrolizumab was capable of causing Intreleukin-2 and Interferon-γ production. Thus it is confirmed from this study that plants as transient systems for monoclonal antibody production are not only efficient but provide good results and are worthy opponents to industrial production technologies.

Industrially established systems of production are prone to human and animal pathogenic contamination whereas plants provide production platform free from this type of contamination. Plants are capable of producing large amount of product rapidly

[18]. All these advantages indicate plants as cheap optional sources for the production of complex proteins. This recent research on the development of an ICI Nivolumab supports this point. Nivolumab is an anti-PD1 IgG4 monoclonal antibody (mAb) and is used to treat high metastaic colorectal cancer following treatment with fluoropyrimidine, oxaliplatin etc. Currently, Nivolumab is produced from Chinese hamster ovary (CHO) cells which in turn is highly expensive process and expected to be up to 1 million US dollars per patient [19]. In this research, plant platform is used for its production. Plant of interest in this study was *Nicotiana benthamiana.* The result shows that plant produced Nivolumab was comparable to industrially produced Nivolumab. Both the antibodies had similar protein structures and the plant specific structures were absent in N-glycans on plant produced antibody. Plant produced Nivolumab was tested for PD-1 binding efficiency and showed comparative activity of binding to PD-1 protein inhibiting PD-1/PDL-1 interaction with high specificity to CHO produced Nivolumab. This confirmed that plant produced Nivolumab can be a potent anti- cancerous drug for effective immunotherapy [20].

Another study dedicated to cost effective production of monoclonal antibodies through plants as production systems provided good results. Hydroponically grown *N.benthamiana*plants were used for agroinfiltration for the production. The system was set up to examine the total investment, operative cost and cost of products sold as function of monoclonal antibody expression level in plants and production capacity. For the basic design, model predicts about $122 million dollars of total capital investment with cost of product sold of $121/g. Comparative analysis to traditional production platforms shows notable decrement in investment and >50% reduction in cost of products with the published value. This model can be a great alternative and with profitable modifications can be used for production purposes [14].
Plant cells are capable of assembling complex proteins, and perform necessary post translational modifications [21]. Hence plants are attractive alternative for producing complex proteins in form of medicinal drugs for developing countries.

Conclusions

Immunotherapy is known as a recent therapeutic option against cancer that can be a successful alternative to traditional cancer treatments. Immune checkpoint inhibitors are boon in this line of treatment. These monoclonal antibodies are reliable and only target cancer cells thus eliminating the side effect of killing healthy cells. Immune checkpoint inhibitors are produced in mammalian cells in bioreactors which is economically very costly. Thus plants can be used as reliable transient expression systems. *Nicotiana benthamiana* is one such plant which has been used worldwide for the production of complex protein molecules. Recent studies shows that complex proteins like monoclonal antibodies can be produced in this plant and have shown good results. With development and modifications in this line of therapeautics, plant based production of these complex proteins can be a good source to provide an easily available medicine to all to fight cancer.

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Nipah: the virus with pandemic potential

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Abstract: While the world grapples with Covid-19, there is another virus that could cause the next pandemic in Asia. Nipah is one of the most lethal viruses, with a high death rate and potential of causing a pandemic. The first outbreak of Nipah virus happened about 22 years ago in Malaysia, leading to the virus's identification in 1999. Following that, outbreaks have been observed practically annually, implying that the virus has been infecting humans unnoticed for many years. The high mortality rate of the Nipah virus, which ranges from 40% to 75%, plus the lack of therapy make it a major worry. World Health Organization (WHO) review each year, the large list of pathogens which pose greatest risk to Human health. Nipah virus is among top ten in their list, which focus on viruses that have no vaccines. This review elaborates about the past outbreaks of Nipah virus, mode of transmission, clinical symptoms, variables that contribute to virus emergence and spread, as well as preventative and control strategies to ensure a decrease in number of incidents in future.

Keywords: Nipah, fruit bats, primary host, encephalitis.

Introduction

Severe Acute Respiratory Syndrome (SARS), Influenza, Middle East respiratory syndrome coronavirus (MERS-CoV), Zika, Ebola, Nipah, and COVID are only a few of the viral infections that have arisen as a global public health issue. [1]. Nipah is one of these deadliest viruses which have caused various outbreaks in past and have the potential of causing a pandemic in future [2]. The first case of outbreak of Nipah virus was recorded in Malaysia in the year 1998. In the year 1999, scientists established the presence of the Nipah virus. The three nations where Nipah outbreaks have previously been documented are Malaysia, Bangladesh, and India [3]. Nipah virus is a highly lethal zoonotic virus belonging to Paramyxoviridae family and order Mononegavirales [4]. It is a member of the Henipavirus genus, which also includes Cedar virus (CedPV) and Hendra virus (HeV) [5]. Nipah virus is named after the place where it had first emerged in, i.e., Sungai Nipah, a village in Malaysia [6]. The fruit bats of Pteropodidae family particularly belonging to Pteropus genus are the natural reservoir of Nipah virus. However these bats do not show any signs of disease caused by this virus. Pigs and horses are the intermediate hosts of the virus [7]. Coming in contact with these bats and intermediate hosts is the main cause of spreading infection in Humans. Virus transmission occurs through contact, inhalation, or consumption of virus contaminated food [8]. The reason for scientists' concern about the Nipah virus is that it is on the World Health

Organization's list of viruses that pose the greatest risk to human health and for which no vaccination exists [9]. The recent case of a boy who died due to Nipah virus infection in Kerala, a southern state in India, has made officials to take prompt actions to prevent the spread of this deadly virus with potential of causing a pandemic. Another reason why Nipah is so sinister is its long incubation period which ranges from 6-21 days. Its long incubation period therefore gives ample opportunity to an infected host to spread it without even knowing it that they are ill [10].

Several factors are responsible for the outbreak of various zoonotic viruses [11]. By introducing a massive environmental and ecological disequilibrium, anthropogenic influences have contributed to the growth of viral illnesses [12]. In this context, the mode of transmission, prevention and management efforts, along with variables which might have contributed to the outbreak have all been explored.

Nipah Virus

Nipah virus is an RNA virus that belongs to the Paramyxovirinae subfamily of the Paramyxoviridae family of viruses; order Mononegavirales, and genus Henipavirus. It is closely related to the Hendra virus which also belongs to the same genus [13]. The genome of the virus is made up of an 18.2 kb negative sense single-stranded RNA with no segments. Matrix protein (M), phosphoprotein (P), nucleocapsid (N), glycoprotein (G), fusion protein (F), and long polymerase (L) are six structural proteins encoded by the RNA genome. The virus ribonucleoprotein is formed when the Phosphoprotein, Nucleocapsid and long polymerase connect to the viral RNA (vRNP) [14]. The fusion protein and glycoprotein are in charge of the virion's cellular attachment as well as subsequent host cell invasion. By interacting to the Ephrin-B2 and -B3 receptors, the Glycoprotein facilitates virus binding on host cell membrane, whereas the Fusion protein stimulates viral-cell membrane attachment, allowing virion penetration [15].

Past outbreaks of Nipah virus

There are three countries in which outbreaks of Nipah virus have been documented: Malaysia, India and Bangladesh. Out of the total incident cases of Nipah virus worldwide, 43% cases are of Malaysia, 42% are of Bangladesh and 15% are of India [16].

The first case of the outbreak occurred in the year 1998 in Malaysia. After that the next two outbreaks also occurred in the same year in the month of December [17]. However initially, the cause of outbreaks was thought to be a virus known as Japanese encephalitis. But the presence of novel virus, Nipah was confirmed by the scientists in the year 1999 [18].

After almost two years of the past outbreak of Nipah virus in Malaysia, Bangladesh recorded its first outbreak in the year 2001. From 2001 to 2015, there were numerous outbreaks of the Nipah virus in various locations of Bangladesh [19].

India reported Nipah virus first outbreak in the year 2001 in West Bengal. The mortality rate of this outbreak was 68%, as 45 deaths were recorded from total of 66 confirmed patients [20]. In 2007 West Bengal recorded its second outbreak of Nipah virus. This time the fatality rate was

100% as all the five Nipah virus patients died [21]. India reported its third outbreak in the year of 2018 in the state of Kerala which was the most intense one. This time the mortality rate was 91% [22]. A recurring incidence of Nipah virus was reported in 2019 in Kerala, with one patient positive for Nipah virus. The most recent case has been recorded in which a 12 year boy died in Kerala who tested positive for the virus. This caused panic and havoc among the scientists and officials. However, no more case has been reported after it.

Nipah Virus Transmission Modes

Interaction with the bats that are the natural host of Nipah virus and pigs, the intermediate host, is the main reason of infection in Humans. Although these fruit bats are symptom free but they are able to shed the virus through their urine, saliva, excreta and semen [18]. Mainly the cases of Nipah have been mostly occurring in regions where bats, pigs and humans live next to each other. People in many Asian countries depend upon animal rearing for their source of income [23]. They also cultivate fruit bearing trees like date palm in and around their farms. Flying foxes which are the primary reservoir of Nipah virus, get attracted by fruits, and then lead to the spillover of virus to the pigs/animals and humans also. Mainly the Nipah virus transmission occurs through the ingestion of virus infected fruits, interaction with contaminated animal effluents or secretions or infected human body fluids [24].

It's important to understand the propagation of virus between flying foxes, pigs and humans. From the past outbreaks it's recorded that the pigs in the farms got infected by consuming the fruits partially chewed or tainted by flying foxes infected by Nipah virus. After which the spillover from pigs to humans occurred via direct exposure to sick pigs and human to human transmission of virus was through direct contact, fomites or aerosols [25].

Clinical Symptoms

The virus is known to affect the both the respiratory and central neurological systems in humans. It takes about 3-14 days post exposure, to show the signs and symptoms. The infected person may experience drowsiness, headache, and respiratory problems with high temperature during the initial phase. In severe cases Nipah infection causes encephalitis, a swelling of the brain which can cause seizures turning fatal [26].

While in pigs, there is a progression of respiratory ailment along with weakness of hind legs, twitching of muscles because of the neurological system's involvement. In case of acute infection, there may be emergence of small vessel vasculopathy in Central Nervous System and other important organs like lungs and kidneys [27].

Factors that play a role in virus initiation and transmission

Asia is home to nearly 15% of the world's tropical region, which means it has rich array of biodiversity. But along with that it is home to large pool of pathogens, increasing the chances for emergence of novel virus [28].As the population of Humans is expanding, they are changing the planet and destroying habitats of wild animals in order to meet their increasing demand for resources. Due to this they are increasing the risk factor of spreading zoonotic diseases as human and wild animal

interactions have also increased [29]. Destruction of the bat habitats due to urbanization, deforestation, climate change, agricultural intensification has forced bats to search for alternative roosts. They have started living on the fruit trees grown on the same farms as pigs and hence do the spillover of viruses. Bats harbor number of nasty diseases like Nipah, Covid-19, Ebola, Sars etc [30].

Does the eradication of bats is the solution for our above mentioned problems? No, as bats play hugely important role in our ecology and human health. Bats are known to pollinate more than 500 plant species. Along with that they are important for human health because they control the diseases by, for example, reducing malaria by eating mosquitoes. Therefore we can't think of eradicating bats.

Prevention measures

Other than vaccination there are other strategies which can play an integral role in prevention and management of Nipah infection in Human [31]. Because livestock are intermediate hosts, preventing infection in them could be a successful method. Fruit trees along with bat roosting trees should be kept away from virus-prone cattle farms. Increasing awareness among people about the prohibition of procurement and consumption of raw date palm sap or bat bitten fruits is necessary [32]. Proper washing of fruits and vegetables before consuming them is important to remove the bat effluent. The skirts are used to enclose the sap generating regions of date palm trees, a measure to control the access of fruit bats to the sap stream of date palm [33]. Human-to-human transmission of infection could be controlled by reducing the contact between the patient and caretaker. All sort of patient aid material must be handled only while wearing gloves and a mask [34].

Conclusion

Over the past two decades, the outbreaks of Nipah virus have been documented in several regions of the world posing threats to the global health security. The outbreaks could be controlled and managed by providing proper awareness among people. Due to extensive research, the pathogenesis and spread of the Nipah virus are well understood. Despite of this, a deeper knowledge of basic facts of Nipah virus biology, epidemiology and the development of effective treatment is needed.

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Biology of oyster mushroom fly, *Bradysia asiatica* and its management using sticky traps and repellent effect of eucalyptus extracts

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Abstract: The present investigation was carried out to study the life history and development characteristics of oyster mushroom fly, *Bradysia asiatica*. It has a high reproductive potential and a short life cycle of 23±1 days. It can complete many generations on a single crop. Therefore, its severity can be increased manifold. Different colored sticky traps (red, green, blue, yellow, white) with/ without bulbs were used to capture the adult flies. The results obtained depicted that yellow (156.5) sticky traps gave the maximum number of adult fly count while white (22.5) traps were the least efficient in capturing flies. Diethyl ether extract of eucalyptus showed maximum repellency rate of 70.78 per cent against the adults of *B. asiatica*.

Keywords: *Bradysia asiatica*, biology, sticky traps, repellency, eucalyptus leaf extracts.

Introduction

The oyster mushroom (Pleurotus spp.) of the Class Basidiomycetes and Family Agaricaceae, widely known as 'Dhingri' in India, grows naturally on dead and rotting wooden logs in temperate and tropical woods [1]. Oyster mushroom ranks second among the cultivated mushrooms in the world [2]. Mushrooms are attacked by a number of pests from spawning to harvest. Various insects, mites and nematode pests feed on oyster mushroom in India at different growth stages and cause extensive losses in the yield and even sometimes cause total crop failure [3]. Among insect pests; sciarid fly, phorid fly, cecid fly, nematodes, springtails, mites, beetles, moths etc. cause damage to the crop [4]. Mushroom flies are commonly found in mushroom cultures, but they may also be found in rotting wood, decaying potatoes, and decomposing crops [5]. Their females may lay eggs in the compost or casing layer, and cause yield losses from their larvae feeding on the mycelium and creates tunnels into the caps and stems of the mushrooms. Larval feeding of second and the subsequent generations can be very destructive. The larvae can derail the product for consumption, leading to losses of around 20 per cent. In addition, adults of mushroom flies serve as vectors for pathogenic agents, nematodes, mites, and other contaminants [6,7]. Uncontrolled populations of any species of mushroom fly can result in significant production losses owing to both direct larval activity and disease transmission by adult flies. The continuous use of insecticides has resulted in resistance development in mushroom fly populations [8], and additional tactics or methods need to be evaluated [9]. In various

ways, with the use of repellents, antifeedants, pesticides, growth regulators and oviposition deterrents can be implemented on adult population [10].

Materials and Methods

Mushroom fly culture

The population of *B. asiatica* encountered during survey was collected and maintained in the laboratory by keeping them in plastic cages by providing 500 g insect pest free spawned mushroom compost and the culture was used for further experiments.

Biology of *B. asiatica* on oyster mushroom (*Pleurotus* spp.)

Biology of dipteran fly collected during the survey was studied in the laboratory.

Observations recorded:

i. Fecundity rate- Freshly emerged one pair of adult fly (male and female) was released in the insect cage having fresh spawned compost in Petri plate. The same set was replicated thrice. No. of eggs were counted every day and fresh spawned compost was provided everyday till the female fly stopped egg laying.

ii. Pre-oviposition period- Time of emergence of adult female fly from pupa was recorded and after emergence the female fly was transferred to the insect cage and was provided with Petri plate having fresh insect free spawned compost. The same set was replicated thrice. The female fly was observed for egg laying at 12 hours interval till the egg laying started.

iii. Oviposition period- The time period from first to last egg laying was recorded. Petri plates for observation were replaced every day and were replicated thrice.

iv. Incubation period- Days from egg laying to hatching were counted. On an average 20 eggs were taken.

v. Larval period- The time period from 1st to last instar was recorded.

vi. Pupal period- The time period from pupa formed to emergence of adult was recorded.

vii. Adult longevity-The time period for which the adult remained alive was recorded.

viii. Male: female ratio- 20 pupae were kept in petri plate and proper moisture was maintained. Males and females emerged were counted randomly.

ix. Total life cycle- The total time taken from egg to adult emergence was recorded.

Collection of plant leaves for extraction

Leaves of *Eucalyptus globulus* were collected from the surroundings of Hamirpur (H.P.) and were dried under shade before being pounded with a wooden stick to make a coarse powder and were stored in a cool and dry place in the room.

Preparation of aqueous, ethanolic and diethyl ether leaf extract

Shade dried leaves were powdered with the help of electric grinder. A sample of 20 g powder was weighed and soaked in 200 ml distilled water / ethanol / diethyl ether overnight in a beaker. Solvents were evaporated and extract was further dried. The extract obtained after drying was again dissolved in 200 ml distilled water and the contents were filtered through the Whattman No. 1 filter paper. This stock solution was designated as 10 per cent leaf extract and was used for further experiments.

Effect of different colored sticky traps against *B. asiatica* on oyster mushroom

Double sided different colored sticky traps (10×20 cm) *viz.* yellow, blue, green, red

and white with bulbs (0.5 Watt) and without bulbs were placed inside the cages. The traps were divided into squares and coated with mustard oil. Adult pests were introduced into the cage. Color of the trap preferred by the pest was noted down and number of insects that were found sticking to the trap in each square was calculated.

Repellent effect of leaf extracts of *E. globulus* on *B. asiatica*

100 ml capacity beakers were filled with spawned compost. In total twelve beakers having compost were placed in the insect cage. Out of twelve, nine beakers were treated with the formulations under study and these beakers were placed randomly in the insect cage. Three sets of replication were made for observation in the laboratory. Twenty number of adult flies were released in the cage having beakers. The observations were recorded after 24 hours and the number of flies landed on the compost kept in the beaker were counted.

Percent repellency= $\frac{UT-T}{UT} \times 100$

Where,

T = average number of flies on treated bags

UT = average number of flies on untreated bags

Results and Discussions

Biology of *B. asiatica* on oyster mushroom

The life cycle of *B. asiatica* was studied under the laboratory conditions at COHF, Neri. Three sets of replicates were taken under each head. The eggs of *B. asiatica* were oval, smooth, shiny and translucent and were laid singly or in small clusters of 3-4 eggs. Eggs were laid singly as well as in groups selectively on the mycelium. The incubation period was 3.33 ± 0.33 days (Table 1). Similar observations about the eggs and incubation period were made by Katumanyane [11] for *Bradysia* spp. (Diptera: Sciaridae). The larvae were apodous, slender with strongly sclerotized head capsule of black to brown color and translucent body. Their skin was semi-transparent which revealed all the contents of the digestive tract. The larvae fed on the mycelium agar plate and went through four developmental stages before turning into a pupa. Initially the larvae were too small but with every moult, the instar increased in length. The time taken for larval deelopment was 10.67 ± 0.33 days. Similar results were recorded by Katumanyane [11] for *Bradysia coprophila* and *Bradysia impatiens*. Choi [12] also recorded mean larval period of 10.5 days at 28°C for *Coboldia fuscipes* (Diptera: Scatopsidae).

The fully grown larva after completing its development stopped feeding for some time and became sluggish. It moved to a certain depth into mycelial threads and spun a cocoon for pupation. The newly formed pupa was white which later turned yellow and finally to golden brown color. The pupa had visible appendages that were close to the body and resembled the adult fly. The pupal period was completed in a lapse of 4.33 ± 0.33 days. Lee [13] reported that the growth period of pupa lasts about 5 days depending on the increasing temperature. The variance in length, on the other hand, is primarily dependent to temperature, with the average being around 3 days. The adults that emerged from the pupa were small, brown colored flies with prominent eyes, transparent wings and thread like antennae. Adults did not feed on the mycelium. The females were longer than the males and had an ovipositor at the end. The males possessed a pair of claspers for holding the female during mating. The duration from the date of emergence to death of adults was considered as the adult longevity. The longevity period was 4.67 ± 0.33 days for *B. asiatica*. Katumanyane [11] reported adult longevity to

be 4-7 days long and longevity of males was short as compared to females.

Male: female ratio was 6.66: 13.33 for the adult fly. Nigro [14] found that the sex-ratio of *Sciara ocellaris* was symmetrical (*i.e.* about 50% males) at 18-20°C. At higher temperatures of 24-28°C, the distributions were biased toward a large number of females, with the mean proportion of males dropping to around 30-37 percent. The total life cycle was completed in 23 ± 1 days. Choi [12] noted that the total time period for complete development for *Coboldia fuscipes* is dependent on the temperature and varies as 24.5 and 18.9 days at 25 and 28°C, respectively. The average number of eggs laid per female were 28.67 ± 2.03. Pre-oviposition and oviposition period lasted for 1.67 ± 0.33 and 3 ± 0.58 days, respectively.

Table 1. Biology of *B. asiatica* on oyster mushroom (*Pleurotus* spp.)

Developmental stage	Mean	S.E.
Ovipositional period (days)	3.00	0.58
Pre-oviposition period (days)	1.67	0.33
Incubation period (days)	3.33	0.33
Larval period (days)	10.67	0.33
Pupal period (days)	4.33	0.33
Adult longevity (days)	4.67	0.33
Total life cycle (days)	23.00	1.00

Efficacy of different colored sticky traps against *B. asiatica* on oyster mushroom

Mushroom bags infested with flies were kept in a room for a period of about 10 days. The traps were randomly hung at different locations on the walls of the room. The efficacy of colored sticky traps was evaluated by counting the number of flies that were stuck to the trap after 10 days.

The results depicted significantly greater number of flies sticking to the traps with bulb (91.80) in comparison to the traps without bulb (62.60). Yellow was the most preferred color as maximum flies were found sticking to it (156.50). Not much difference was observed between blue (82.50) and red traps (73.50) while white traps were the least effective and caught minimum number of flies.

Sahin [15] performed a similar experiment to elucidate the effectiveness of yellow and blue colored sticky traps in capturing the mushroom flies. Similarly Manzin [16] also studied the activity and population of mushroom phorid fly, *Megaselia halterata* using yellow sticky traps.

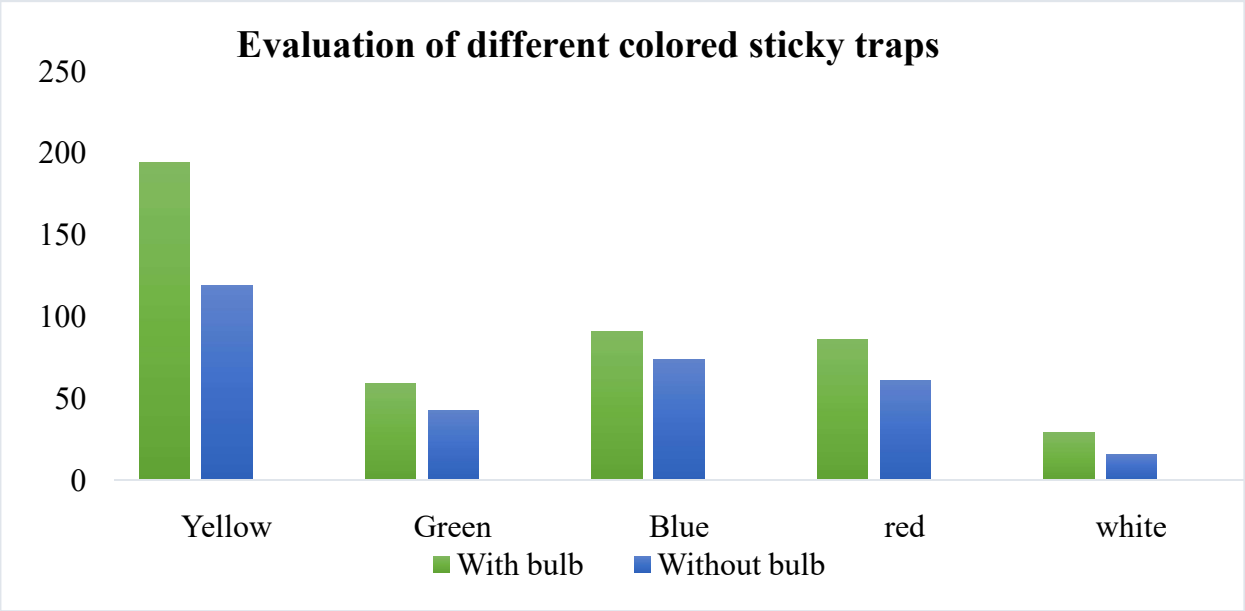

Figure 1: Effect of different colored sticky traps against *B. asiatica*

Repellent effect of leaf extracts of *E. globulus* on *B. asiatica*

Repellent effect of different eucalyptus formulations was studied under laboratory conditions. 100 ml beakers containing compost treated with 10.0 per cent aqueous/ diethyl ether/ ethanol were kept in a cage. A control without any treatment was also kept along with it.

Twenty number of flies were introduced into the cage and after 24 h repellency was checked by observing the number of flies that landed on the treated beakers and control. The same experiment was replicated thrice. The best repellent rate was shown by eucalyptus diethyl ether leaf extract (70.83%) followed by ethanolic extract (54.17%). Aqueous leaf extract of eucalyptus was found to be least effective (25.00%) for repellency against mushroom flies. Akob and Ewete [17] performed a similar experiment and tested ethanolic extracts of *E. grandis* along with 3 other plants against *Sitophilus zeamais* and found varying percentage of repellency by all four plants.

Conclusion

From the present investigation, we can conclude that the development (life cycle) of *B. asiatica* was short and was completed in 23 ± 1 days. It can complete more than one generation on a single crop and has high reproductive potential. The adult flies can be best monitored with the help of yellow sticky traps while white colored traps were the least effective in capturing the flies. Eucalyptus diethyl ether extract provided best repellency rate of 70.83 per cent.

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Original Research

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Optimization of iron and calcium rich formulation using response surface method

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Abstract: In the current examination endeavors have been made to create a ready to use iron and calcium rich "Nutrimix" and evaluating its physiochemical properties and proximate composition with respect to optimize process variables such as particle size, moisture content and anti-caking agent using response surface methodology (RSM).The experiment was designed to analyze water activity (a_w), oil holding capacity (OHC), water holding capacity (WHC), density, colour index (CI) and overall acceptability (OAA) as a response variables. Optimization techniques were chosen to obtain the optimum levels of process variable for the development of iron and calcium rich nutrimix. The optimum conditions were found at 250 micron particle size, 13% moisture content and 5% anti-caking agent of formulation that gives 304.22%±0.015 for WHC, 146.87%±0.177 for OHC, 0.702±0.010 for a_w, 0.454±0.016g/ml for density, 27.2±0.568 for colour index and 7.6±0.321 for overall acceptability of nutrimix.

Key words: Nutrimix, iron, calcium, partical size, anti-caking agent.

Introduction

Malnutrition simply means bad nutrition. This condition happens when the body is denied of least every day sustenance. Malnutrition for the most part happens in youngsters and pregnant ladies, which results in the underweight of the kid. Be that as it may, it is additionally present in the grown-ups, where the essential explanation is Malabsorbtion[1].Generous enhancements has been endeavored in wellbeing and prosperity of unhealthiness, yet the greater part of all kids younger than 4 are malnourished whereas, thirty percent of children are fundamentally underweight and sixty percent of ladies are anemic[2,3]. "As assessed by the World bank, India is positioned second in malnourished children populace after Bangladesh" [4,5]. According to the recent research found that worldwide most commonly found deficiencies of micronutrient are iron, vitamin A and iodine deficiency. Out of that, lack of iron causes anemia is the most common basis of malnutrition in the society [6]. Micronutrient deficiencies can be addressed by using food dependent policy. These policies can enhance the content of calcium and iron available for human body development. These strategies enhance the bioavailability of foods that rich in calcium and iron, changing eating behavior by consumption of these foods through nutrition education programs, increasing bioavailability of calcium and iron in the body through breeding of new plant foods [7]. This can be achieved through many natural bioresources rich in iron, calcium and vitamin A available such as herbs and spices, pulses, legumes, cereals, green leafy vegetables and nuts for regular diet of malnourished population.

World food programme (WFP) fights against micronutrient malnutrition by using a wide scope of specific food sources to work on the healthful admission of individuals. They fostered a reach from fortified blended foods (FBFs) to micronutrient powders for diet and high-energy biscuits (HEBs) to combat this problem in any part of the country. A large part of the WFP's was initiated for upgrade the nutritional status of mothers and young children. These programs highlighted specialty of various food products in their regular diet such as pastas for mothers and children of malnourished inhabitants[8]. The survey found that among children, adolescent girls, expectant and nursing mothers, the median admission of the relative nutrients is low when contrasted with the suggested dietary recompenses (RDAs). On the other hand, the mean admission of this load of supplements is just 32–40 mg [9]. This needs quick improvement for the development of any country. In this study, our aim is to develop iron and calcium rich powder premixes as "Nutrimix" using response surface methodology for malnourished society. The final product will be composed of various underutilized sources and food crops that can be available at low and affordable cost for ready to use in various food preparations.

Materials and Methods

Raw Materials

Natural food bioresources such as cauliflower leaves, oregano, thyme, basil, and pipali were collected from the local region of Himachal Pradesh, India. Sesame seed as well as coconut powder were purchased from the local market. The AR grade reagent and chemicals of Sigma Aldrich were used for analysis.

Preparation of nutrimix

All food bioresources and other procured ingredients were pre processed such as placed under hot air oven for drying. After drying these dried bioresources milled to powder and kept under cool and dry place for further use. The formulation below was prepared by blending desirable quantity of each powdered ingredient. This formulation was optimized further using statistical technique for various end use food applications. Composition of different nutritionally rich ingredients as follow:

Ingredient	Quantity
Oregano (*Origanum vulgare*)	4gm
Thyme (*Thymus vulgaris*)	4gm
Basil (*Ocimum basilicum*)	4gm
Pipali (*Piper longum*)	2gm
Cauliflower leaves (*Brassica oleracea*)	2gm
Sesame seed (*Sesamum indicum*)	2gm
Coconut powder (Cocos nucifera)	2gm

Design of Experiment

The experimental plan for this study was carried out using "central composite rotatable design". "Response surface methodology (RSM) is found to be generally embraced apparatus for the nature of improvement processes of optimization processes"[10]. The number of independent variables decides the number of design points in the experimental plan. The RSM, initially depicted by Box and Wilson[11] In the CCRD designs all factors were fluctuated inside a picked limit[12]. The 3 independent variables were partical size (X_1), moisture (X_2) and anticaking agent (X_3). Every optimizing parameter was designed at five levels and total twenty experiments were set in which the combination number 15, which is also called the centre-point, was rehashed 6 times. The levels of every factor were set up as indicated by writing data and starter preliminaries. The coded and natural values of each experimental variable is introduces in Table 1. "Analysis of variance (ANOVA)" was carried out to decide the

significance of the design experiment. The multiple regression i.e. R^2 values were also analyzed. To estimate partical size, moisture content and anticaking agent effect on various responses such WHC, OHC, bulk density, a_w, CI and OAA, the quadratic polynomial regression equations were designed[13]. The quadratic equation for the response Y was as following:

$$Y= \beta^0+ \beta^1X^1+ \beta^2 X^2+ \beta^3X^3+ \beta^{11}X1^2+ \beta^{22}X2^2+ \beta^{33}X3^2 + \beta^{12}X1X2+ \beta^{13}X1X3+ \beta^{23}X2X3$$

Where Y = the response, X1= partical size, X2 = moisture content, X3 = anticaking agent, β^0= intercepts, β^1, β^2, β^3 were the linear regression coefficients. β^{11}, β^{22}, β^{33} were the quadratic regression coefficients, whereas, β^{12}, β^{13} and β^{23} were the interaction regression coefficients. The sufficiency as well as accuracy of the model was determined using P value, F value and Lack of Fit. The different experiment run were performed as well as further analyzed for its optimize conditions of process variables.

Proximate evaluation

According to the design 20 different batches of formulation were prepared by blending of weighed amount of these ingredients and were passed through the different sieve size at required moisture content. The experiment run were analyzed for different responses.The moisture content of each nutrimix was assesed using standard AOAC [14] methods. Water holding capacity of each formulation (nutrimix) was carried out in accordance to Robertson et al. [15]. Approximately 2gm of fine ground nutrimix was weighed and allowed to rehydration overnight in excess water after draining, it was reweighed and WHC calculated on dry basis. Oil holding capacity of each formulation was determined from 2gm of fine ground nutrimix sample was allowed to rehydration overnight in excess oil and OHC was calculated by increase the initial sample weight. Density of the each formulation was calculated using standard units of measurement for mass and volume of preweighed samples [16]. Hunter colour values and colour index of different formulation of experiment design was determined by colour lab (CR 400, Konica Minolta).

Water Activity (aw)

Water Activity of the different formulations can be calculated by water activity meter (4TE Aqualab, USA) using sample cups. This is based on the vapour equilibrium under infrared beam focused on a dew point temperature and finally dew point temperature is translated into water activity.

Iron and calcium content

"Atomic absorption spectrometry (AAS) is a scientific method that measures the concentrations of elements". "Atomic absorption is excessively touchy such that it can gauge down to parts per billion of a gram (µg dm-3) in an example". Atomic Absorption (AA) happens when a ground state molecule assimilates energy as light of a particular frequency and is raised to an energized level. The connection of the light consumed as well as the concentration of substance present in samples can be utilized in this experimentation. The Atomic absorption spectroscopy depends upon the similar trends as the flame test used in qualitative analysis [17]. The test was performed using 1gm nutrimix sample that digested first with nitric acid for 2 hour. After digestion the content was filtered, cooled and make up to 100ml with distilled water. The iron and calcium content was analyzed using atomic absorption spectroscopy (Shimadzu Model AA6300, Tokyo, Japan).

Table 1: Experimental Design

Run	Coded Values			Actual Values		
	X1	X2	X3	X1	X2	X3
1	1.00	-1.00	1.00	250.000	9.000	15.000
2	0.00	-1.68	0.00	215.000	7.636	10.000
3	-1.00	1.00	-1.00	180.000	13.000	5.000
4	-1.00	-1.00	-1.00	180.000	9.000	5.000
5	-1.00	-1.00	1.00	180.000	9.000	15.000
6	0.00	0.00	0.00	215.000	11.000	10.000
7	0.00	0.00	0.00	215.000	11.000	10.000
8	0.00	0.00	-1.68	215.000	11.000	1.5910
9	0.00	0.00	1.68	215.000	11.000	18.409
10	0.00	0.00	0.00	215.000	11.000	10.000
11	1.00	-1.00	-1.00	250.000	9.000	5.000
12	1.68	0.00	0.00	273.863	11.000	10.000
13	0.00	0.00	0.00	215.000	11.000	10.000
14	0.00	0.00	0.00	215.000	11.000	10.000
15	0.00	0.00	0.00	215.000	11.000	10.000
16	-1.00	1.00	1.00	180.000	13.000	15.000
17	1.00	1.00	-1.00	250.000	13.000	5.000
18	1.00	1.00	1.00	250.000	13.000	15.000
19	-1.68	0.00	0.00	156.137	11.000	10.000
20	0.00	1.68	0.00	215.000	14.363	10.000

X1= Partical size, X2=Moisture Content, X3= Anti-caking agent

Sensory evaluation

The prepared nutrimix formulation of experiment design was analysed for the colour, aroma, taste, and overall acceptability at 9 point hedonic scale by the panel of 15 people at different concentration of nutrimix in different foods. The prepared formulation was incorporated into soup, noodles and snacks to accesses its applicability at maximum acceptability by taste panel.

Statistical analysis

In statistical ANOVA was utilized to determine the significance of the experiment. The values obtained at $p<0.05$ was assessed and utilized to discover critical significance of the overall experimental design.

Result and Discussion

The nutrimix formulations were prepared in this study to perform the design of experiment. It was further subjected for determining proximate composition as well as organoleptic characteristics. In the analysis of design WHC (Y1), OHC (Y2), water activity (Y3), Density (Y4) and colour index (Y5) and overall acceptability (Y_6) were assessed as responses. The results of proximate analysis of nutrimix formulations revealed that protein content of 0.545, 1.148, 0.808, 0.126, 0.167, 0.765 mg/gm and fat content of 2.76, 2.4, 5.5, 6.2, 58.28, 4.20% present in oregano leaves, basil, thyme,

pipali, sesame seeds and green cauliflower leaves respectively. The nutritional data found that oregano, thyme and cauliflower leaves had highest range of 79.17, 78.67, 72.64 mg/kg of iron content and 658, 417, 304 mg/kg of calcium content respectively.

Results of regression analysis indicated that coefficient of determination (R^2) for fitted model was 86.9% for WHC, 76.5% for OHC, 92.5% for a_w, 82% for density, 94.7% for CI and 74.8% for overall acceptability of developed nutrimix formulations. The water holding capacity of the developed nutrimix was ranged from 248.0 to 335.3%. The multiple regressions analysis of experimental data for response variable and process variables is shown in second order polynomial equation as:

$$Y1\ (WHC) = 291.859+6.765X1-7.315X2-0.378X3–2.964X1^2+1.809X2^2+7.662X3^2+6.455X1X2-3.205X1X3+1.547X2X3$$

The squared effect of partical size has negative effect on the WHC of nutrimix formulation. The interactions of partical size and moisture has the positive effect while the partical size and anticaking agent has the negative effect on the WHC. The results of other study also supported the similar observation of particle size effect on water holding capacity in lime and cabbage by-product powder [18]. Overall results from analysis of variance revealed that squared effect is nonsignificant, but linear regression is found to be significant at value $p \leq 0.001$ in case of WHC of ready to use "nutrimix". Oil holding capacity of the developed formulations was ranged from 125.33% to 202.86% with significantly coefficient of determination of fitted regression model. Phatcharaporn et al. [19] also observed the same effect of particle size on oil holding capacity of banana peel concentrate. Using multiple regressions analysis on the experimental data for regression equation integrates the process variable and their responses in the second order polynomial equation as:

$$Y2\ (OHC) = 166.17+5.01X1-4.69X2-11.46X3-7.26X1^2-8.60X2^2+2.08X3^22.93X1X2+0.47X1X3+12.0X2X3$$

The polynomial equation indicated process variables $X1^2$ & $X2^2$ showed negative squared effect on OHC while interaction model of X1 X3 and X2 X3 have the positive effect on OHC. The impact of partical size, moisture content and anticaking agent on the OHC has been studied. Results from overall analysis showed both linear and quadratic regression model fitted insignificantly. The water activity of the developed nutrimix formulations was ranged from 0.429 to 0.851. The analysis of variance results showed that squared effects showed significant values in the quadratic equations with the overall positive effect on water activity. The similar trends were also observed in other study of partical size on water activity of soy flour [20]. The interaction of X1 X2 and X1 X3 has the negative effect while X2 X3 has the positive effect on the water activity, whereas interaction model X1 X2 fitted more significant at $p \leq 0.001$ of nutrimix formulations. The second order regression coefficients were used to develop equation as follows:

$$Y3\ (a_w) = 0.67747+0.00223X1+0.08920X2-0.02023X3+0.00188X1^2 - 0.02516X2^2+0.00312X3^2 - 0.08325X1X2 - 0.03350X1X3+0.01550X2X3$$

The individual effect of partical size, moisture content and anticaking agent on the water activity has been studied. The R^2 values indicates the significant contribution of process variables of formulations. The ANOVA findings revealed that linear regression model is

significant that $p \leq 0.001$, whereas quadratic regression model is insignificant. The density of the developed formulations was varied from 0.318 to 0.471gm/ml. The R^2 value showed that regression models fitted significantly with their F value of 5.05. The quadratic equation of various parameters was as follows:

$$Y4\ (density) = 0.37830+0.00341X1+0.01926X2+0.01244X3+0.02514X1^2-0.00632X2^2+0.02284X3^2-0.01050X1X2+0.00175X1X3-0.00975X2X3$$

The polynomial equation indicated that moisture content showed negative quadratic effect while interaction model between partical size and anticaking agent has the positive effect on density. Gustaeovo et al. [21] also obtained same conclusions of moisture content and particle size on density of food powders. The colour index was ranged from 24.00 to 29.34. The F value was found to be 19.98 and lack of fit is insignificant. The polynomial equation as follows:

$$Y5\ (CI) = 26.2779+0.6017X1+0.6137X2+0.5629X3-0.1542X1^2-0.3257X2^2+0.8711X3^2+0.1900X1X2-0.7050X1X3-0.2300X2X3$$

The quadratic models $X1^2$, $X2^2$and interaction model X1 X3, X2 X3 fitted insignificantly with their negative effect on colour index. The same effect of moisture content on colour index during sorghum flour processing was also found by others [22]. Results of analysis of variance showed that both linear regression and squared effect is significant at $p \leq 0.001$. The OAA results were varied from 6.8 to 7.6 with R^2 fitted in regression model. Multiple regression equation for OAA was as follows:

$$Y6\ (OAA) = 6.79087+0.00732X1+0.13679X2-0.00732X3+0.09501X1^2+0.21875X2^2+0.09501X3^2+0.01250X1X2-0.01250X1X3-0.01250X2X3$$

The squared effect of different variables was found to be significant in the regression model having positive impact on OAA. But the interaction of X1 X3 and X2 X3 put negative effect on OAA.

Conclusion

The nutritionally rich formulation was optimized using response surface methodology for ready to use "Nutrimix" based on natural bioresources that rich in iron and calcium. The analysis of variance showed that particle size (X_1) and anti-caking agent (X_3) exhibited more significant effect on the development of nutrimix formulation. The results of this study found that optimum process conditions for nutrimix were obtained at 250 micron particle size, 13% moisture content and 5% anti-caking agent with higher acceptability and shelf stability that can be use as food ingredient. However, this nutrimix formulation can be used in different food matrix or preparations in regular diet, which will improve the health and wellness of society. The outcome of this study explores the avenue for further assessment of market potential and consumer preference of optimized formulation as food ingredient.

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Original Research

Sharma et al

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Improved bacoside biosynthesis in micropropagated *Bacopa monnieri* grown in liquid medium with different support systems

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Abstract: *Bacopa monnieri* (Brahmi) is a well-documented memory enhancer plant of high commercial global demand. This study demonstrated the liquid medium based micropropagation of Brahmi. The agar culture systems (ACS) was compared to various static liquid culture having different support systems: liquid medium with submerged explants (LCS), with filter paper (LFP), with glass bead (LGB) and Growtek® membrane raft culture system (LGK). The fresh and dry weight of shoots was found to be highest in liquid culture systems. The growth index on the basis of dry weight was found to be 4.66 in LCS which was ~ 2 fold higher than in ACS (2.53). The Bacoside content was also evaluated in all culture systems. LFP showed marked increase in shoot biomass and Bacoside production (7.02 mg/gm DW) in comparison to ACS (5.18 mg/gm DW). The results of this study suggest the use of liquid media as a potential way to improve the shoot biomass and Bacoside production.

Key words: Brahmi, Liquid medium, Support matrix, HPLC, Bacoside

Introduction

The physical environment of the culture medium, as well as its content, has a significant impact on plant growth *in vitro*. The most often used agar based method causes heat to build up and prevents dissolved oxygen from reaching the cultured tissue [1]. Because liquid cultures have better growth rates due to more water and nutrient availability to the tissue in touch, they are preferred over solid support media and can be used in bioreactors for multiplication of plant at larger scale [2]. However, the plants which are completely submerged in liquid medium led to the hyperhydricity of the tissue [3]. To overcome this problem, several effective approaches have been reported using inactive support matrixes such as cotton fiber, foam, Whatman filter paper, glass beads and rock wool. [4,5]. Few reports are also available for *in vitro* Bacoside production in organized or unorganized agar based culture medium [6,7,8] and the suitability of shoot multiplication in liquid culture systems *in vitro* [9,10,11] of *B. monnieri*. The impact of different support matrix in liquid medium for the stimulation of shoot growth and Bacoside biosynthesis has been studied.

Material and Methods

Explant selection and growth conditions

In vitro proliferation of shoot organ cultures of *B. monnieri* was achieved on Murashige and Skoog medium [12] fortified with 1.0 mg/L 6-benzyladenine (BA), 30 gm/L sucrose, solidified with 7.0 gm/L agar. The pH of the growth medium was calibrated to 5.8-

6.0 before autoclaving at 121^0C for 15 minutes. All aseptic cultures were incubated in the growth room at 24 ± 2°C under white light with a total irradiance of 3000 lux under 16 hr photoperiod. The shoot culture grown on agar medium served as a control for the various culture systems (Figure 1A). Excised shoots from the control flasks were also inoculated on liquid medium of same nutrient composition for their multiplication and maintenance.

Experimental culture system

The shoot explants taken from the control flask were inoculated onto the various static liquid culture systems. The support is placed at the bottom of the culture vessel so that cultures grown in the liquid medium are only partially exposed to the medium. The Erlenmeyer flasks were used for all culture systems. In the LFP system, analytical qualitative filter paper discs (diameter 12.5 cm), were folded and placed at the bottom of the flask as a support matrix. For the establishment of LGB system, the glass beads of size 1.5 mm were steeped in chromic acid overnight before being rinsed with 1% Teepol and sterile distilled water. Prior to use, the glass beads were dried in a hot air oven. As a support matrix, 80 gm of glass beads were added to the culture system.

Measurement of growth parameters

The shoot biomass was carefully taken from the flasks after 4 weeks of cultivation, blotted dry, and the fresh weight (FW) determined. The shoot biomass was dried under shade condition at room temperature to a constant weight to measure dry weight. The growth index (GI) and shoot multiplication rate was also determined for all the media which depicts plant growth.

Quantitative determination of Bacoside

The shoot biomass of each treatment was air dried, finely powdered with the use of mortar pestle and analyzed for Bacoside content as reported earlier [13].

Statistical analysis

The data generated was analyzed using SPSS software by analysis of variance along with Duncan multiple range test (DMRT) at the significance level of $P \leq 0.05$. Data was indicated as mean ± standard error (SE) of three separate analyses (from three flasks). Each experiment was carried out twice.

Result and Discussion

Effect on growth and shoot proliferation

The various liquid culture systems differently to the agar based control culture system (ACS) (Table 1). The LCS showed shoot multiplication rate of 8.83 which was ~ 3 fold higher than in control ACS (3.45) (Table 1, Figure 1). This could be due to the close and broader contact of the shoot with the medium thus, increasing the capability of shoot culture for the absorption of nutrients and hormones in better way. The beneficial effect of liquid over agar media on micropropagation of *B. serrata* has also been studied earlier [14]. Out of various culture systems, elevated GI (DW) was recorded in LCS (4.66), then LGB (4.37), LFP (3.93), LGK (3.79) and ACS (2.53). The application of liquid media for boosting shoot propagation has also been described earlier for *Allium sativum* [15]. The use of gelling agents for solidifying the medium can limit the hydraulic conductivity; therefore, reduces the availability of required nutrients to the plant tissue [16].

Table 1. Shoot biomass and Bacoside production on different support matrix

System/ Volume	Shoot multiplication rate (SMR)	Growth index		Bacosides (mg/ gm DW)		Total Bacosides (mg/ gm DW)
		FW	DW	A_3	A_2	
ACS/ 250ml	3.45± 0.17**a**	4.71±0.53 **a**	2.53±0.48 **a**	2.99±0.44 **a**	2.18±0.45 **a**	5.18±0.89 **a**
LCS / 250ml	8.83 ±0.33 **d**	7.03±0.35 **b**	4.66±0.66 **b**	3.05±0.4 **a**	2.89±0.4 **a**	5.94±0.78 **a**
LGB/ 250ml	7.55 ± 0.2 **c**	6.64±0.32 **b**	4.37±0.51 **b**	3.48±0.78 **a**	2.62±0.69 **a**	6.1±1.47 **a**
LFP/ 250ml	6 ± 0.17 **b**	6.45±0.57 **b**	3.93±0.12 **b**	3.47±0.45 **a**	3.55±0.41 **a**	7.02±0.86 **a**
LGK/ 1L	6.94 ± 0.22 **c**	6.17±0.32 **b**	3.79±0.23 **ab**	3.14±0.62 **a**	3.36±0.75 **a**	6.49±1.38 **a**

Mean within a column followed by common letters are not significantly different at P≤ 0.05, according to DMRT

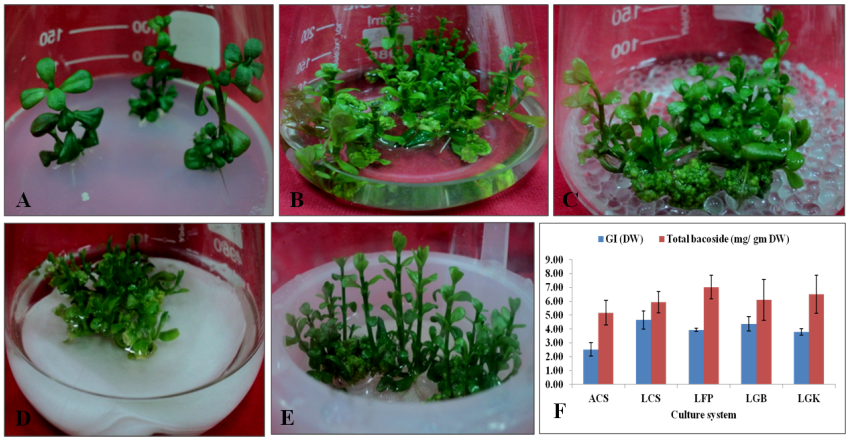

Figure 1. Shoot culture growing in all systems *in vitro*: (A) ACS, (B) LCS, (C) LGB, (D) LFP, (E) LGK, and (F) Graphical depiction of GI and Bacoside production in all systems. Bars indicates SE.

Effect on bacoside production

Bacoside production by the shoot cultures of various treatments were evaluated after 4 weeks of cultivation (Table 1). The accumulation of total Bacoside (sum of bacoside A_3 and A_2) was significantly higher in shoot biomass grown on different liquid culture systems: LFP (7.02); LGK (6.49); LGB (6.10) and LCS (5.94) in comparison with ACS medium (5.18). The data obtained showed the feasibility of various culture or growth systems for enhancing the production of memory enhancing Bacoside from *B. monnieri*. Reports are available wherein maximum secondary metabolite production in liquid medium has been studied in comparison to agar culture system [17]. The absence of agar from the culture media also resulted in considerable cost reduction.

Conclusion

The present work conclude that shoot biomass of *B. monnieri* can be enhanced in liquid medium without agitation of the culture

vessel. This can be employed as one of the approach for considerable cost reduction during *in vitro* plantlets regeneration.

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Original Research

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Effect of plant conformations and training techniques on Sweet pepper growth and yield under polyhouse conditions

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Abstract: Protected cultivation has gained momentum in Himachal Pradesh during last few years due to technology advancement and various interventions by the state funded schemes. More and more areas are being brought every year under polyhouse cultivation. Sweet pepper is a principle potential money spinner crop grown under naturally ventilated polyhouse conditions due to introduction of its new color variants and their off-season production and availability during lean periods. However, improved suitable production technologies under protected structures in mid hill conditions of the state do not yield optimum results. Among different agro-techniques, proper spacing and pruning of greenhouse grown capsicum will help in improving the canopy, fruit set, fruit quality and fruit yield. The current study was done with the objective to determine appropriate spacing for various training systems. The investigation was performed in Factorial Randomized Block Design with triplicates and information was collected on various growth, yield and yield contributing parameters. It was observed that 60 cm × 30 cm spacing and four stems trained plants yielded maximum amount of fruits/plant (21.5), yield/plant (1.7 Kg) and yield/m^2 (11.6 Kg), whereas 60 cm × 30 cm spacing along with two stems training produced exceptionally tall plant of height of 184.2 cm with mean fruit weight, length and width of 88.6 g, 7.8 cm and 7.6 cm, respectively. However, spacing and training have insignificant effects on days to 50% blooming, duration of first harvest, and reaping period. On the basis of overall performance, the 60 cm × 30 cm spacing and training to four stems are recommended for obtaining higher yields in capsicum.

Keywords: Protected cultivation, capsicum, training, spacing.

Introduction

Sweet pepper (*Capsicum annuum* L. var. *grossum* Sendt.), a most important suitable principle crop for polyhouse cultivation in Himachal Pradesh, as it is grown year-round as an off-season vegetable and exported to distant markets, bringing remunerative returns to the farmers. Earlier, the crop was being grown under open field conditions where harsh weather followed by varying temperatures, was a common factor affecting crop productivity and quality thereby reducing producer's profit. In such circumstances, polyhouse capsicum farming has the potential to produce higher yields and better-quality fruits than open-field cultivation. In India, it is being grown in open fields over an area of 7,92,100 ha, with an annual production of 12,23,400 MT and productivity of 1.5 MT/ha [1], while in Himachal Pradesh, it is widely grown as a

cash crop (June - October) in zones 1, 11 and 111 in open fields distributed over an area of 2,447 hectares (ha), with the production of 31,810 tonnes including hot peppers [2].

Training and pruning are important cultural operations under polyhouse cultivation for quality production. Capsicum is indeterminate in growth habit and initially develops into single stem. After emergence of about 10-13 leaves, a typical flower, known as crown bud develops, and thereafter, the stem divides into two and occasionally three or four shoots may develop. Sometimes, there may be more than one crown bud at bifurcation of the main stem. The crown bud should be removed immediately and either four or two main stems are trained of each plant, depending upon the period of the growing season and height of the greenhouse. The desired stems are maintained on each plant by pruning or pinching off the unwanted branches, by retaining two leaves and one flower at each node. The desired stems are trained upon cords and secured with them using plastic clips. The plant will regularly produce terminal flower and two side shoots at every inter-node, and from every four or two new shoots, one is retained to continue the stem and other is removed or just pruned by leaving one flower and two leaves. Interval for training and pruning is two weeks during the period of active growth.

The growth and yield attributes of capsicum are profoundly influenced by the other cultural practices, especially proper plant spacing and fertigation for better quality fruit production. As a result, the current study was undertaken to determine the most appropriate plant spacing and training strategies for producing large quantity and high-quality bell peppers under protected habitat in Himachal Pradesh's mid-hills.

Materials and Methods

The study was performed in Vegetable Research Farm, Department of Vegetable Science and Floriculture, CSKHPKV, Palampur in a naturally ventilated polyhouse in year 2012. It is located in Himachal Pradesh's mid hill zone at an elevation of 1,290.8 m amsl having 32^0 $6^{'}$ N latitude and 76^0 3' E longitudes. The region's overall climate is partially temperate and sub-humid distinguished by chilled winters. In general, the highest temperature has reported in the months of May and June, while extreme low temperature is recorded in December and January. The annual rainfall is 2,500 mm, with 80 per cent falling between June and September. During crop season, the average temperature varied from 15 ^{0}C to 35 ^{0}C, with relative humidity ranging from 40-70%.

The nursery was raised in plastic plug trays in the Department of Vegetable Science and Floriculture's growth chamber using soilless media containing coco-peat, vermiculite, and perlite in a ratio of 3:1:1, respectively to produce healthy and disease-free seedlings. All important precautions for raising a healthy nursery were taken. The seedling transplanting beds were thoroughly prepared inside naturally ventilated or low cost polyhouse. The beds were sterilized by drenching with 5% formalin (*i.e.* 1L of 40% commercial formalin diluted with 7L of water). Whole of the vermicompost @ 10 tonnes ha^{-1} and chemical fertilizers NPK @ 50:50:50 $Kgha^{-1}$ were applied in pits before transplanting.

Healthy hybrid capsicum seedlings were transplanted in Factorial Randomized Block Design in triplicates. The plot dimensions of 1.9 m × 0.9 m, two plant spacing *i.e.* 60 × 30 cm (S_1) and 45 × 30 cm (S_2), and three training systems *i.e.* double stems (T_1), three stems (T_2) and four stems

(T_3) were tried. Plant height (cm), fruit counts per plant, days to 50% blooming, duration of first harvest, harvesting/reaping period, weight (g), width (cm) and length (cm) of fruit, yield/plant and yield/m^2 (Kg) all were measured of 5 randomly chosen plants in every single plot. Factorial Randomized Block Design consisting of three replicates of 3×2×2 spacing, trained to three levels with two levels of fertigation in each was tried at CSKHPKV's Vegetable Research Farm in Palampur. The treatments were administered in 12 modules. The training system, fertigation, and spacing used for each module are given in table 1.

Table 1. The training system, fertigation, and spacing used for each of 12 modules.

S. No.	Modules	Trainings	Spacing	Fertigation
1	$S_1T_1F_1$	2 stems	60 cm × 30 cm	Twice a week @ 2g/m^2
2	$S_1T_1F_2$	2 stems	60 cm × 30 cm	Thrice a week @ 2g/m^2
3	$S_1T_2F_1$	3 stems	60 cm × 30 cm	Twice a week @ 2g/m^2
4	$S_1T_2F_2$	3 stems	60 cm × 30 cm	Thrice a week @ 2g/m^2
5	$S_1T_3F_1$	4 stems	60 cm × 30 cm	Twice a week @ 2g/m^2
6	$S_1T_3F_2$	4 stems	60 cm × 30 cm	Thrice a week @ 2g/m^2
7	$S_2T_1F_1$	2 stems	45 cm × 30 cm	Twice a week @ 2g/m^2
8	$S_2T_1F_2$	2 stems	45 cm × 30 cm	Thrice a week @ 2g/m^2
9	$S_2T_2F_1$	3 stems	45 cm × 30 cm	Twice a week @ 2g/m^2
10	$S_2T_2F_2$	3 stems	45 cm × 30 cm	Thrice a week @ 2g/m^2
11	$S_2T_3F_1$	4 stems	45 cm × 30 cm	Twice a week @ 2g/m^2
12	$S_2T_3F_2$	4 stems	45 cm × 30 cm	Thrice a week @ 2g/m^2

Results and Discussion

Remarkable differences were noticed for every single character studied, except days to 50% blooming, duration of first harvest and reaping period.

Growth characters

The important growth characters recorded were plant height (cm), days to 50 % blooming, day counts to first harvest, and reaping/harvesting period. It was reported that there was no significant effect on these last three parameters. This may be due to fluctuations in temperature, but plants trained up to two shoots along with 60 cm × 30 cm spacing resulted into significant enhancement in their height (184.2 cm) (Table 2). This can be explained by the fact that compactly placed plants have less space for elongation and thereby leading to reduction in their height and vice-versa. In case of more spacing and pruning of lateral branches, nutrients rush to axillary branches has reduced, probably shifting the nutrients flow to the apex regions causing stem elongation. These findings were according to the results of Aliyu and Yusuf [3] in capsicum and Afzal *et al* [4] in chilli.

Yield characters

Spacing and training systems (Table 3 and Table 5) were responsible to bring out better expression of all the four yield attributes *i.e.* fruit counts/plant, weight (g), length and width of fruit in centimeters.

The fruit counts per unit plant had a direct contribution to fruit yield. The average number of fruits was 18.22 and 17.33 in S_1 and S_2, respectively (Table 5). This can be attributed to the fact that compactly placed plants have less space for their overall growth than loosely spaced plants which have large area per unit plant for their growth. So latter strategy decreasing the competition within the plants, hence, increasing fruit numbers per plant at their widest space area up to 15.75, 16.67, and 20.92 in T_1, T_2, and T_3, respectively (Table 5). It might be possible due to increase in branches number per plant which tends to increase number of fruits/plant. These observations were in accordance with those of Lee *et al* [5], who found that increasing row spacing increased the fruit counts and yield/plant.

Fruit yield per plant

Fruit yielding capsicum number was increased along with the increase in spacing and training to four stems and the differences were highly significant. The treatments- S_1T_1, S_1T_2, S_1T_3, S_2T_1, S_2T_2 and S_2T_3 produced on an average 1.5, 1.4, 1.7, 1.2, 1.4 and 1.5 Kg/plant yield, respectively (Table 3). These results were in accordance with Lee *et al* [5]; Mishrinky and Alphonse [6]; Maya *et al* [7]; An *et al* [8].

Fruit yield (Kg/m^2)

Fruit yield/m^2 was highest at S_1 (60 X 30 cm) spacing and training to four shoots. The contribution of wider spacing to higher yield was due to increased light penetration, proper aeration into the canopy, which resulted into production of large quantity of fruits/plant, and four shooted plants produced the highest amount of fruits/plant, eventually increased yield per square meter. These findings were consistent with those of Gowde *et al* [9] and Maya *et al* [7]. Plants which received fertigation three times a week at the rate of 2g NPK (19:19:19)/m^2 (F_2) produced the highest (1.63 Kg) yield per plant, which was 0.35 Kg higher than the plants who received

fertigation twice a week (F_1) at the same rate. This could be due to increased carbohydrate translocation because of the better nutrient availability with high fertilizer doses. The present findings were also supported by Shrivastava [10] in capsicum.

Table 2. Interaction effects of training and spacing on growth characters of capsicum.

Treatments	Days to 50% flowering		Days to first harvest		Plant height (cm)		Harvest duration	
S×T	S_1	S_2	S_1	S_2	S_1	S_2	S1	S2
T_1	40.5	41.5	72.0	72.8	184.2	172.4	151.0	152.3
T_2	40.7	40.3	74.2	72.3	161.6	154.9	153.0	151.5
T_3	40.5	41.0	72.2	72.3	141.4	128.2	151.0	152.0
CD (P=0.05) **NS**			**NS**		**3.22**		**NS**	

NS = non-significant

Table 3. Interaction effects of training and spacing on yield and yield contributingcharacters of capsicum.

Treat-ments	Yield/plant (kg)		Yield/m^2 (kg)		Fruit length (cm)		Fruit width (cm)		Fruit weight (g)		No. of fruits/plant	
S×T	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
T_1	1.5	1.2	10.2	8.7	7.8	7.4	7.6	7.2	88.6	82.5	16.7	14.8
T_2	1.4	1.4	10.1	9.8	7.3	6.9	7.2	7.1	82.4	82.1	16.5	16.8
T_3	1.7	1.5	11.6	10.7	6.9	6.8	7.1	7.0	75.6	68.8	21.5	20.3
CD (P=0.05) **0.06**			**0.48**		**0.12**		**0.12**		**2.20**		**0.64**	

Table 4. Effect of training and spacing on growth characters of capsicum.

Treatments	Days to 50% flowering	Days to first harvest	Plant height (cm)	Harvest duration/Reaping period
S1	40.56	72.78	162.41	151.67
S2	40.94	72.50	151.83	151.94
CD (P=0.05)	**NS**	**NS**	**1.86**	**NS**
T1	41.00	72.42	178.32	151.67
T2	40.50	73.25	158.25	152.25
T3	40.75	72.25	134.80	151.50
CD (P=0.05)	**NS**	**NS**	**2.28**	**NS**

Table 5. Effect of training and spacing on yield and yield contributing characters of capsicum.

Treatments	Number of fruits/plant	Yield/plant (Kg)	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Yield/m^2 (Kg)
S1	18.22	1.52	7.3	7.3	82.20	10.64
S2	17.33	1.39	7.0	7.1	77.80	9.71
CD (P=0.05)	**0.37**	**0.04**	**0.1**	**0.1**	**1.27**	**0.28**
T1	15.75	1.35	7.6	7.4	85.58	9.47
T2	16.67	1.42	7.1	7.2	82.23	9.93
T3	20.92	1.59	6.8	7.0	72.20	11.14
CD (P=0.05)	**0.45**	**0.05**	**0.1**	**0.1**	**1.56**	**0.34**

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Network-based prediction of drug biomarker targets in lung cancer and brain cancer

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Abstract: Cancer leads to abnormal growth in cells and disrupts the process of a healthy cell life cycle. Many of the cancer types may now be treated to eradicate, minimize, or slowdown the impact of disease impact on patient's lives. In this work, we attempt for network-based prediction of drug-target biomarkers associated with the glioblastoma multiform brain cancer and lung adenocarcinoma. TCGA data portal was used to extract the clinical data related to both of these cancers. Drug targets and associated pathways were retrieved from DrugBank and KEGG databases. Biomarker selection is performed on the basis of hops values, that are primarily the shortest distances between one node to other nodes in a bipartite network. In this study, biomarkers are identified as the protein targets having hop values less than the four in the drug-target network. We report the HDAC1 (histone deacetylase 1) and PRKCA (protein kinase C alpha) as the potential drug targets in case of lung cancer and brain cancer, respectively.

Keywords: Brain cancer, Lung cancer, Biomarkers, Drug targets, Pathways

Introduction

The human body is a multi-cellular organism that is made up of millions of microscopic cells, each of which is a self-contained living entity. Every cell in our body normally collaborates with the others to form tissues and organs. Cell division is one such example of how this coordination works. Normal cells in the body grow and divide for a short amount of time, and are reproduced as per the need to replace the damaged or dying cells. When this cellular reproduction process becomes uncontrollable, cancer develops [1]. Cancer is frequently known as an incurable, excruciatingly painful disease with no cure. However, several types of cancer can now be successfully treated to eradicate, minimize, or slow down the impact of disease on patients' lives [2]. While a cancer diagnosis can still make people feel helpless and powerless, there are more reasons for hope than hopelessness in many situations today.

Biomarker is a very broad term for something we can test to confirm the presence or absence of a disease [3]. A biomarker is a natural atom or molecule present in the blood, other body liquids, or tissues. Biomarkers are used to assess the patients in a variety of clinical situations, such as evaluating disease risk, screening for primary tumours, identifying malignant signs, or separating one form of malignancy from another, assessing prognosis and prediction for cancer patients, and monitoring the disease's course, either to detect or to determine response to

progression of therapy [2]. Biomarkers show that a treatment is working, or could be a red flag to warn of side effects. Cancer biomarker may be used to forecast how aggressively cancer will develop, which is important for determining prognosis. Moreover, one of the most promising applications of biomarker is to determine the side effects of medicines given to cancer patients.

To check the efficacy of drug molecules, an important step is to identify the information about interactions of drugs with protein targets. With the availability of big data in biology, large number of drug-protein interactions is known [4]. In recent years, analysis and visualisation of interaction of drug with its targets using the network theory plays a significant role due to its ability to express complex information in a simple form. Network medicine is an important area of research that contributes in drug designing by studying the interactions of drugs with their targets, involvement in pathways, effectiveness in comorbidities, unintended side-effects etc. [5]. Network pharmacology is among one of these rational approaches that is used for detailed analysis of interactions among the drugs and their target proteins [6], [7]. Advancement in the computational methods has paved ways for researchers to devise and apply new innovative methods to predict unknown interactions among drugs and their targeting proteins. Network proximity evaluates the topological connections between the two nodes of a network [8]. In this study, we identified the drug biomarkers in lung cancer and brain cancer using the shortest path by a limited breadth first search approach in a bipartite network.

Material and Methods

Collection of clinical data

TCGA is a comprehensive and coordinated effort to accelerate our understanding of the molecular basis of cancer through the application of genome analysis technologies, including large-scale genome sequencing [9]. Various kinds of information (Whole genome progression (WGS), whole exome gathering (WES), methylation, RNA explanation, proteomics, and clinical datasets) were gathered from the TCGA information gateway. The clinical data of lung cancer (TCGA-LAUD) and brain cancer (TCGA-GBM) were collected from TCGA data portal.

Identification of drugs and protein targets

The drugs and their protein targets were downloaded from the DrugBank database [10]. DrugBank easily explained asset that consolidates point-by-point drug information with thorough medication target and medication activity data. Since its first release in 2006, DrugBank has been broadly utilized to work with in silico drug target revelation, drug configuration, drug docking or screening, drug digestion forecast, drug connection expectation, and general drug pharmaceutical education.

Retrieval of pathways for drug-targets

Using the KEGG database [11], pathways associated with protein-targets of lung and brain cancer were accessed. All the pathways in which protein targets associated with a particular drug compound were retrieved.

Network generation and visualization

Various networks, drug- drug target interaction, drug target-pathway interaction and tripartite network of drug-drug target-pathways, were developed and visualized by using the Cytoscape [12].

Identification of drug biomarker

Drug targets having hop value less than four in the drug-target association network were considered as a drug biomarker for a particular disease. Shortest-path-bfs-master tool which converts the bipartite network to unipartite for hop value calculation was used.

Results

Drugs, protein targets and pathways of lung cancer

Identification of the drugs and their protein targets of lung cancer was done by clinical data which was downloaded from the TCGA data portal. Out of 1,505 drugs, only 16 unique drug molecules are related to lung cancer (LAUD) disease. 62 protein targets of these drugs were extracted from the DrugBank database (Table 1). These 62 drug targets of lung cancer were found to be involved in 402 different pathways. While most of the protein targets were involved in multiple pathways, some targets, like, O00244, P29372, P01023 were found to be just in a single pathway.

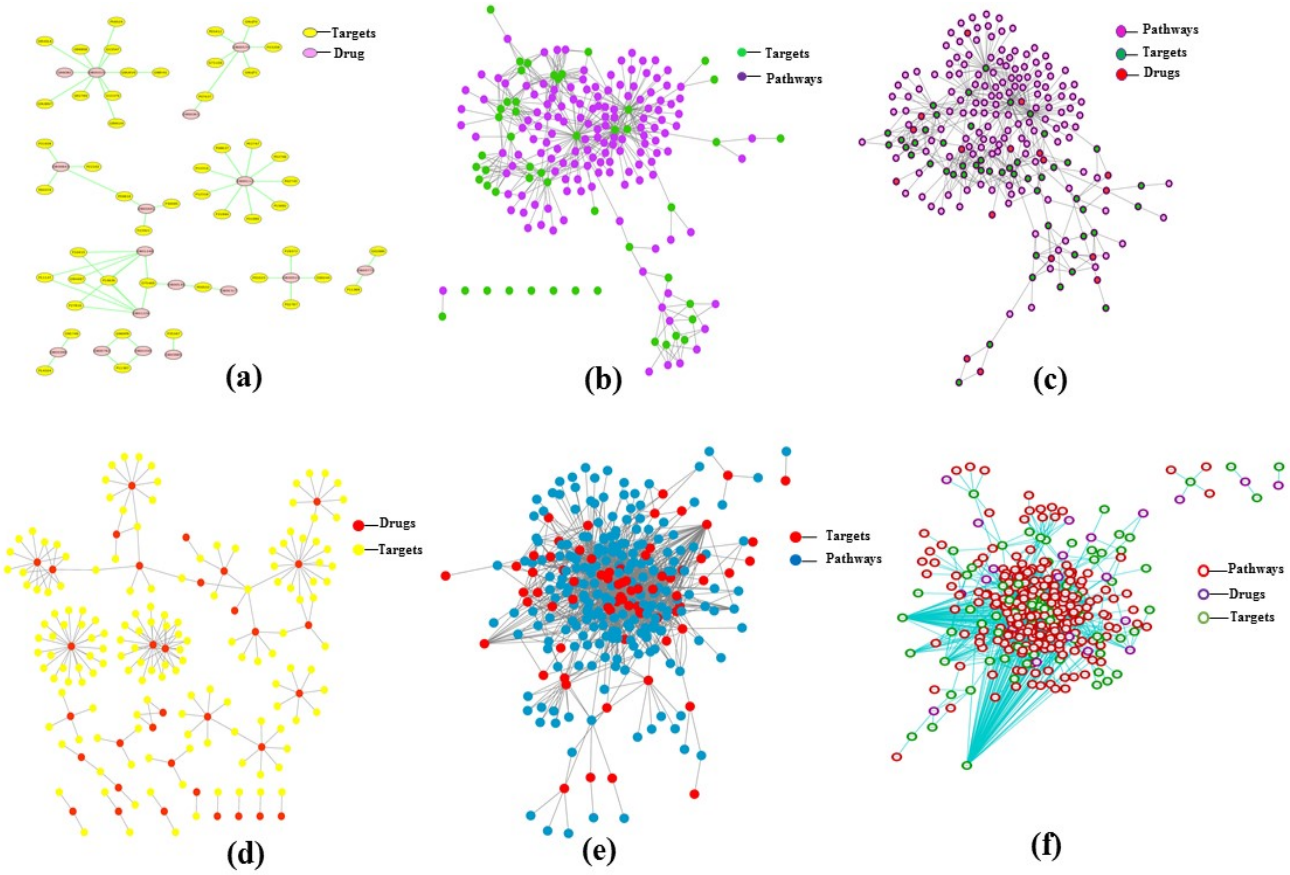

Figure 1: (a) Drug-drug target network for lung cancer. (b) Drug target-pathways network for lung cancer. (c) Tripartite network of drug-drug target-pathway for lung cancer. (d) Drug-drug target network for brain cancer. (e) Drug target-pathways network for brain cancer. (f) Tripartite network of drug-drug target-pathway for brain cancer

Drugs related to Brain cancer	Targets	Drugs related to Lung cancer	Targets
DB00675	P10275, Q12809, O75469, P62508, P04278, P45983, P05129	DB05015	Q13547, Q969S8, Q96DB2, Q92769, O15379, P56524, Q8WU14
DB01041	Q96SW2, P01375, P19838, P21802, P35354	DB00112	P15692, P02745, P02747, P02746, P08637, P12314, P12318, P31994
DB01005	P21397, P27338	DB00985	P35367
DB00112	P08637, P12314, P12318, P31994, P31995	DB00515	P29372, P01023, P02787, O0024
DB00541	P07437, P68366	DB01248	Q9H4B7, P10415, P11137, P27816, P10636, O75469
DB01168	P41231, Q9NXA8, P23945, P21817	DB00530	P00533, O75469
DB04786	P21817, P00734, P14555, P68638	DB00773	P11388, Q02880
DB00339	P95029	DB00317	P00533
DB00515	P29372, P01023, P02787, O00244	DB00441	P23921, P04818, P30085
DB00262	P00390	DB00762	Q969P6, P11387
DB01229	Q9H4B7, P10415, P27816, P11137, P10636, O75469	DB01229	Q9H4B7, P10415, P27816, P11137, P10636, O75469
DB01206	Q9H169	DB00642	P04818, P31939, P00374, P22102
DB00762	Q969P6, P11387	DB01030	P11387, Q969P6
DB00398	P09619, P1072,1 P11362, P07949, P17948	DB00570	Q71U36, P07437, Q9UJT1, P23258, Q9UJT0, P05412
DB00773	P11388, Q02880	DB00361	P07437
DB00530	P00533, O75469	DB00399	P14324, O95749
DB01196	P03372, Q92731, P11137		
DB08875	P08581, P35968, P07949		
DB05294	P15692, P00533, Q13882, Q02763, P07949		
DB06486	P31749, Q96Q40, P05771, P27986, O14965		
DB00786	O96017, O14757, Q96GD4, P03956		
DB00877	P42345, P62942, P09038		
DB01030	P11387, Q969P6		
DB00531	O75469		
DB00608	P01375, P09210, Q9NR96, Q8MU52, P09429, P09488		
DB00111	P08637, P12314 , P12318, P31994, P31995		
DB01234	P04150, P51843, P04083, P35228, O75469		
DB00428	P11168, Q89ZI2, O60502		
DB00635	P04150		
DB00776	Q9UQD0, Q15858, Q07699, O60939, Q9NY72, Q8IWT1		
DB04106	Q16881		
DB01202	Q7L0J3, Q00975		
DB00997	P11388, Q14978		
DB00313	P45954, Q9UKV0, Q02218, Q92769, Q07869, Q03181, P37231		

Table1: Drugs and their pathways associated with Brain cancer and Lung cancer

Drugs, protein targets and pathways of brain cancer

Extraction of cerebrum malignant growth clinical information was performed utilizing TCGA. 1,508 drug molecules were found in this clinical information. Out of these 1,508 medications, just 35 are found in the DrugBank (Table 1). These 35 drugs are found to be associated with 193 protein targets. These 193 drug-targets of brain cancer are found to be involved in 1,504 different pathways.

Network analysis

Drug-target (Fig. 1a), target-pathway (Fig. 1b) and tripartite network (drug-target-pathway) (Fig. 1c) of lung cancer and Drug-target (Fig. 1d), target-pathway (Fig. 1e) and tripartite network (drug-target-pathway) (Fig. 1f) of brain cancer were generated, visualized and analyzed using Cytoscape.

Biomarker protein-targets

In this study, we considered a protein target to be a biomarker of the selected disease if its hop value is less than four in the drug-target association network. In case of lung cancer, on the basis of hop value we have identified P15692 as a biomarker target of drug DB05015 whereas, Q13547 was identified as biomarker target of drug DB00112. On the other hand, P05129 has been identified as the biomarker target of drug DB00675 on the basis of the hop value criterion.

Conclusion

In this study, we used network-based prediction method for the identification of biomarkers on the basis of hop values in the drug-target bipartite network, and propose one protein target as a biomarker for lung cancer and another for the brain cancer. In this work, we identified that HDAC1 (histone deacetylase 1) is the target of lung malignant growth infection and PRKCA (protein kinase C alpha) is the target of brain carcinoma, that are proposed to be considered as biomarkers. Leucovorin is a drug corresponding to the protein target HDAC1 which is used for brain cancer and Belinostat is a drug of the protein target PRKCA which is used for lung cancer disease. Belinostat has demonstrated to be successful as a solitary specialist or in the mix with other anticancer specialists. The anti-neoplastic movement of Belinostat seen in pre-clinical examinations has brought about just moderate poisonous.

The biomarker prediction can be attributed to the development of new strategies with increasing knowledge about druggable targets. The target prediction innovates drug discovery, drug repositioning, and other fields with a purpose to discover new drugs and the novel usage of existing drugs. We hope that the proposed biomarkers may help for further researches to work on restoring the illness of malignant growth because these effectively cooperate with the medications of lung and brain cancers, respectively.

Authors Contribution

VS deigned the research framework and supervised the study. Priyanka and AP performed the computational experiments. All the authors analysed results and wrote the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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Original Research

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Phytoremedial effect of ginger on liver function test of diabetic mice

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Abstract: Now a days diabetes mellitus is leading non-communicable disease increased many folds in last few decades worldwide. It occurs due to insufficient and non secretion of insulin. Diabetes is associated with other macrovasular and microvascular diseases. In this way it not only affect pancrease function but it also acts on liver, kidney, neuronal and reproductive system. This study is designed to investigate the effect of *Zinger officinale* on Liver Function test of mice. In this present study, animals were divided into three groups according to the treatment schedule. Alloxan was introduced by intra-peritoneally @120 mg/kg/bw once to induce diabetis in mice. Normal mice received distilled water *ad libidum*. While mice induced with diabetes were administrated with aquous rhizome extract of *Zinger officinale* @ 150mg/kg/bw 20 weeks. In diabetic mice glucose level were increased many folds and persists upto final sacrifice. SGPT and, bilirubin level were increased in diabetic mice. In ginger administered group glucose level were restored to normal level and SGPT and bilirubin were restored in ginger treated group effectively. It is concluded that *Zingiber officinale* causes restoration in glucose level and long term administration of ginger also restores liver function test in diabetic mice.

Key words: Alloxan, hepatocytes, *Zingiber officinale and SGPT*.

Introduction

Diabetis is a common metabolic disease characterized by chronic hyperglycemia [1]. It arises due to endocrine and metabolism anomalies of glucose, protein, and lipids due to insulin deficiency and insulin resistance or both. [2]. The Prevalence of diabetes increases day by day all over the globe at an alarming rate. Now it becomes a major cause of morbidity and mortality. International Diabetes Federation (IDF) identified that 1 in 11 adults above age 20–79 years had chances of development of diabetes globally according to 2015 study. This evaluation was predicted to exceed 645 million upto 2040[3]. The most common clinical symptoms are polyphagia, polyuria, and polydipsia [4]. Chronic hyperglycemiais is associated with lots of disease including nephropathy, stroke, neuropathy, retinopathy and CVD etc. It is associated with various liver diseases such as abnormal glycogen formation in non-alcoholic fatty liver, and viral hepatitis [5]. It imposes a huge expense on society that in almost every country around the world accounts for 5 to 13% of the costs set aside for the treatment of diseases. These treatments are mostly dependent upon chemically synthetic drugshaving harmful side effects [6]. Hence there is a need for an alternative to prevent the detrimental response.Sciencemedicinal Herbs presents valuable therapeutic agents, in both traditional systems and modern medicine. More than four hundred traditional herbal remedies for diabetes have

been described, However, a small number of these have gained medical and scientific assessment to assess their usefulness and safety [7].

Zingiber officinale (Willd.) is used as a traditional spice [8]. Ginger is used as a traditional medicine since very long back for treatment of many diseases including vomiting, pain, indigestion, and cold. It has anti-inflammatory, antioxidant, anti-hyperglycemic, and anticancer properties [9]. It carries numerous bioactive components such as gingerols, shaogaols, Zingerone and some volatile oils including sesquiterpene, like b-bisabolene and zingiberene, and monoterpenes, mostly geranial [10]. Since very long times ginger is used in treatment of many traditional disease including cold and cough as well as many other inflammetery disease. This study is tend to illustrate hypoglycaemic and hepatoprotective effect of *Zinger officinale* on *Mus musculus*.

Materials and Methods

The healthy *Mus musculus* (BALB/c) were brought up in the animal house the age group of 12 weeks old with 30±5 gm body weight.The mice were kept at standard environmental conditions 20±2ºC, relative humidity were maintained at 50 to 60 %, and 12h dark and light period. All experiments was conducted on standard ethical guidelines. IAEC approval were obtained for the study through Animal Ethics Committee.

Chemicals

Alloxan, manufactured by Lobachem Pvt.Ltd., Mumbai was utilized for the experimental design. It is used to make a diabetic model in the experimental animal. It was injected intraperitoneally @ 120 mg/kg/b.w/day to overnight (12h) fasted mice. Gluecose level is tested after 72 hours of alloxan induction to confirm its diabetic potential. After four weeks of continuous monitoring if animals were ready as diabetic model on getting persistant elevated glucose level.

Medicinal plant used

Aquous rhizome extract of *Zingiber officinale* (150 mg/kg/BW) orally administered to a diabetic group of mice for 20 weeks.

Study Design

The animal included in the experiment were dived in three groups with contain 6 animals in every group, which are as given below:

Group 1 (Control group) it received DW and standardized food orally

Group II (Diabetic) not treated received standardized food only.

Group III (diabetic + ginger administrated group) received an aquous extract of ginger 150mg/kg/b. worally by gavage method once a day for 20 weeks

Mice in groups I and II were sacrificed in twenty weeks. Animals in group III sacrificed at the interval of4th, 8th,12th, 16th and 20th weeks, after the termination of the last dose. Serum was collected for glucose (GOD/POD method), SGPT (Reitman Frankel method), and bilirubin(Mod. Jendrassik and Grof's Method) analysed.

Statistical analysis

All the results were analysed as mean ± SD. The data was analysed for one way analysis of variance (ANOVA) through graph pad prism 5.03.

Results

Serum glucose level in ginger administrated group

The Glucose level was 90.00±0.5774 mg/dl in control group; while in diabetic group the

level was 222.0±0.5774 mg/dl. It was 127.3±17.68 mg/dl in 4 weeks, 93.92.50±13.56 mg/dl in 8 weeks, 93.67±4.177 mg/dl in 12 weeks, 112.0±1.732 mg/dlin16 weeks and 92.51±10.73 mg/dl in 20 weeks *Zingiber officinale* administered group. (Table- 1).

Serum SGPT level in ginger administrated group

SGPT level was 21.30 ± 1.120 U/l inthecontrol group; while in the diabetic group it was 226.0 ± 1.042 mg/dl. It was 141.3 ± 1.497 U/l in 4 weeks, 57.9 ± 1.272 U/l in 8 weeks, 28.0 ± 0.3640 U/l in 12 weeks, 16.0 ± 0.7108 U/l in 16 weeks and 18.0 ± 0.7025 U/l in 20 weeks ginger administered group. (Table -1).

Serum Bilirubin levelin ginger administrated group

Bilirubin was 0.6730 ± 0.1183 mg/dl incontrol group; while in diabetic group it was 1.400 ± 0.2464 mg/dl. It was 0.987 ± 0.14449 mg/dl in 4 weeks, 1.330 ± 0.1633 mg/dl in 8 weeks, 0.7700 ± 0.1130 mg/dl in 12 weeks, 0.839 ± 0.1742 mg/dlin16 weeks and 0.788 ± 0.1005 mg/dl in 20 weeks *Zingiber officinale* administered group. (Table-1).

Discussions

Elevated glucose level produces oxidative stress, which damages the tissue and alters their functions. Since alloxan is β-cytotoxic that induces diabetes by damaging the β-cell due to structural similarity of glucose it enters into β-cell by a transporters known as GLUT-2 transporter. It leads to glucokinase inhibition and ROS generation [11]. Several studies reported that aqueous extract of ginger effectively reduces the blood glucose level [12]. It takes place due to the increase of glycogen synthesis, glucose uptake, and due to increase phosphorylation of the insulin receptor [13]. In this study ginger reduces the blood glucose level in 4th weeks. It consistently maintained their glucose level in 8^{th} ,16^{th} and 20^{th} weeks of *Zingiber officinale* administration. Flavenoids and polyphenols found inginger is required for many pharmacological activity including hypoglycemic mechanism [14]. Liver marker enzyme SGPT,SGOT and bilirubin levels were significantly higher in diabetic induced mice [15].The present research also showed that diabetic group has an elevated level of SGPT and bilirubin. 6-shogaol an active component of ginger which significantly reduced the glucose levels, SGPT, and SGOT levels in streptozotocin induced mice [16]. SGPT and SGOT level was decreased in diabetis induced mice in aquous rhizome extract of ginger administred group of mice [17]. Aqueous ginger extract decreased the SGPT and SGOT levels as well as ameliorates the damaged liver tissue [18]. Aqueous extract of ginger restores the SGOT and SGPT levels in the guinea pig [19]. Present study reveals that aquous extract of ginger is very effective on SGPT and bilirubin. It was restored upto normal level in mice after 20^{th} weeks of administration.

Conclusion

It was concluded from entire study that aquous rhizome extract of ginger reduce the blood glucose level effectively and ginger consistently maintains the normal range glucose very effectively. SGPT level was also restored effectively in ginger 150 mg/kg.BW/day administered group after 12 weeks of administration. Billirubin level was also restored to normal range in 12 weeks ginger administered group.

Table 1: Effect of aquous extract of ginger on Glucose and Liver function parameters

Group	Glucose mg/dl	SGPT u/l	Bilirubin mg/dl
Control	90.00±0.5774	21.30 ± 1.120	0.6730 ± 0.1183
Diabetic	222.0±0.5774##	226.0 ± 1.042	1.400 ± 0.2464
4 week ginger administrated	127.3±17.68*	141.3 ± 1.497	0.987 ± 0.14449
8 week ginger administrated	93.92±13.56**	57.9 ± 1.272	1.330 ± 0.1633
12 week ginger administrated	93.67±4.177**	28.0 ± 0.3640	0.7700 ± 0.1130
16 weeks ginger administrated	96.67±10.19**	16.0 ± 0.7108	0.839 ± 0.1742
20 weeks ginger administrated	92.51±10.73**	18.0 ± 0.7025	0.788 ± 0.1005

Data expressed as mean ±SD; n=6 in each group, at the significant level < 0.05

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Original Research

Pathania and Nair

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Ameliorative effect of Sulphosalicylic acid and Mannitol in rice varities (Swift Gold and BH-21) under salinity stress

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Abstract: Plants grow on soil which assist root anchorage and also behave as a pool of water and nutrients that are needed for plant's growth and evolution which may be disturbed by different sorts of plant stresses. Salt stress (abiotic stress) is one of the main reasons that lead to low productivity. Rice is hypersensitive to salt stress. In the experimental setup, four pots were taken for each variety and 10 seeds were sown in each. Various parameters were calculated on the 14th day. Then for the treatment, one pot per variety was taken as control. In the second pot a solution of 100mM NaCl was added. 5mM Mannitol and 5mM Sulphosalicylic acid were respectively added in the latter two pots along with 100mM NaCl. The main aim was to determine the harmful effects of salt stress on growth and other morphological parameters in rice and to study the ameliorative effect of Sulphosalicylic acid and Mannitol on salt stressed rice varieties (Swift Gold and BH-21).The results shows that under saline conditions the growth parameters of rice reduced. The growth of plants was considerably improved when the rice plants were treated with these chemicals.

Keywords: Salt stress, stress amelioration, sulphosalicylic acid and mannitol.

Introduction

The word stress in plants refers to any external condition that hampers the structural, physiological and biochemical performance of plants, unfavourably altering their growth and maturation [1]. There are two kinds of stresses biotic (caused by insects, weeds, diseases, anthropogenic) or abiotic [2]. Various abiotic stresses involves extreme temperature (cold, frost and heat), water logging or scarcity (drought), salinity and mineral toxicity [3,4,5] bear devastating impact on crop metabolism by altering the biosynthesis, transmission, amount and storage of primary and secondary metabolites and thus influencing their growth, development and productivity.

Amongst all the abiotic stresses, soil salinity is the prime cause of reduced crop production throughout the world [6]. Saline soil contains high amount of soluble salts like NaCl, Na_2CO_3 and $CaCl_2$ which may enter soil by irrigation [7]. NaCl is the most harmful salt and is mostly used to induce stress in controlled experiments. Soil salinity reduces crop yield [8]. According to the latest studies it is estimated that 1,128 Mha of land cover is salinity hit worldwide [9]. In India, 6.727 Mha land which covers 2.1% of the total geographical area is salinity affected [10]. States of Gujarat, Uttar Pradesh, Maharashtra, West Bengal and Rajasthan accounts for 75% salt affected areas in India [11]. It is found that salinity affects more than 7% of total land cover and 20% of the cultivated land with 1-2%

increasing annual rate of affected area [12,13]. Salt-affected soil is divided by USDA into saline, sodic and saline-sodic and they show stresses in plants variously by different mechanisms. By 2050, it is anticipated that in crop plants 50% of the arable area will be rendered barren because of salinity stress [14,15]. At high salinity, glycophytes (consisting of most food crops) would not thrive at higher levels of salt concentration. They may be killed at 100-200mM NaCl. Halophytes can tolerate 300-400mM of salinity. Soil salinity is of two types i.e. primary salinity (caused by natural accumulation of salt by processes like weathering) and the cause of secondary salinity is poor irrigation [16], overuse of fertilizers, pollution, etc. Many crop plants are glycophytes while some of them such as *Beta vulgaris* (sugarbeet), *Hordeum vulgare* (barley), *Triticum aestivum* (wheat), *Gossypium* spp. (cotton), *Brassica napus* (canola), *Glycine max* (soybean),*Olea europaea* (olives) are considered as salt tolerant whereas *Solanum lycopersicum* (tomato), *Daucus carota* (carrot), *Solanum tuberosum* (potato) and *Brassica oleracea* (cabbage) are regarded as moderately tolerant crops.

More salt concentration in the soil gives rise to osmotic stress, ion-toxicity and even causes oxidative stress [17]. Under salinity stress the osmotic pressure in plant cells is lesser than the soil solution because of the occurrence of more salt which limits the capability of plants to uptake water and minerals like K^+ and Ca^+. The secondary effects includes assimilate production, decreased cell expansion and reduced cytosolic metabolism [2]. The osmotic stress due to salinity stress causes several physiological variations like membrane interruption, disturbance in nutrient balance, disrupts the capacity to remove reactive oxygen species (ROS) and lowers photosynthetic rate [18]. Improper photosynthesis is due to reduction of leaf area, chlorophyll content and exchange through the stomata and decrease in photosystem II performance due to high salt levels [19]. Reactive oxygen species (ROS) oxidizes lipids, proteins and DNAs [20].

Rice (*Oryza sativa*) is the dominant food crop for about 50% population throughout the globe. It belongs to the family Poaceae. China is the largest producer of rice (30% production) followed by India (24% production) in the year 2019/2020. West Bengal is the major producer of rice in India. *Oryza indica* is thought to have emerged in the Indian Himalayas whereas *Oryza japonica* was first cultivated in southern China [21]. Asian rice (*Oryza sativa*) yield is highly susceptible to salinity [18]. Arize Swift Gold and BH-21 are hybrid cultivars of rice are grown throughout India and in Himachal they are grown in the Kangra district. Rice is observed as a salt-susceptible species with a threshold ranging between 0-8 dSm^- for most cultivated species [22]. Rice has more susceptibility at the seedling [23] and reproductive stage [24] and is tolerant to salt stress at the time of germination [25], tillering and while approaching maturity. Rice yield may reduce by the addition of as little as 50mM NaCl. Under salt stress conditions, rice uptakes more Na^+ which interferes with the K^+ and Ca^{2+}. In rice salt stress leads to low photosynthesis and growth which ultimately leads to loss of biomass and cause partial sterility, finally causing yield loss [26]. Salt stress in rice affects panicle length, number of spikelet in each panicle, rice yield [27]. It also obstructs the appearance of panicle and flowering [28].

Salinity stress feedback in crop plant depends upon the amount and type of soil along with the genotype of plant [20]. In response to salinity stress, plants have evolved numerals of methods to prevent high salt stress like restriction of uptake of

toxic ions, biosynthesis of osmoprotectants, sequestration of vacuolar toxic ions, regulation of stomata to maintain water. Other tolerant mechanisms include hormone modulation, lodging of compatible solutes like proline [29]. Osmotic tolerance includes the plant's quality to endure the dry spell condition of salinity stress and to sustain leaf expansion and stomatal conductance [30]. Another tolerance mechanism in salt stressed plants is the minimization of Na^+ concentration in the cytosol of the cell. In *Oryza sativa, Triticum aestivum, Triticum duram* and *Hordeum vulgare* Na^+ is excluded from the leaves [31].

Several techniques have been developed to combat salinity stress. Genetic enhancement of salt tolerance in present varieties has been practiced but none of these techniques has been found to be fully effective [32]. This may be because the reaction to salinity stress is distinct at cellular position, tissue or complete plant level, complicate tolerance mechanisms and environmental conditions. So it becomes compulsory to advance new techniques and procedures to mend the harmful results of salinity stress. Some efficient methods are organic amendment, chemical priming or exogenous treatment with metabolites. Some natural metabolites/compounds or synthetic compounds like amino acids, antioxidant enzymes, hormones, sugars, vitamins, polyamines act as priming agent and enhance salt tolerance in many crop plants. These agents prevent from salt stress by osmoregulation, and ROS and detoxifying methylglyoxal [33]. Some examples are sugar alcohols like Pinitol and Mannitol, Proline [34], Glycine betaine [35], Nitric oxide (NO), Silicon [13], Salicylic acid (SA) and brassinosteroids (BR), ABA, putrescine and many more [20].

Salicylic acid (SA) is a phytohormone and is a phenolic compound which helps to combat all types of stresses in plants [36]. Exogenous use of SA has an ameliorative impact in salt stress. It can elevate H_2O_2 contents of tissues, accumulation of soluble phenolics and carotenoids [37], antioxidant enzymes are induced to express [38]. Proline content is also increased. Mannitol is a sugar alcohol, which also increases tolerance against NaCl and osmotic stress [39]. It acts as osmoprotectant, scavenger of ROS, stabilizer of proteins and membrane structure during salinity stress [20].

Materials and methods

Plant materials

Two hybrid rice varieties namely BH-21 and Swift gold were chosen for this study. Seeds for these varieties were obtained from a local seed store at Ichhi (Kangra, Himachal Pradesh). Seeds were cleaned with normal tap water to get rid of dirt. 40 seeds of each variety were separately soaked in water on 28-April-2021 for 48 hours to speed the germination process. 8 inches plastic pots were taken for this experiment. The pots were filled with the potting mix. The potting mix was made of 50% garden soil and 50% farm yard manure.

Experimental design

For the experimental setup four pots were taken for each variety. One was used as control and rest three were used for treatment. After filling the pot with potting mix or rice substrate, they were watered properly. 10 seeds per pot for each variety were sown in line on 30-April-2021. The pots were labelled with the pot number and variety name. The seeds germinated after three days. After the germination, the seedlings were watered daily. Weeds were removed regularly and proper care of pests and insects was taken.

Germination studies and plant growth analysis before treatment

On 14th day after sowing, germination percentage along with the other growth parameters like the total number of leaves and the leaf length were checked and recorded.
The germination percentage was calculated per pot with the formula given below:-

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated per pot}}{\text{Total number of seeds sown per pot}} \times 100$$

Total number of leaves in all the plants was counted per pot, for both the varieties.
The average leaf length of the plants per pot was calculated in centimetres by using the following formula:-

$$\text{Average leaf length} = \frac{\text{Sum of length of all the leaves per pot}}{\text{Total number of leaves per pot}}$$

Preparation of chemical solutions

Before treating the plants, three different chemical solutions were made separately for both the varieties. High concentration stock solutions were made ready and then they were diluted to reach the desired concentration. The desired concentration was calculated using the formula $N_1V_1=N_2V_2$. The chemicals were taken from CENRAL UNIVERSITY OF HIMACHAL PRADESH. All the chemicals used were of laboratory grade. The chemicals were weighed using a weighing machine. 1% NaCl solution was prepared by dissolving 1gm of NaCl in 100mL of water. Similarly, 2% Sulphosalicylic acid solution was formulated by mixing 2gm of Sulphosalicylic acid with 100mL of water and 2% Mannitol solution was also prepared by dissolving 2gm of Manniol in 100mL of water.These solutions were serially diluted and 100mM of NaCl, 5mM of each Sulphosalicylic acid and Mannitol was taken for treatment.

Treatments

On 14-april-2021, the plants were treated with the chemicals by soil drenching method. By this method the diluted chemicals were directly added to the base (roots) of the rice plants. Treatments were given in three pots and the remaining one pot was taken as control. The plants were treated exogenously to check the consequence of NaCl (salt stress) on rice and to check the ameliorative effect of Sulphosalicylic acid and Mannitol on NaCl stressed rice. It was made sure that there was no contact with the other parts of the rice plant. On the day of treatment the plants were watered properly so that the plants do not come into immediate shock after treatment. The pots were labelled according to the chemical treatment given to the plants. Same treatment was given to both the varieties. Out of the four pots, one was taken as control for each variety with no chemical treatment. In the 2nd pots, the plants were treated exogenously with 100mM NaCl solution. In the 3rd pot, the plants were firstly treated with 100mM NaCl and then immediately after the first treatment they were treated with 5mM Sulphosalicylic acid solution. In the 4th pot also, the plants were treated with 100mM NaCl solution and then 5mM mannitol solution was immediately poured into the drench. The pots were labelled according to the treatments given to them.

Measurement of growth parameters after treatments

To investigate the effects of the treatments on rice, the plant growth characteristics were again analysed on 15th

day of treatment. Three plants were uprooted from each pot to study the shoot and root length, count of leaves, leaf area, fresh weight and dry weight of rice seedlings. The pots were watered properly before removing the plants to prevent the roots from damage while uprooting. One large, one medium sized and one small plant were selected for the analysis. The plants were uprooted gently and then they were washed to remove the soil particles from the roots. The morphological parameters studied are given below:-

Shoot length

The shoot length of the selected plants was measured in centimetres by measuring scale above the root zone. Average shoot length per pot was calculated as shown below:-

$$\text{Average shoot length} = \frac{\text{sum of the shoot length of the 3 selected plants}}{3}$$

Root length

Root length of the three plants was measured in centimetres with a measuring scale by placing them on the floor. Average root length per pot was calculated by:-

$$\text{Average root lengt} = \frac{\text{sum of the root lengths of the 3 selected plants}}{3}$$

Leaf area

The area of the leaf is measured by grid square method**.** In this method the leaf is laid on the graph paper. The outline of the leaf is traced using a pencil/pen. The number of full and half squares are counted and the leaf area was recorded by counting the grid cover of the leaf. The average leaf area was calculated by taking the sum of the leaf areas of all the plant leaves dividing by the number of leaves.

Leaf number

Number of leaves was counted in the selected plants.

$$\text{Average number of leaves} = \frac{\text{sum of the number of leaves present in the 3 selected p}}{3}$$

Fresh and Dry weight of the plants

For measuring the fresh weight of the plants, the rice plants were washed to remove any loose soil. Remove the free surface moisture by blotting plants with soft towel. The plants were weighed immediately to get accurate results. For measuring dry weight, the plants were dried at a temperature slightly higher (oven) than the normal temperature, to drive away the water. After this the plants were weighed on a weighing machine.

Results

Observations before and after treatment

On 14^{th} day, the primary growth of plants in all the pots was checked as shown in Figure 1 (a) and the germination percentage, total number of leaves per pot and average leaf length was calculated shows the primary growth of Swift gold and BH-21 rice varieties (Table 1). The graphs representing these parameters are shown in Figure 2. These observations help in understanding the after effects of the treatment.

Various morphological changes such as shoot and root length, number of leaves, leaf area, fresh weight and dry weight were studied under different treatments after 15 days of treatment (Table 2). The graphs representing these parameters are shown in Figure 3. Applications of test chemicals adversely influenced these growth characteristics on corelating to the control plants of rice. Figure 1(b) shows the effect

of chemical treatment on rice. In the present study, NaCl induced salt stress and significantly affected the different morphological factors. In the pots which were treated with NaCl showed slow growth and leaf rolling which ultimately led to leaf dying in few plants. The results clearly show that the plants treated with Sulphosalicylic acid and Mannitol had less effect on the growth, as correlated to the NaCl treated plants. General observations show that Sulphosalicylic acid had a better ameliorative effect than Mannitol with more survival rate. The comparative account of growth in all the treatments along with the control plants is given in Figure 1(c).

Effect on number of leaves

The results show that there was less effect of salinity on the number of leaves. The plants selected for observations had maximum of three leaves with a few having two. The plants treated with NaCl only had two leaves each. Salinity had more effect on the leaf morphology than its number.

Effect on shoot and root length

The observations show that the shoot length in NaCl treated plants was least among all in both the varieties. It was also observed that after the treatment there was very less increase in the shoot length. The plants treated with Sulphosalicylic acid and Mannitol had shown a better result. A considerable decrease in the root length has been remarked in NaCl treated rice plants. In both the varieties control plants showed the highest value of root length. Sulphosalicylic acid and Mannitol treated plants had showed a better response which show that they were successful in ameliorating the harmful impacts of salt stress on root size.

Effect on leaf area

Leaf area is an important growth parameter while studying the plant response to salinity and other environmental changes. Leaf area of the control plants was the highest in both the varieties.

Effect on fresh weight and dry weight

The observations show that the wet and dry weight of NaCl treated plants was the lowest in both the various.

Discussions

The chief target of this work was to assess the probability of decreasing the negative result caused by salinity stressed rice by the application of Sulphosalicylic acid and Mannitol. To survive in the changing environment generated by salt stress, plants alter their metabolism. At a higher concentration of salt there is decline in the growth of rice which may be due to the reduction in the expansion of leaf area [40]. In sugarbeet, it was revealed that salinity (NaCl) causes a sharp decline in growth of the plants [41]. Shoot and root lengths may be reduced due to the harmful effect of salt and also by disturbed nutrient uptake by the rice seedlings. The observations clearly show that the growth of NaCl treated plants was the slowest in both the varieties. From the result it was also found that the plants showed leaf rolling and tips of the plants had turned yellow. The leaves rolling ultimately led to the death of the entire plants. Drop in the photosynthetic activity under NaCl stress conditions led to the reduction of growth or leaf dying. Amid salt stress the amount of chlorophyll in the plants decreases due to which the photosynthetic activity gets impaired.

According to a recent study it was found that the germination percentage inversely correlated with the amount of salt in the soil [42]. The root and shoot length was also

reduced under salt stress. The height of the rice plants reduced in soil having 0.5% salinity [43]. Exogenous application of Sulphosalicylic acid (NaCl + SSA) and Mannitol (NaCl + Mannitol) for salt treatment at the time of primary growth showed that the reduced rates of growth by salinity were greatly improved by their use.

Conclusion

From many years, various methods for increasing the tolerance of plants have been used in different stress conditions. One of such methods includes the use of exogenous chemicals like phytohormones and sugar alcohols which act as osmoprotectant. From the above discussions it is clear that salt (NaCl) has a negative influence on the growth of rice varieties (Swift Gold and BH-21). On comparing the treated plants with the control it is concluded that the exogenous application of SSA and Mannitol during salinity stress positively influenced the growth parameters in rice. Growth in plants treated with NaCl was slow as compared to rest of the plants. The reason for slow growth can be due the osmotic effect of salinity and reduced photosynthesis. The outcomes in the above examination clearly show that SSA and Mannitol can upgrade the growth of rice plants under salinity conditions by providing tolerance to stress.

Table 1. Observations of primary growth parameters of Swift Gold and BH-21 rice after 14th day of sowing (before treatment)

Variety	Pot number	Germination percentage (%)	Total number of Leaves per pot	Average leaf length (cm)
SWIFT GOLD	POT 1	60 %	11	6.87
	POT 2	80%	14	5.57
	POT 3	60%	12	7.17
	POT 4	40%	7	6.64
BH-21	POT 1	90%	18	7.32
	POT 2	100%	21	5.80
	POT 3	90%	18	5.5
	POT 4	100%	19	6.71

Table 2. Effect of treatments on different morphological parameters like number of leaves, shoot length and root length, leaf area and fresh weight and dry weight in Swift Gold and BH-21 varieties of rice

Variety	Treatment	Average number of leaves	Shoot length (cm)	Root length (cm)	Leaf area (cm^2)	Weight (gm)	
						Fresh weight	Dry weight
SWIFT GOLD	Control	2.67	16	5.17	1.29	1.95	0.98
	100mM NaCl	2	13.19	3.33	0.97	0.98	0.45
	100mM NaCl+5mM Sulphosalicylic acid	2.67	15.17	4.20	1.13	1.60	0.80
	100mM NaCl+5mM Mannitol	2.33	15.5	4.11	0.99	1.30	0.69
BH-21	Control	2.67	15.5	3.73	1.17	1.70	0.91
	100mM NaCl	2	12.83	2.63	0.88	1	0.40
	100mM NaCl+5mM Sulphosalicylic acid	2.67	14.33	3.57	1.10	1.25	0.75
	100mM NaCl+5mM Mannitol	2.67	13.6	3.40	0.98	1.05	0.59

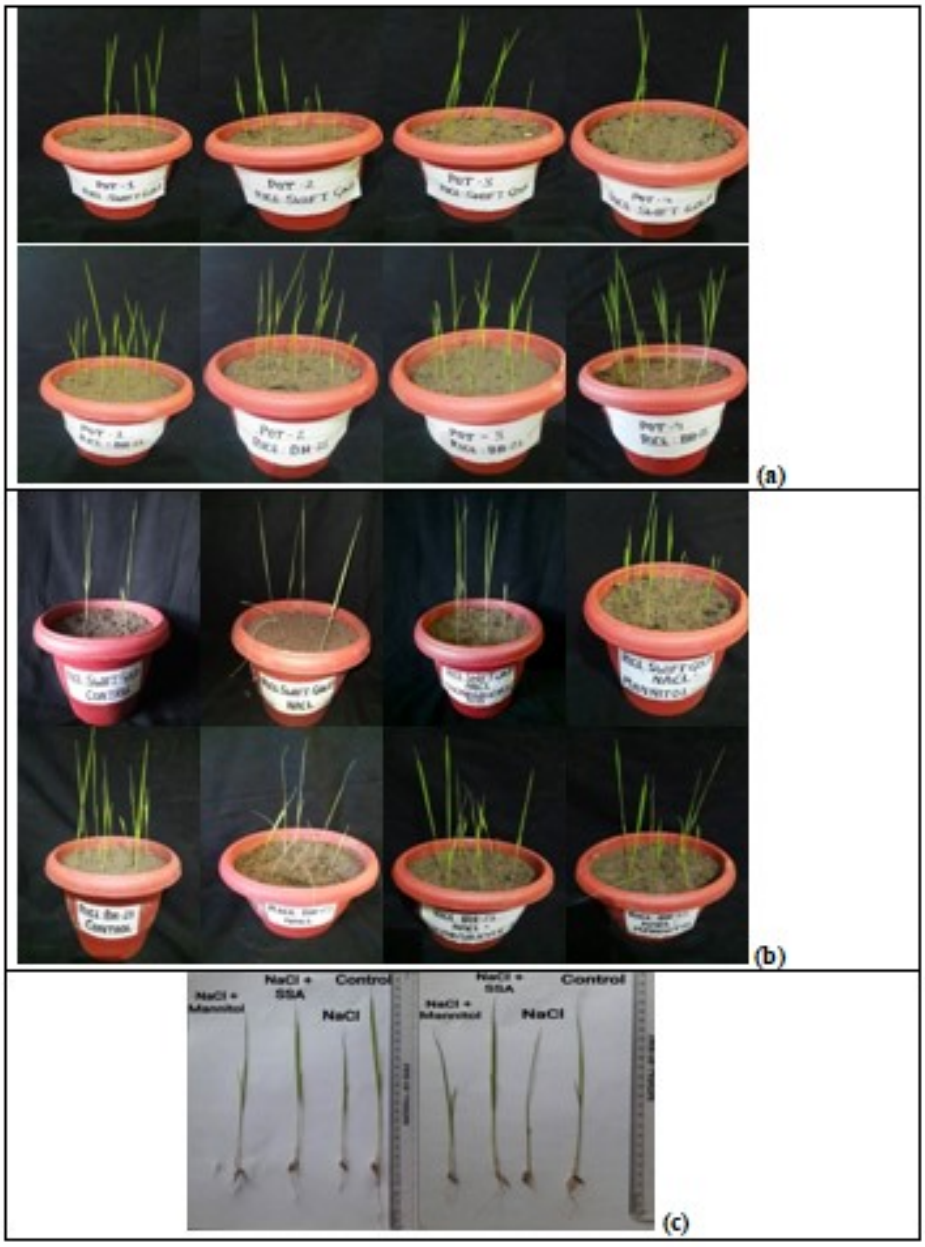

Figure 1. (a) Pictures showing the primary growth of Swift Gold and BH-21 rice after 14th day of sowing (before treatment). (b) Pictures showing the response of Swift Gold and BH-21 varieties of rice to different exogenous chemical treatments. (c) Pictures showing comparative account of different treatments on Swift Gold and BH-21 variety.

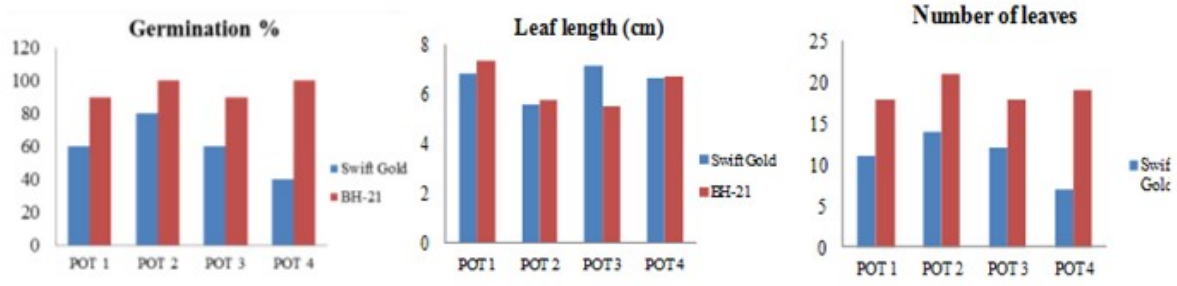

Figure 2. Graphs showing the germination %, length (cm) and number of leaves in Swift Gold and BH-21 before treatment.

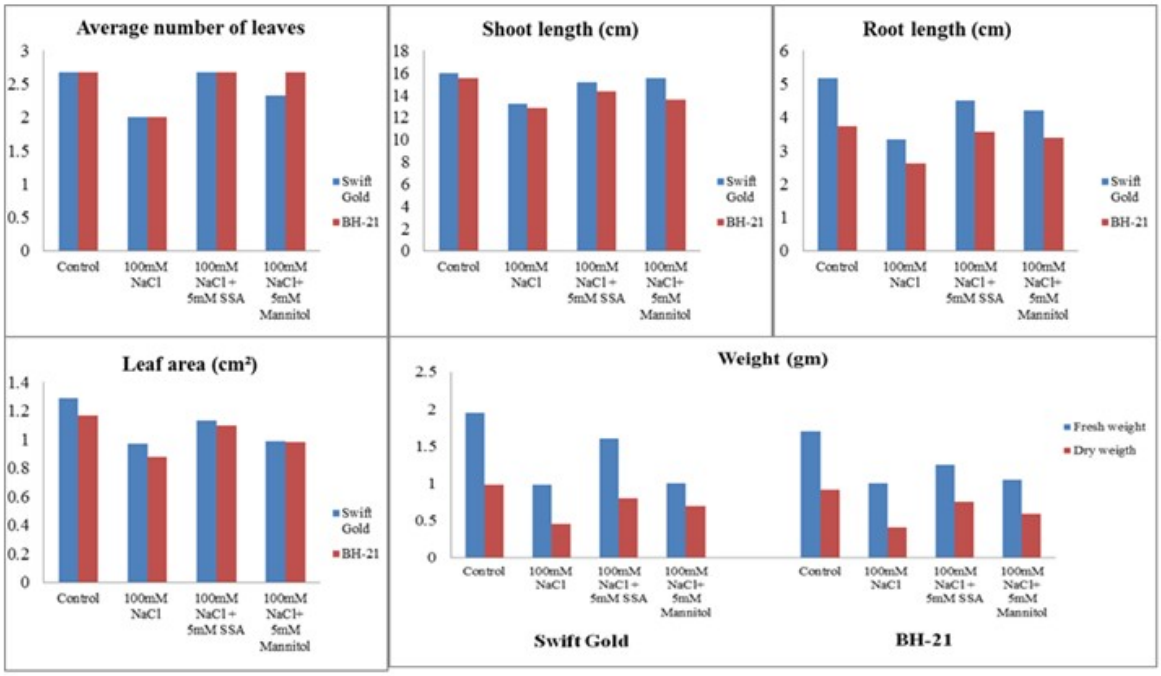

Figure 3. Graphs showing the average number of leaves, shoot length (cm), root length (cm), leaf area (cm²), fresh and dry weight (gm) of Swift Gold and BH-21 varieties after treatment.

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Original Research

Sharma et al

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First report of a persistent pest in Kangra tea, *Pulvinaria floccifera* (Westwood, 1870) and its phenology

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Abstract: *Pulvinaria floccifera* (Westwood), commonly called as cottony camellia scale is reported to infest 'Kangra tea' for the first time. The phenological detail of the scale insect was reported in the paper. It was noted that this scale insect completes one generation in the field and overwinters as 2nd nymphal stage. Number of eggs ranged from 236-627 eggs per sac. While the incubation time lasted for 12-16 days and the oviposition period ranged upto 5-7 days per female. Data showed the growth phase for first, second and third nymphal stage for 12, 18 and 16 days in field conditions, whereas under controlled conditions it was 15, 17 and 19 days. This study indicated that cottony camellia scale reproduced by parthenogenesis as no males were recorded during this work.

Key words: Cottony camellia scale, tea, life cycle.

Introduction

Tea, *Camellia sinensis* (L.) O. Kuntze is one of the significant agro-industrial commodity. It is grown within the tropics and in diverse agro ecological conditions. Tea is indigenous to India as it is a major producer in the world with more than 13,000 gardens spanning over the area of about 5,07,000 hectares. Tea trade is perhaps the biggest supporter of the nation's economy. It has been grown by many states yet Assam, West Bengal, Tamil Nadu, Kerala, Tripura, Arunachal Pradesh, Himachal Pradesh, Karnataka, Sikkim, Nagaland, Uttarakhand, Manipur, Mizoram, Meghalaya, Bihar and Orissa are the significant patrons in India. Himachal Pradesh is famous for its 'Kangra tea' has an area of 2,348 hectares under tea production, which contributed 1,625 thousand kilograms to the total production in 2008 [1].

As it is being grown on well drained medium loamy soil with pH and annual rainfall ranging between 5-6 and 175-375 cm annually with 1000-1700 masl in mid Himalayan range, it becomes a suitable host for the growth of number of insect pests [2]. Each geographic area has its own unique pest fauna although many species have been recorded from more than one region. So is true for this plantation crop. It was found that nearly 1031 arthropod species attack the tea crop globally [3]. More than 300 types of insect pests and mites have been accounted for to influence the tea estates in Indian mainland. The North Asian countries have the longest history of tea cultivation have the largest number of pests. Kangra tea is

reported to be attacked by mites, scales, aphids, thrips, leaf minors and some caterpillars. About, twenty-four arthropod pests were reported to infest tea plantation of Kangra valley [4].

Scales are one of those delicate bodied insect pests assaulting all pieces of the plants viz. leaves, stem, bark, crowns and root making it as a major pest to farmers as well as the gardeners alike. They damage the plant directly as well as indirectly by feeding on the plant sap and transmitting various pathogens causing severe economic damage [5]. This was trailed by the presence of honey dew on the leaves. Resulting in spread of sooty mould and other serious symptoms appear, for example, the fall of leaves stretching out steadily to a practically finish defoliation and whole branch dryness. Severe infestation do not result in the death of tree, but cause the reduction or even absence of yield for a number of years [6] .

It has been recommended that among the scale bugs, it is the delicate scales (Coccidae), heavily clad scales (Diaspididae) and mealy bugs (Pseudococcidae) who affect the fauna of the world and having the status of most common and serious insect pests [7]. Soft scales are the third largest family of scale insects, with 1148 species known till now in the world [7]. In the Oriental region, there are forty-four genera and 126 species of scale insects explored by Ben-Dov [8] and Varshney [9,10]. Similarly, Tang [11] reported 39 genera and 83 species from China and its territories. In addition, the Oriental region has several species-rich genera - *Ceroplastes* (8 species), *Coccus* (23 species), *Paralecanium* (13 species), *Pulvinaria* (16 species) and *Saissetia* with 5 species [7]. However, only China, India and Sri Lanka have been more or less explored [9, 10, 11].

The name of Pulvinariini tribe was ascertained by Ashmead [12]. Targioni Tozzetti [13] gave the name to the genus *Pulvinaria* on the basis of the characters found in the adult female of *Coccus vitis* Linnaeus, which was producing the floccose material, i.e. ovisac. However, it was Signoret [14] who acknowledged the genus for first time in his work in 1873. Apart from this, he discovered 18 species of *Pulvinaria*. With the passage of time, few more species in the genus were added from the various parts of the world [15, 16, 17, 18, 19]. It was Steinweden [20] who redefined the genus *Pulvinaria* based on adult female's characters.

The soft scales under tribe pulvinariine are those scales in which the adult female lays eggs in an ovisac and 'cushion bears', 'cottony scales' and 'cottony soft scales' are terms used in casual conversation for this genus [21,22,23,24]. In 27 genera, more than 200 species of pulvinariine scales are being distributed all over the world. Earlier, it was documented that genus Pulvinaria (Sternorrhyncha: Coccidae) contains more than 100 species which are described in details, out of which 25 species have been recorded from the New World [5, 8, 26]. Recently, Gracia Morales [27] reported 144 species of Pulvinaria all over the world. Being so many different species, every coccid was reported to be unique or unusual, structurally as well as biologically [28].

Till date, about 54 genera of plants distributed among 35 families were found to be infested by *P. floccifera* worldwide. Though polyphagous in nature, infests various plants including economical important ones as well as horticultural crops such as mango, citrus, guava, fig [29, 30, 31]. While discussing the feeding behavior of soft scales, they were found to be monophagous, polyphagous and most

frequently oligophagous in nature [32]. The polyphagous species attained the status of major pest. Though, the coccids are present worldwide, but few of them were reported in restricted region. Waatt and Mann [33] have reported few coccids *Fiorinia theae* Mask in the restricted area like Kangra region and *Eriochiton theae* (Green) in Darjeeling tea. Joshi [34] has stated that 11 different species of *Pulvinaria* were present in India prior to this study.

Many authors such reported this particular species in other crops in the world [29, 30, 31]. From very few years, the trend of scale insect population in the world has appeared to risen in the worldwide. It was found that being in abundant state; it started to extend its geographical distribution from Britain to other regions [35]. Likewise in Netherlands, it has got the status of important pest of ornamental plants and drifted from the cultivated areas to the natural woodland [36]. For Poland, it is an outsider obtrusive species [37].

After reviewing the literature, it was found that the cottony camellia scale *P. floccifera* isn't the scale found in tea in India yet other scale bugs species were reported from tea Of Kangra and Darjeeling region [33]. Some other important species of scale insects including *Coccus hesperidum* L., C. viridis (Green), *Aonidiella aurantii* (Mask.), *Fiorinia theae* Green, and *Ceroplastodes chiton* (Green) were reported to infest tea gardens [38]. In Darjeeling tea diaspidid scale *Hemiberlesia rapax* was reported [39]. Although, Reddy and Sharma [40] reported the incidence of few other species of scale insects viz. *Coccus viridis* (Green), *Saissetia coffeae*, *Coccus hesperidum* (Linnaeus) and *Aonidiella citrina* (Conquillett) in the Kangra tea of Himalayan region. No such records were found for the cottony scale in the region.

The work done on the cottony camellia scale included so many aspects like its biological parameters as well as spatial distribution documented by so many authors in the world [29, 30, 31, 41, 42] forced us to find the possibility of finding the species-specific infestation in the Himalayan tea. For highlighting the specie, it was necessary to describe the scale insect taxonomically. Earlier the emphasis on the description of this particular scale was done on the basis of no-type material which led to mixture of more than one species of this scale. However, Tanaka and Amano [43] solved the problem by redescribing *Pulvinaria floccifera* syntype.

We submit the first report of *P. floccifera* recognition in tea in the Himalayan region. Being reported as an injurious pest worldwide covering a wide range of hosts, the biological data on this coccid in the Himalayan region is scanty or negligible

Materials and Methods

Species identity was very necessary for further description of the pest on tea. An intensive work was done for the detailed description of *Pulvinaria floccifera* collected from the Himalayan region. A continuous survey at regular time interval was done in Kangra tea growing regions. The scale insects from the infested area were collected from the field and green-house cultures and kept in the plastic jars with wire mesh. In the laboratory they were transferred in the vials and were preserved for further morphological study and identification. For each scale insect sample, mature females were collected. A camel-hair brush was used to transfer the specimens gently into a 10 ml sample vial of 70% alcohol. The specimens were sent to Dr Sunil Joshi, Senior scientist, National Bureau of Agricultural Insect Resources, Bengaluru (NBAII) for identification.

Apart from this, the life cycle study was done to understand the further biology of the insect and have relevant data for the making various useful and effective control measures avoiding further infestation with related climate conditions. Likewise, the elaborated details of the emergence of nymphal stages, high peaks with number of generation's plays an important role in control measures.

The experimental data was collected for two consecutive years. For this, a plot with size 3m X 3m was selected and 10 plots were considered for the study. Observations were taken on 10 bushes selected randomly from an unsprayed area of the field at weekly interval for two successive years. The biology of scale insect was studied in the laboratory under at 25±2^{0}C and 50±10% RH. For this, they were allowed to grow on the tea plants developed in the pots.

As *P. floccifera* has different nymphal stages, it is necessary to review its population for studying various phenological stages for effective management. Various parameters were studied viz. life cycle, reproductive and developmental stages including fecundity, ovipositional period, incubation time and hatching ratio. This can be helpful in estimating the infestation level and the damage caused by it.

Life cycle

The first instar stages, the "settlers" was collected from the field and were allowed to settle on the plants potted under the laboratory conditions. They were maintained at 25±2 ^{0}C, 50±10% RH with a photoperiod of 16:8 (L: D). After 24-48 hours, they got settle down on the feeding site. Hardly any kind of movement was noticed. They were marked for avoiding any kind of inconvenience. Any alteration in shape or morphology was noted down daily and total number of days taken by females to complete its life cycle was recorded. The data was calculated on the Microsoft excel spreadsheets.

Reproductive and Development Stages: For studying reproductive and development changes, it was necessary to monitor the infestation of the scale insect with damage symptoms. The changes that took place in the field were noted down and reconfirmed in the laboratory. The numbers of days taken by the subsequent nymphal stages including the other parameters were recorded.

Fecundity

Ovisacs from tea fields were collected to count the number of eggs per ovisac. Ten ovisacs were selected randomly and total numbers of eggs were counted under microscope. The experiment was repeated over through the egg-laying season for two years and results were statistically analyzed for further interpretation. Data was collected and analyzed for following months: May-June and June July for two consecutive years.

Ovipositional period

To study the ovipositional period, five mature females were selected and kept at 25±2 ^{0}C and 50±10% RH. They were observed daily for their egg laying activity. Freshly laid eggs were removed daily with the help of fine hair Camlin brush.

Incubation period

The newly laid eggs were oval shaped and translucent in color. The color of the egg changes from translucent to pinkish towards hatching. For studying the incubation period, one day old eggs were collected and kept individually on the tea leaf kept on 1% agar agar in plastic dishes for hatching. Besides this, they were also kept on blotting paper spread on the Petri plates.

Hatching percentage

Eggs in different numbers (15-28) were kept on moist filter paper kept in the Petri plate for studying the hatching percentage.

Results

The coccid *Pulvinaria floccifera* has three nymphal stages and all of them take particular time duration for growth. All the three nymphal stages L1, L2 and L3 took 12.3±0.57 days, 18±2.64 and 16.6±1.15 days under field conditions with 9.2-34.40 C, 39.4-63.0% RH with 0-63.6 mm rainfall 4.6-11.7 h of sunshine respectively. While the growth rate was noticed to be somewhat different under the laboratory conditions i.e. 15.3 ±1.5 days for L1, 17.6 ±2.08 days for L2 and 19.6 ±1.5 days for L3 with 25±2 ^{0}C and 50±10% RH. In our research, the camellia scale showed incubation period ranged from 12 to15 days during first year of study and for the consecutive year, it was noted to be of 12 to 16 days with an average of 12.9 ± 1.3 and 13.50±1.1 days, respectively. However, in our study the longevity of an adult female was recorded to be 60.8± 8.52 days under the lab conditions. The life cycle lasted for 66.67 ± 7.76 days respectively. The generation time for this scale was noted to be 108.33± 7.094 days under the lab conditions of 25±2 ^{0}C and 50±10% RH, while it was found to be of 119.2 ± 3.701 days under the field conditions.

The fecundity ranged from 315 to 627 eggs with an average of 408±100.44 eggs in May-June for first year. During second year (in June-July), the eggs loads per ovisac ranged from 236 to 451 with an average of 352±63.38 eggs. In the present study, it was seen that the present scale species completed only one generation per year and it was the first instar that overwinters. Oviposition period ranged from 5 to 7 days with an average of 4.86 ±2.41 days.

Discussions

The recent study represents that the specie *P. floccifera* was reported for first time in Kangra tea of the Himalayan terrain. It showed that the population remained in the field for whole year with severity for few months from April to July as its peak time. In countries like Slovakia, Virginia, France, Iran and Netherlands reported one generation per year [24, 31, 36, 44, 45]. As in Japan, it showed two generations per year [30].

On basis of our observation, it was observed that *P. floccifera* lie dormant in form of first nymphal stage on leaves and on woody part of the tea plant. However, in Poland it was second and third nymphal stage, second or third stage in Slovakia who overwintered [36, 44]. In Egypt, it was only adult female who pass through the situation [31]. Unpublished data of our observation revealed only one generation per year for *P. floccifera*. Mohamed [46] studied the behavior of the camellia scale on the sago plant and found only one generation per year in each of the two studied consecutive seasons.

So far data available regarding the biology of *P. floccifera*, a limited set of research papers have been published. It included the work done by El-Minshawy and Moursi [41]. He studied the biological behavior of *P. psidii*, *P. floccifera* and *C. elongatus* under field and lab conditions. In case of *P. psidii*, the egg hatched after 11th day upto 28 days and 2nd and 3rd nymphal stages were able to settle down on the pumpkin fruits. Meantime, the total life cycle lasted for 180-210 days and fecundity of adult female was found to be 200.4 eggs. The phenological parameters for *C. elongatus* were to be found as follows, the average number of 475.4 crawlers in its life time with birth of 11.8 per day crawler in life span of 134.4 days (being viviparous in nature). In case of *P. floccifera,* fecundity was found to be as 857 eggs per adult female with incubation period of 5 days at 27 ^{0}C. But same parameter gave different

value at 17-20 ^{0}C *i.e.* 23.3 days. The time duration of three nymphal stages at 22.5 ^{0}C was 11.3, 9.7 and 15.7 days but it was 10, 19 and 14 days at 27 ^{0}C, respectively.

However, previously reported fecundity for the respective scale showed 857 eggs per adult female on guava tree in Egypt [41]. Elborolosy [47] reported the fecundity for the same species on citrus tree as 875 eggs per adult female. Abd-Rabou [29] showed the incubation period of 9.6±0.30, 11.1±0.55 and 7.3±0.15 days at 18 ^{0}C for the same scale grown on citrus, fig and guava. The scale gave different values when growth was noted at 24^{0}C, *i.e*. 7.4±0.20, 8.7±0.35 and 4.5±0.10 days while at 30 ^{0}C, the values were recorded as 4.5±0.20, 5.6±0.30 and 2.3±0.15 days on citrus, fig and guava plants. For *P. floccifera,* the incubation time was found to be of 11.1 ± 0.7 days and the duration of an adult female was observed in the range of 56-61 day with an average of 58.2±2.1 days under the laboratory conditions [31]. Adult endurance was evaluated on citrus, fig and guava at three different temperatures (18, 24 &30 ^{0}C) [29]. It was found that adult lasted for 67.6±1.00, 69.1±2.50 and 62.5±0.50 days on three hosts at 18^{0}C. At 24^{0}C the values were found to be 58.5±1.50, 62.8±0.40 and 52.7±1.00 days and for 30^{0}C the durations of the adult longevity were 47.9±1.5, 53.1±1.55 and 44.1±0.05 days. However, the generation time for same scale infesting citrus tree was found to be 134.4 days [47].

Conclusion

In the present study, *P. floccifera* is identified and revealed as a significant nuisance of tea in the Kangra Valley. Information on different stages of this scale insect's life cycle, their durations and appearance in tea were generated. The results of the study can be used to evaluate the potential threat of this particular scale insect to the tea plants of the area in relation to number of overwintering stages and most effective stage for further management. Such studies will be helpful in the prediction and management of this pest in tea.

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Original Research

Sapna et al.

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Functional classification of hypothetical proteins from the proteome of *Chlamydia pneumoniae* to characterize potential drug targets

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Abstract: *Chlamydia pneumoniae* is an intracellular gram-negative bacterium. *C. pneumoniae* is non-motile and has a spherical shape and causes serious respiratory illnesses has already been linked to atherosclerosis, asthma and arthritis, along with other chronic conditions. Regardless of the fact that therapies exist to cure chlamydial infections, no drug has still proved to be value enough to eradicate the bacteria in developing nations, and an effective vaccine is far from certain. Functional characterization is critical for establishing the function of proteins during proteome analysis. Meanwhile, the function of a large number of proteins remains unknown. As a result, these molecules are referred to as fictitious proteins (HPs). *C. pneumoniae* has a proteome of 1,028 proteins, including 245 hypothetical proteins (HPs) that have been functionally classified by using Pfam and CDD tool. We are able to identify functions of 24 HPs having function in secretion system, flagellar synthesis and some are involved in lipopolysaccharide synthesis. The proposed study also identified the Physico-chemical properties of putative HPs and prediction of localization of HPs in the cell was done by using CELLO tool. Further analysis leads the current study to the conclusion that out of 24 HPs only 19 proteins are found to be virulent. The human proteome was also cross-referenced with these HPs to check for the similarity using NCBI BLASTp. This research implies that these hypothetical proteins are important for the organism's proliferation and pathogenicity. As a result, they can be utilized to forecast probable medication interactions.

Keywords: *Chlamydia pneumoniae*, Pfam, Hypothetical Proteins, Virulent, Homology.

Introduction

Bacteria can survive in varied environmental conditions, to acquire nutrients and avoid other effects such as cells affecting immune systems. *Chlamydia* has been the most common bacterial communicable disease in the world and it particularly affects women. *Chlamydial* bacteria usually reside within the cells [1]. They lack a plethora of metabolic and synthesis pathways, and hence rely on the host cell for intermediates like ATP [1]. *Chlamydia*can be split into two phases: infection particles which are particularly known as elementary bodes and intracytoplasmic reproductive forms known as reticulate bodies [2]. *C. trachomatis, C. psittaci* and *C. pneumoniae* are the three species of Chlamydiae [2]. Many serovars are discovered in the first two due to variations in cell wall and outer membrane proteins [2]. The *Chlamydia* was once believed to be viruses which reveal that they need host biosynthetic machinery to multiply, but after

several studies it has been shown that they have cell wall and contain DNA, RNA and ribosomes, therefore they are called as bacteria [3]. Human sickness is caused by all three species [4]. *Chlamydia psittaci* infects a wide range of birds and mammals, but *Chlamydia* is primarily seen in humans [4]. Only humans have been detected with *Chlamydia pneumoniae* (The TWAR organism) [4].

Chlamydia pneumoniae is a gram negative bacteria of bacillus shape causes respiratory infection. Furthermore, recent genomic research has indicated that both *C. trachomatis* and *C. pneumoniae* gene for proteins [5]. Chlamydiae get a group-specific lipopolysaccharide antigen and produce chlamydial protein using host adenosine triphosphate (ATP) [5]. Regardless of the fact that Chlamydiae are auxotrophic for terms of three nucleoside triphosphates, they do encode active glucose-catabolizing enzymes that could be used to produce ATP [5]. *C. pneumoniae* induces respiratory infections and is associated to a number of chronic problems, including asthma, atherosclerosis and arthritis [6]. It contributes for 10% of peer pneumonia [6]. Although drugs can treat chlamydial infections, no drug has now become cost-effective enough to wipe the bacteria in developing countries, and a feasible vaccine remains a mystery [6]. In 2018, the Centers for Diseases Control and Prevention estimated that four million people infected with *Chlamydia* (www.cdc.gov.in). In the United States, chlamydia is the most commonly observed bacterial sexually transmitted disease (www.cdc.gov.in). Unfortunately, because most individuals with chlamydia are asymptomatic and do not seek testing, a substantial proportion of infections go unidentified [7].

During proteome analysis, functional characterization is important for determining the function of protein. In the meantime, the role of a huge number of codons currently unexplained [8]. As a response, these molecules are considered to as hypothetical proteins (HPs) [8]. The majority of these proteins are considered to make a significant contribution in the cell, and their identification could lead to additional insights into their structures, roles and pathways [9]. Due to the obvious link with known proteins, template gene identification can also be used to identify undiscovered function to proteins [10]. Several bacterial species, especially *Vibrio cholerae, Clostridium difficile, Neisseria gonorrhoeae* and *Staphylococcusaureus*, have successfully used in silico strategies to predict HP function [11]. As a consequence in finding chlamydia; virulence markers or biomarkers of host disease that may just predict probability of severe reproductive sequel and improve specific screening and treatment is a problem [12]. With the development of molecular genetic tools, abundant genome sequences, and innovative ideas for appropriate genetic manipulation, chlamydial biology is entering a new era of significant development [12]. We hope to see better progress towards a more viable treatment with unique capabilities to analyze the pathogenic pathways producing chlamydial illnesses. The objective of this research is to assign roles to the hypothetical proteins that are involved or helping this particular bacterium to survive in this severe environment and discover potential biotechnological targets.

Material and Methods

In this work, we have taken *Chlamydia pneumoniae* and the proteome of the *C. pneumoniae* has been retrieved from the NCBI RefSeq (https://www.ncbi.nlm.nih.gov/). After retrieving the proteome, we dissected the hypothetical proteins (HPs) from whole proteome of organism. The whole methodology of this work is shown in Figure 1.

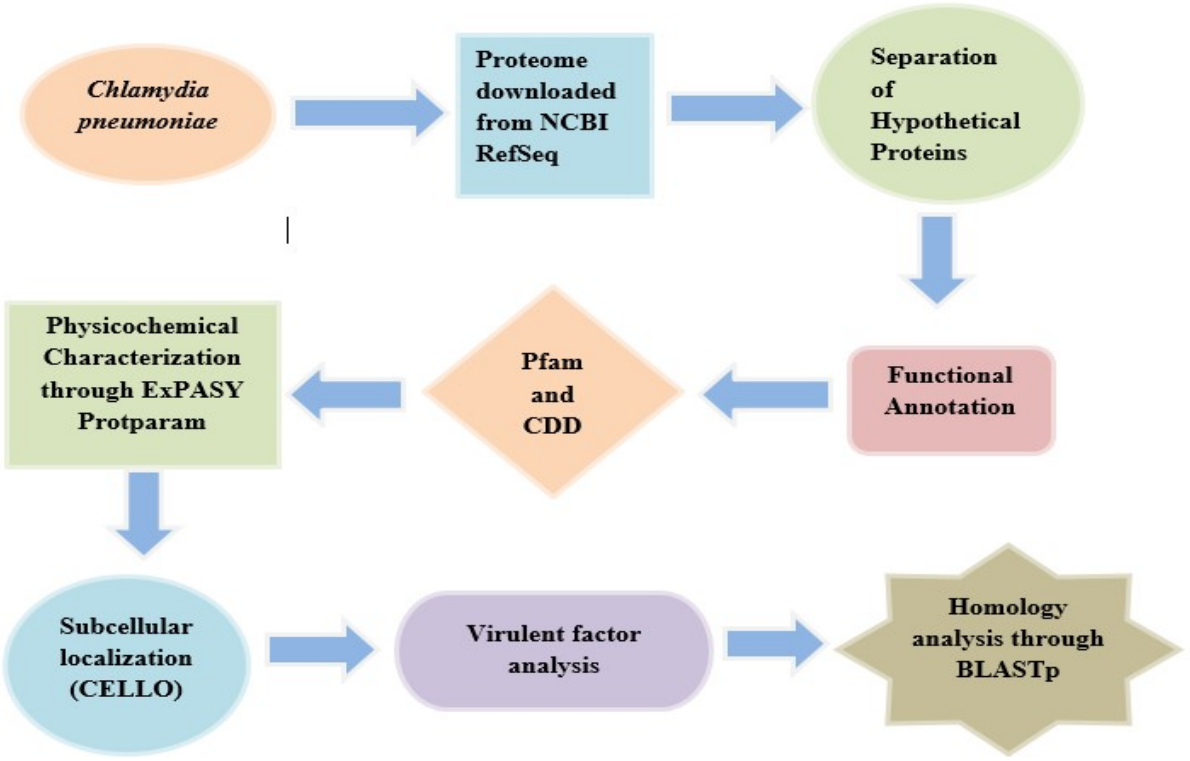

Figure 1. The overall strategy of the work

Functional characterization of hypothetical proteins

Hypothetical proteins' functions were discovered by utilizing databases that has been used to generate a profile Hidden Markov Model (HMM) also with HMMER software [13]. Pfam is used to predict the domain identification of hypothetical proteins [13]. The conserved domain database is a database that provide data from domain-based operations but sometimes manually annotated molecular structures [14].

Characterization of physicochemical properties

For the screening of physicochemical properties of the HPs, we utilized ExPASY Protparam [15]. The physicochemical parameters of proteins yield evidence about their biological nature that can be used to predict biological function [15]. We projected the HPs' aliphatic index, instability index, theoretical pI and hydropathicity index using the accurate parameters.

Sub-cellular location of hypothetical proteins

The activities of proteins are often believed to be associated with their location in the cell, identifying the sub-cellular localization of a protein well with functions of HPs is crucial to determine whether these HPs are vaccine or drug targets [16]. The localization of the HPs was discovered using CELLO. The CELLO tool is used to determine bacterial subcellular distribution throughout the cell [17].

Virulent proteins identification

In the drug discovery and development, virulent proteins in any

bacteria work as a strong drug target. We utilized VirulentPred to predict virulent factors in all functionally classified HPs for potent drug target purpose [18]. To detect pathogenic proteins from all these hypothetical protein sequences, VirulentPred uses the Support Vector Machine (SVM) algorithm [18].

Homology analysis

BLASTp used to perform homology analysis with the human proteome [19]. It is the most widely used homologous analysis tool [20]. This analysis help us in analyzing sequence similarity (forecast homologous and non-homologous sequences).

Results and discussion

Retrieval of proteome of c. pneumoniae

The proteome of *C. pneumoniae* has been retrieved from NCBI RefSeq database. After downloading the proteome set, we found that there are total of 1028 proteins present in this organism. Out of 1,028 proteins, 245 were listed as HPs. These proteins are further used for next screening process.

Functional annotation of HPs

This analysis is used to predict the cellular function of all extracted HPs. In this process all 245 targeted HPs are subjected against these tool (Pfam and CDD) to analyze the functional domains and their cellular function prediction. We pooled the results of both tools to arrive at a consensus result. Only 24 proteins out of 245 HPs have been classified as having their function.

Characterization of physicochemical properties

After functional classification, HPs have been physicochemical determined by using ExPASY Protparam tool. In this step, we described the amino acid length, instability index, number of positively and negatively residues, theoretical pI and grand average of hydropathicity index (GRAVY) of 24 HPs. The hydropathicity value of a peptide is displayed by the GRAVY, which calculates the sum of the hydropathy values of all the amino acids divided by the sequence length [21]. Hydropathicity value less than 0 indicate that proteins are most probably globular proteins and value greater than 0 indicate membranous proteins [21]. The current screening process of all the functionally classified HPs showed GRAVY value less than 0 which represent the globular nature of proteins. The instability index is an indicator of protein stability in vitro condition, if the index is less than 40, than protein will be most probably stable in nature or vice-versa.

Localization of hypothetical proteins

We determined the position of the protein in the cell, including such as extracellular, cell membrane or intracellular. We went across all 24 HPs and find out their location in the cell. On examination of distribution of these hypothetical proteins in the cell, we analysed that majority of HPs are found in the cytoplasm. The subcellular location of HPs is depicted in below diagram (Figure 2). Figure 2 shows that most of the query HPs are located in the cytoplasm.

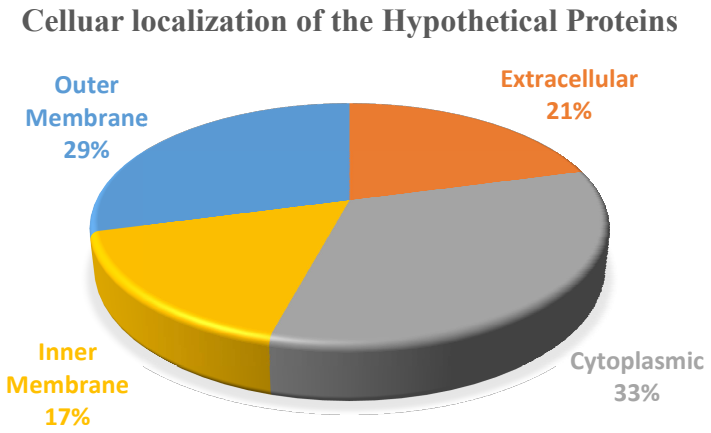

Figure 2. Location of hypothetical proteins in the cell. Blue colourrepresents the location of proteins in outer membrane, orange colour represents extracellular, grey colour represents cytoplasmic and yellow colour represents inner membrane location along with their percentages.

Virulent factor analysis

In this step we checked the virulence of HPs. By using this tool, we predict whether the HPs are virulent or non-virulent. We found that out of 24 HPs, only 20 HPs are virulent and these proteins are mostly used by bacteria for infection. Bacterial pathogen uses a number of active and passive methods to penetrate host cells. They create different virulence factors that help in the transmission of the infection across the host's body. Bacterial virulence is facilitated by microbial products such as adhesions, toxins, porins etc. They all aid the bacteria in host cell invasion, adhesion and eventual spread throughout the host body. From such a strategic point of view, we believed that all these virulence factors can used as a possible therapeutic target for the development of effective antibiotics to counter the bacterial pathogenesis.

Homology analysis

In this step we checked the similarity of predicted virulent hypothetical proteins with human proteome by using NCBI BLASTp with expected value (0.00001). We dissected all non-homologous proteins so that in future they can use as a probable drug target. During this analysis, we found that out of 20 HPs, no protein show homology with human proteome.

Conclusion

In the current pilot study 245 HPs were extracted from the proteome of *Chlamydia pneumoniae*. Only 24 HPs were functionally classified and remaining 221 proteins were rejected. In the next step, functional analysis of putative HPs was done using Pfam tool. Using ExPASY Protparam Bioinformatic tool characterization of physicochemical properties of targeted 24 HPs was done. For the classification of drug targets and vaccine targets of Physico-chemically characterized proteins, we used CELLO tool to localize their position in the cell. VirulentPred tool is utilized to predict the virulence factors of subcellular localized HPs and it predicted that out of 24 putative HPs, only 4 HPs showed non-virulent characters and remaining 19 are virulent proteins in nature. As a result, the bacteria

may exploit these predicted pathogenic proteins to increase its virulence. These proteins can be used as drug target to treat bacterial infection. Further analysis of predicted virulent HPs proteins was done to identify the similarity with Human proteome using BLASTp tool, and result shows no similarity with human proteome which indicate that all predicted virulent HPs are non-homologous in nature. After examining proteins functions, location and pathogenicity with numerous tools, it will be simple to predict the possible treatment targets for this type organisms. These findings could enhance and facilitate drug development, perhaps leading to effective antibiotics to tackle C. pneumoniae pathogenesis.

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Author contributions

Sapna performed all the work with Ms. Shilpa Chauhan. Manuscript edited by Ms. Shilpa Chauhan and Mr. Virender Kumar and reviewed by Ms. Himisha Dixit.

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Original Research

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Habitat Types and its impact on Macrobenthos Assemblies Structure in Gharana Wetland

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Abstract: The Gharana, a dying wetland, is a semi-arid wetland adjacent to agricultural areas merely 500m from the international Indo-Pakistani border. The main goal of the current research was to inspect the types of habitat and its impacts on macrobenthos assemblies. Four stations were identified based on different types of habitat i.e. kind of substrate and quality of water. Species richness, abundance, and three diversity indices (Shannon- Weiner, Simpson and evenness index) for macrobenthos were evaluated. Total 29 genera were observed during the current research. Ephemeroptera (8 genera) were the dominant followed by Diptera in comparison to all other orders. The outcomes of the current research clearly specified the populace of macrobenthos species in diverse habitats was pointedly dissimilar. Also, the results obtained by the CCA showed the maximum number of species, their richness, and abundance in zone 1. The diversity indices showed a higher overtone with the types of the habitat of silt-gravel bed substrate followed by sand, and open lotic and seasonal lotic habitat types.

Key words: Macrobenthos, habitat type, water quality, Gharana wetland, Jammu.

Introduction

Wetlands have unique ecological landscapes that offer several products and services to humankind and acts as an intermediate region between aquatic and terrestrial bionetworks wherever the water level is almost at the superficial or the land is encased by low water [37]. Wetlands play a vital role to regulate the regional hydrological cycle. The Gharana (a dying wetland) is a semi-arid adjacent to agricultural areas just 500m from the international border [39]. It is a notified in almost wetland preservation hold under the J&K Wildlife Protection Act 1978. Microbenthic fauna assumes a significant part for the both ecosystem in the earthbound and aquatic. Mainly it lives in the deep or bottom of any water body. Macrobenthic diversity has its significance like any other aquatic and terrestrial animals. Macrobenthos acts as a link between trophic level by feeding phytoplankton & then act as food source for larger organisms such as fish. Macrobenthos fauna is particularly appropriate for long haul virtual analysis as a portion of the genera are sessile or partake squat motility and are fairly long live and united the consequence of ecological alteration for an extensive time (e.g. dredge material, carbon-based enrichment, collective extraction and weather change as reported by [10; 28; 29;9; 4; 22]. In lentil water bodies, the macrobenthos plays a very significant part in crucial ecology processes like natural way of life elements, supplement cycling, efficiency

and disintegration [7; 15; 19]. The circulation and modicum of benthos diversity are directly correlated to various environmental factors like food utility, sediment type, the substrate as well as water quality as per [24]. Habitat structure is the main aspect defining the existence and dispersal of macrobenthos in any freshwater ecosystems. Mainly in riverine ecosystem the substrate edifice is a crucial feature for the gratitude of the probable status of habitation edifice to microbenthic animals is long-lasting [12]. Substrate structure becomes an essential motivation in the supervision and refurbishment of any riverine system [26; 9; 18] and also the civic of macrobenthos is prejudiced by the intricacy and heterogeneity of habitat types [40]. Many researchers have exasperated to relate habitat ecological factors such as substrate type, water quality parameters to influence the macrobenthos community in water ecosystems mainly rivers and lakes worldwide [14, 1, 20]. Several macrobenthos species develop variations emphatically connected with territory conditions, for example, the environment type and size of the substrate arrangement [31]. The shape and size of the substrate along any aquatic ecosystem can differ from smaller to greater and also more intricate, such as boulder, Cobbles, deferred leaves, pebbles and other constituents that sustenance an excessive diversity, abundance and distribution to a well substrate bed alike grit having scarce species [21]. The substrate gives spots to food and shelter for macroinvertebrates [23]. Besides the ecological role, macrobenthos also acts as indicators of water eminence in freshwater ecosystems due to partial mobility, variation in size, abundance, ubiquity, and also the determinate duration of life cycles [11]. The sand extant in the river bed of any riverine body is first glance appeared to the benthic fauna and having huge diversity and is the best natural habitat of the benthic fauna. Different aquatic ecosystems can have diverse ecological gradients and also have different habitats, which regulates the benthic assemblage structure. The present study aims to investigate how habitat types influence the microbenthic assemblage structure in Gharana wetland. Thus, the primary goal of the research work stood to analyses the assemblage's edifice of macrobenthos among different types of the habitat.

Materials and Methods

Study area

The exploration was engrossed on the Gharana wetland from the period of one year from January 2019 to December 2019. An extensive field survey was conducted for collecting the primary data. The stations were divided into 4 sampling zones for the assortment of hydrological parameters, habitat type and macrobenthos species.

Figure 1: Overview of selected sampling area in Gharana wetland

Table 1: Classification of selected zones with according to habitat types

Classification basis	Habitat Types	Zones
Substrate	Silt -gravel	Zone 1
	sand	Zone 2
Water state	Open lotic	Zone 3
	Seasonal lotic	Zone 4

The samples for macrobenthos were collected every month by a Surber sampler with an inspecting space of 10×12 inch of the waterway bed and darting silk net with a cross section size of 500µm. The dislodged animals were swept back by the water current and were collected in the net and transferred to a white tray. The macro-zoobenthos were also collected from bottom sediments, stones and pebbles attached to substratum using sterilized forceps, washed and transferred in trays. The benthos was sorted and collected in different sterilized pre-labelled tubes and preserved in 70% ethanol or formaldehyde. For identification, the organisms were examined visually and through the compound microscope. Further identification was done by following the standard keys. The results were reported in individuals per m^2. The temperature, pH, TDS, Conductivity were resolute with the help of digital type instruments. The water temperature was determined with the help of digital thermometer (Testo 1113-TMH) pH by using a pen-type meter (HANNA = HI98107) TDS and Conductivity by using a pen-type meter (HANNA = HI98301). Water transparency is measured with the help of Secchi plate (18cm breadth). Humidity was determined by using digital thermo/hygro meter (288-CTH).

Data analysis

Variety information investigation among the macrobenthos was measured and afterward factual examination was finished with the assistance of Paleontological Statistics (PAST) form 3.1, a product bundle for Paleontological information investigation composed by P.D. Ryan, D.A.T. Harper and J.S. Whalley, was utilized to run examination. Variety among the macrobenthos species was evaluated by utilizing three variety lists viz., Shannon-Wiener variety file [33], Simpson record and Evenness list, consider both the quantity of people and the dissemination example of people.

Results and Discussions

Distinct hydro-graphic conditions of different zones during different seasons (Table 2). Maximum water temperature recorded was 20.1±1.20C at Zone 4 where the minimum water temperature was found 11.3±0.200C at Zone 3 can be due to the direct relationship between bright sunshine, its duration and air temperature. No significant difference was found in temperature among the selected sampling zones. A similar observation was reported by [35] regarding water quality in Gharana wetland. Water pH values range between 7.3±0.25 to 7.6±0.25. Maximum 8.1±0.45 pH value was at zone 4 where the lowest value 7.3±0.25 was recorded from Zone 3 due to the accessibility of carbonates and bicarbonates in water improve break up carbon dioxide level by separation and goes about as a crude material for photosynthesis. TDS ranges from 64.9±1.11 mg/l at zone 4 to 245.1±0.81 mg/l at zone 3. Turbidity ranges from 53±0.35 mg/l at zone 4 to 402.2±2.01 mg/l at zone 3. A similar observation was reported by [35] regarding water quality in various regions of Gharana wetland. DO is the most important indicator for the development of biota, appraisal of water quality and a significant controller of metabolic cycles of living beings and local area. Dissolve Oxygen (DO) ranges from 7.4±0.2 mg/l at zone 3 to 9.2±0.63 mg/l at zone 1. A similar observation was reported

by [28, 17, 13] while studying physiochemical Characteristics of Asan wetland, Doon valley (Uttarakhand). The decrease of DO may be because of natural burden through the civil, homegrown sewage and supplements. The restricting components influencing the DO content are basically temperature, photosynthesis, breath and decay measures. No critical distinction was found in Dissolve Oxygen fixation among the stations. Natural oxygen request implies a fundamental necessity of oxygen by all biotic life forms for their metabolic exercises in the oceanic framework. Natural oxygen request increments as the biodegradable natural substance increments with enormous quantities of purchasers happened in a waterway. The maximum mean in BOD was 2.4±0.31 mg/l at zone 4 and minimum mean 1.1±0.30 mg/l at Zone 4 due to high temperature favour microbial activity.

Macrobenthic fauna – Seven groups of macrobenthos included Ephemeroptera, Hemiptera, Diptera, Coleoptera, Odonata, Mollusca and Annelida, were recorded from zone 1of Gharana wetland (Table 3). In Zone 1, total of 29 genera of macrobenthos in which 8 genera of Ephemeroptera – *Baetis sp., Cloeon sp. Ephemera sp., Emhemerella sp., Ecdyonurus sp., Habrophlebiasp, Siphlonurus sp.,* and *Hydroptela sp.* were present. 3 genera of Hemiptera- *Gerris sp., Corexia sp.,* and *Hesperocorixa sp.* 6 genera of Diptera- *Antocha sp., Chironomus sp., Culex sp., Simulium sp., Phychoda sp.,* and *Tabanus sp.* 5 genera of Coleoptera- *Agabinus sp., Amphizoa sp., Hydaticus sp., Dineutus sp.,* and *Limnius sp.* 3 genera of Odonata- *Agrion sp., Hegenius sp.* and *Ischnura sp.* 2 genera of Mollusca- *Lymnaea sp.* and *Pleurocera sp.* 2 genera of Annelida- *Tubifex sp.* and *Hirudinaria sp.* were found. In Zone 2, total 21 genera of macrobenthos belong to 6 orders in which 6 genera of Ephemeroptera – *Baetis sp., Cloeon sp. Emhemerella sp., Ecdyonurus sp., Habrophlebiasp,* and *Hydroptela sp*. were present. 2 genera of Hemiptera- *Corexia sp.,* and *Hesperocorixa sp.* 4 genera of Diptera- *Chironomus sp., Simulium sp., Phychoda sp.,* and *Tabanus sp.,* 4 genera of Coleoptera- *Agabinus sp., Hydaticus sp., Dineutus sp.,* and *Limnius sp.,* 3 genera of Odonata- *Agrion sp., Hegenius sp.* and *Ischnura sp.* 2 genera of Annelida- *Tubifex sp.* and *Hirudinaria sp.* were found. In Zone 3, total 17 genera of macrobenthos belongs to 7 orders in which 5 genera of Ephemeroptera – *Baetis sp., Cloeon sp., Ecdyonurus sp., Habrophlebiasp,* and *Siphlonurus sp.,* were present. 2 genera of Hemiptera- *Corexia sp.,* and *Hesperocorixa sp.,* 3 genera of Diptera- *Culex sp., Simulium sp.,* and *Phychoda sp.,* 4 genera of Coleoptera- *Amphizoa sp., Hydaticus sp., Dineutus sp.,* and *Limnius sp.* 1 genera of Odonata- *Ischnura sp.* 1 genera of Mollusca- *Lymnaea sp.,* 1 genus of Annelida- *Tubifex sp.* were present during the study period (figure 2). In Zone 4, total of 15 genera of macrobenthos belong to 7 orders in which 3 genera of Ephemeroptera – *Baetis sp., Ecdyonurus sp., and Habrophlebiasp, were present. 3 genera of Hemiptera- Gerris sp., Corexia sp.,* and *Hesperocorixa sp.* 3 genera of Diptera- *Simulium sp., Phychoda sp.,* and *Tabanus sp.* 3 genera of *Coleoptera- Agabinus sp., Amphizoa sp.,* and *Limnius sp.* 1 genera of Odonata- *Agrion sp.,* 2 genera of Annelida- *Tubifex sp.* and *Hirudinaria sp.* were present (figure 2). 57 genera of macrobenthos was recorded from eleven rivers of the North-Western Himalaya as per [32]. [24]reported fifty genera of macrobenthos from Sherkhad stream in Himachal Pradesh and similarly [3] recorded sixty-eight genera of macrobenthos from several rivers of the Kumaon region. [2] reported that species richness and species abundance are directly correlated with

wetland area and water surface area. Whenever dissolved oxygen concentration reduced in any aquatic ecosystem resulted in precipitation, reduction and inclusion of other benthic organisms. Benthic population in zones 1 of Gharana wetland consisted of a rich fauna with excellent oxygen demand. The abundance of Coleopterans decreases with increasing pollution load, also as an indicator of clean, clear waters and thus they can be authentically identified as pollution intolerant macrobenthos.Water contamination led a decrease in Ephemeropteran populace referenced by [25; 30; 23] announced that a few gastropods are additionally contamination markers. [27]reported that as the water volume and water velocity increased, the abundance of benthic fauna was disturbed and also effect on the population composition and their abundance. Gastropoda is useful to actually look at the situation with water contamination and go about as pointers of contamination in certain wetlands at Santhal Pargana by [34]also stated that macrobenthos has high tolerance to a variety of ecological stress and their high abundance represent a good indicator of eutrophication in the water body. [34; 5; 21]also reported that *Chironomus* and *Tabanus* species are indicative of deteriorating water quality in the riverine ecosystem.

Macrobenthos diversity indices

Most noteworthy 0.954 Simpson record (1-D) was found at zone 1 and least 0.943 was found at zone 3. Higher 0.958 Simpson record (1-D) values were found in winter where least 0.950 during Summer. Most noteworthy 3.222 Shannon list (H) was found at zone 1 and least 3.111 was found at zone 3. Higher 3.232 Shannon record (H) values were found in winter where most reduced 3.152 during Summer. Most noteworthy 0.865 equity esteem was found at zone 1 and least 0.774 was found at zone 3. Higher 0.905 uniformity esteems were found in winter where most minimal 0.835 during Summer. No huge contrast was found in mean worth of variety lists esteem among the months and zones (Table 4).

CCA analysis of macrobenthos groups and water parameters

CCA investigation of macrobenthos species plenitude and water boundaries are introduced in (Table 5 and Figure 3). The length of a vector by the given variable demonstrates the consequence of that variable in CCA investigation and the longest vector of water temperature showed positive relationship with zone Z3. Vector extent of broke down oxygen showed positive connection with zone Z1 and where BOD showed positive relationship with zone Z3 and Z4. High upsides of water temperature are positive associated with Ephemeroptera, Hemiptera, Diptera, Coleoptera, Odonata, Mollusca and Annelida. High upsides of DO are positive associated with Ephemeroptera, Hemiptera, Diptera, Coleoptera, Odonata, Mollusca and Annelida. High upsides of turbidity are negative connection with Ephemeroptera, Hemiptera, Diptera, Coleoptera, Odonata, Mollusca and Annelida. Eigen worth of hub 1 (0.004) clarified 73.16% connection between natural boundaries and macrobenthos local area. Though, Eigen worth of hub 2 (0.001) clarified 4.78% relationship as in (figure 3).

Impact of dregs on richness of Macrobenthos:

Ephemeroptera had the largest quantities of genera followed by Diptera in the various sorts of dregs. Table 3 exhibited the Ephemeroptera were present in every silt type. Residue rock deposit (S-G) had 30

Table 2: Annual discrepancy in physico-chemical parameters at selected zones of Gharana wetland

Parameters	Zone 1		Zone 2		Zone 3		Zone 4	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Water Temperature (^{0}C)	11.3±0.04	19.2±1.7	11.5±1.11	19.8±1.2	11.3±0.20	19.9±1.2	11.9±0.42	20.1±1.2
pH	7.5±0.22	8.5±0.15	7.4±0.27	7.5±0.22	7.3±0.52	7.6±0.25	7.3±0.25	7.5±0.35
TDS (mg/L)	67.2±3.11	237.1±2.30	66.2±3.20	235.3±2.59	68.5±2.10	245.1±0.81	64.9±1.11	220.2±1.18
Conductivity(µS/cm)	115.3±0.30	344.5±1.2	113.3±0.27	354.3±0.42	115.2±0.21	362.6±0.32	112.5±0.11	345.2±0.42
Turbidity (NTU)	55±0.8	399.5±0.67	56.4±0.10	390.5±0.60	57.1±0.11	402.2±2.01	53.3±0.35	375.5±0.52
DO (mg/L)	7.8±0.33	9.2±0.63	7.8±0.54	9.0±0.15	7.5±0.32	8.9±1.5	7.4±0.2	9.0±1.31
BOD (mg/L)	1.3±0.20	2.1±0.53	1.3±0.15	2.2±0.55	1.2±0.59	2.3±0.27	1.1±0.30	2.4±0.31

Table 3: Macrobenthos distribution at selected zones of Gharana wetland

Orders	Species	Zone 1	Zone 2	Zone 3	Zone 4
Ephemeroptera	*Baetis sp.*	+	+	+	+
	Cloeon sp.	+	+	+	
	Emphemera sp.	+			
	Emhemerella sp.	+	+		
	Ecdyonurus sp.	+	+	+	+
	Habrophlebia sp.	+	+	+	+
	Siphlonurus sp.	+		+	
	Hydroptila sp.	+	+		
Hemiptera	*Gerris sp.*	+			+
	Corexia sp.	+	+	+	+
	Hesperocorixa sp.	+	+	+	+

Diptera	*Antocha sp.*	+			
	Chironomus sp.	+	+		
	Culex sp.	+		+	
	Simulium sp.	+	+	+	+
	Phychoda sp.	+	+	+	+
	Tabanus sp.	+	+		+
Coleoptera	*Agabinus sp.*	+	+		+
	Amphizoa sp.	+		+	+
	Hydaticus sp.	+	+	+	
	Dineutussp	+	+	+	
	Limnius sp.	+	+	+	+
Odonata	*Agrion sp.*	+	+		+
	Hegenius sp.	+	+		
	Ischnura sp.	+	+	+	
Mollusca	*Lymnaea sp.*	+		+	
	Pleurocera sp.	+			
Annelida	*Hirudinaria sp.*	+	+		+
	Tubifex sp.	+	+	+	+

Table 4: Variation in selected diversity indices of macrobenthos in Gharana wetland

	Z1	**Z 2**	**Z3**	**Z4**	**Winter**	**Summer**	**Monsoon**
Simpson_1-D	0.954	0.952	0.943	0.949	0.958	0.950	0.953
Shannon_H	3.222	3.198	3.111	3.174	3.232	3.152	3.182
Evenness_e^H/S	0.865	0.844	0.774	0.824	0.905	0.835	0.861

Table 5:CCA biplot scores of habitat ecological parameters and macrobenthos species at selected sampling zones of Gharana wetland

	Axis 1	**Axis 2**	**Axis 3**
Ephemeroptera	-0.034	-0.010	-0.003
Hemiptera	0.053	0.026	0.038
Diptera	-0.034	0.050	-0.011
Coleoptera	0.031	-0.060	-0.008
Odonata	0.166	0.023	-0.010
Mollusca	-0.068	-0.014	0.042
Annelida	0.052	-0.030	0.004
Zone 1	1.384	0.407	-0.371
Zone 2	-0.703	-1.032	-1.067
Zone 3	-1.062	1.403	0.280
Zone 4	0.074	-1.031	1.892
Water Temperature (0C)	-0.739	-0.308	0.582
TDS (mg/L)	-0.275	0.804	-0.655
Turbidity (NTU)	-0.016	0.836	-0.646
DO (mg/L)	0.956	-0.117	-0.253
BOD (mg/L)	-0.511	-0.204	0.830
Eigenvalue	**0.004**	**0.001**	**0.000**
%	**73.16**	**22.06**	**4.783**
p	**0.33**	**0.449**	**0.83**

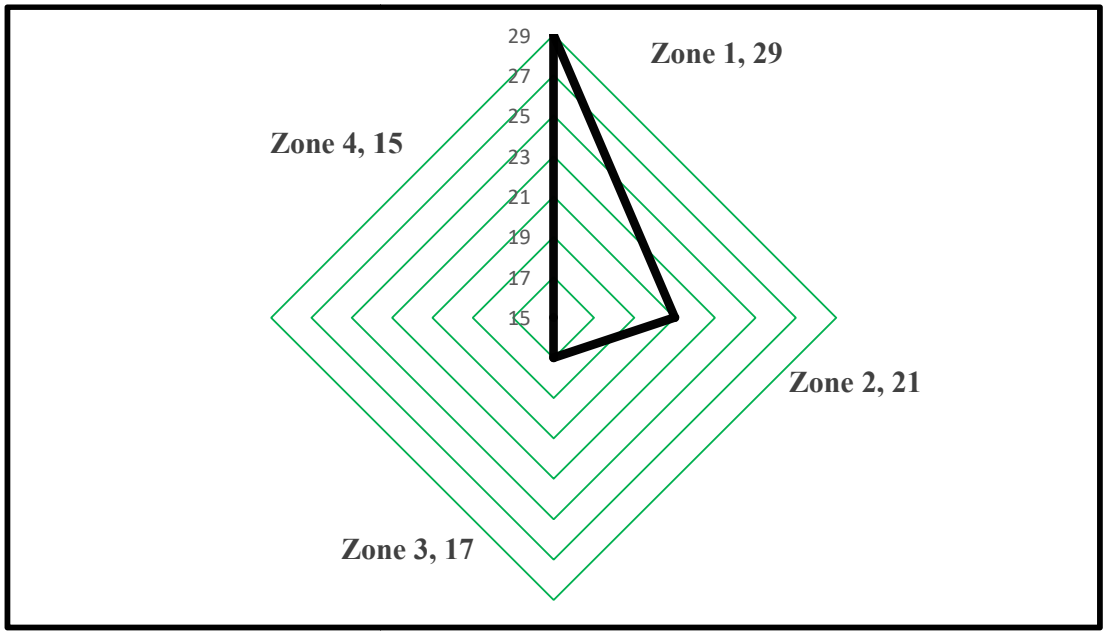

Figure 2: Graph showing total species present at selected sampling zones in Gharana wetland

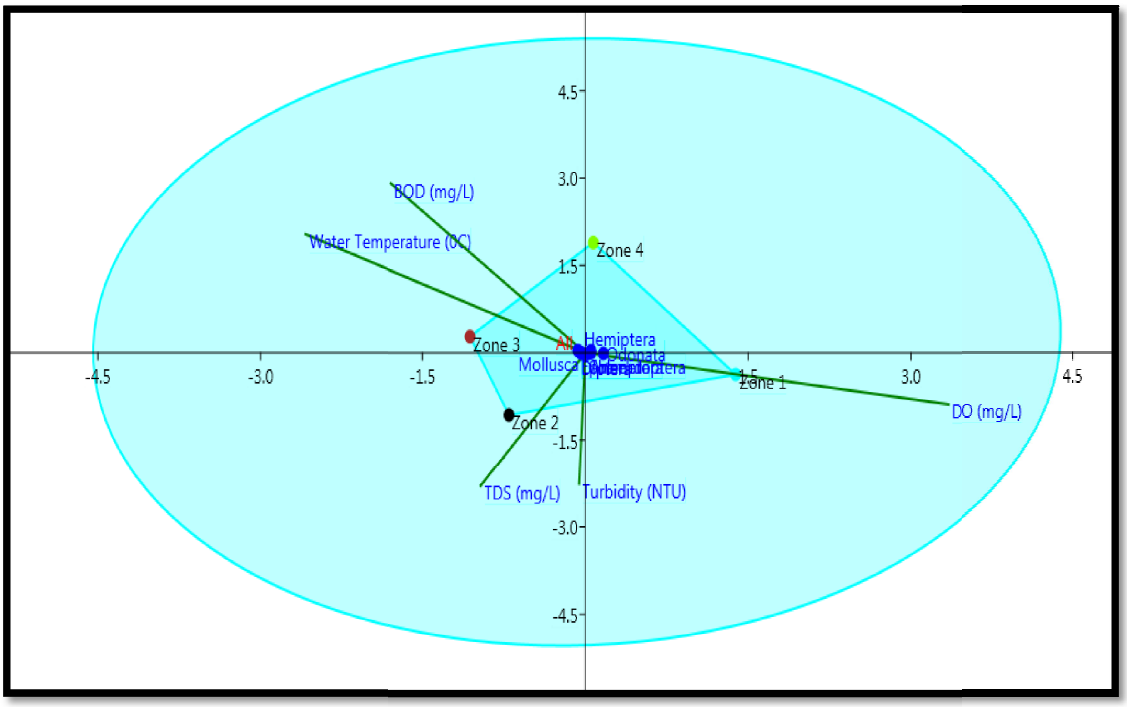

Figure 3:CCA biplot of habitat ecological parameters and macrobenthosspecies at Gharana wetland.

taxa, sand dregs (S) had 21 taxa, and open lotic (O-L) had 17 taxa.The current outcomes likewise showed that the thickness of macroinvertebrates had a more prominent relationship with the residue rock dregs followed by the sand silt and the open lotic residue had the least affiliation. The most noteworthy biomass documented in a residue rock dreg was credited to large number of macrobenthos species.

Relationship between substrate and Macrobenthos:

Macrobenthos people group spend a limit of their life cycle on the bottommost of any sea-going environments and thusly the substrate bed assumes an imperative part in deciding the endurance pace of an assorted local area of macrobenthos. The substrate bed offers an immediate environment and safe house to macrobenthic local area against contenders and predation. Bed dregs, substrate size, and other ecological factors straightforwardly upset the array design of benthic fauna that live in the bed surface [36]. As per [41]substrate having rough rock is entirely reasonable for macrobenthos species as they can connect themselves. The sediment, rock and sand substrate are additionally reasonable for those macrobenthos species, which have tunneling propensities as certain types of request molluscs and crabs [6]. As a general rule, the quantity of species just as the quantity of the people/species are taken to gauge the variety of the macrobenthos local area. The consequences of the current review exhibited that silty-rock bed had most elevated amount of macrobenthos population, their wealth and variety because of the rich natural matter which offers an assortment of diet in an appropriate climate [8; 16; 22]. The types of molluscs in the vast water were recorded not exactly in the sediment or rock addressing that molluscs diversity are more possible to animate in the substrate which has minor molecule extent as residue, rock and so on.

Conclusion

From the present research, it can be concluded that impact of different type of habitat, substrate type, and quality of water parameters on the assemblage's edifice of macrobenthos in the Gharana wetland were assessed and their impact was discussed. The result of the present study revealed that the macrobenthos mostly prefer the bed surface with smaller size sediment i.e. silt, gravel etc, for their feeding and protection against enemies. However, some other factors of water quality such as water temperature, light, water velocity and human disturbance also created the potential impact on the diversity, richness and the abundance of the macrobenthos populace. Thus, additional research is needed, to observed the impact caused by several human interference and physicochemical constraints on assemblage's edifice of macrobenthos.

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Original Research

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Economic analysis of bitter gourd genotypes for open and protected condition

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Abstract: The current experiment had laid out to evaluate the performance of various genotypes of bitter gourdunder open & protected conditions during 2017-18 at Lovely Professional University, Phagwara. The experiment comprised a Randomized Complete Block Design (RCBD) with three replications for evaluating the eight genotypes of bitter gourd in two different conditions. To estimate the performance of genotypes for growth and yield contributing traits to study the economic analysis of bitter gourd for open and protected cultivation. Based on all the aspects related to horticultural parameters, genotypes Prachi and Polo-71 might be recommended to the farmers for commercial cultivation under open and protected conditions and can be used for further study work related to crop improvement programs. In terms of economic benefits, Polo-71 (4.12) followed by Prachi (3.72) was achieved with maximum cost-benefit ratio, whereas among all the genotypes under protected condition Polo-71 showed the highest cost-benefit ratio (1.91) followed by Preeti (0.75). Based on all the aspects related to growth, yield parameters, and economic analysis, genotypes Polo-71, Prachi performed best and can be recommended to the farmers for commercial cultivation and used for further study work related to crop improvement programs. However, all eight genotypes showed the best performance in open conditions based on a cost-benefit ratio.

Keywords: Traits, variance, significant, commercial, yield & economic.

Introduction

Momordica charantia L. acknowledged as the bitter gourd is one of the most important members of the Cucurbitaceae family had chromosome number 2n=22. Indo Burma is the origin of bitter gourd had reported by Garrison in 1977 **(Singh, 2013). It** is monoecious, cross-pollinated, and annual climber in nature **(Dey *et al.,* 2008).** Bitter gourd is widely cultivated in open fields, although the economics of protected agriculture is dependent on the structure used, the crop chosen, and other factors. Protected agriculture has the potential to alleviate the issue of low yield amid harsh weather. The cultivation of vegetables in protected conditions is appropriate for home and export purposes is thus the greatest choice for managing forest resources more efficiently in the current context of the continual need for vegetables and severely diminishing landholdings (Sanwal et al., 2004). The goal of the investigation was to assess the efficacy of protected cultivation to open fields in terms of cost throughout the summer and winter seasons. Protected agriculture has grown in popularity in recent years as a means of increasing agricultural

production. Keep in mind above all discussed fact the present investigation had conducted to find out the beneficial cultivation season based on a comparison among open and protected conditions with the following objective i.e. to analyze the economics of bitter gourd under open and protected conditions.

Materials and methods

The current experimentation was carried outfor the period of the summer season in open & protected conditions during the winter season in 2017-18 at Lovely Professional University, Phagwara (Punjab) in Randomized Complete Block Design with three replications. Total eight genotypes namely Solan Hara, Punjab-14, (varieties) Prachi, Polo-71, Harit, Charu, Preeti, and Arushi-910 (hybrid). For both conditions, the nursery was grown in portrays having cell size 1.5". Seedlings were raised on soilless media then kept in a germination chamber. To maintain the crop regular watering and plant protection measures were adopted. The seedlings were ready for transplanting within 20-25 days. Spacing in open condition was 1.5m x 60cm, whereas under protected conditions, 60cm × 60cm. All the recommended packages and practices were carried out throughout the investigation. Fruits were harvested at marketable maturity level when they were more or less cylindrical and well filled with seeds, and subsequent harvesting had done two-three times a week. From all the varieties/hybrids in each replication, five plants were randomly selected for recording the observations for various horticultural traits. For the economic scrutiny of bitter gourd cultivation in open and protected conditions data was collected and analyzed by using formulas.

Economic analysis

Gross Returns = Net Sales- Cost of Goods and Services

Net Income = Gross Return- Total Cost of Cultivation

$$\text{Benefit cost ratio} = \frac{\text{Net income}}{\text{Total } cost\ of\ cultivation}$$

Economic analysis for open and protected conditions

Table 1 shows the expenses for growing bitter gourd under open conditions the total variable cost was Rs. 44,529.93. The cost patternof the total variable cost determined that the uppermost percentage had used up on weed control(Rs. 9,500.00), followed by manure and fertilizer, plant protection, field preparation, staking, planting and transplanting, bedding preparation, irrigation, and harvesting (9229.93/-, 6425.00/-, 4400.00/-, 4350.00/-, 3575.00/-, 2500.00/-, 2150.00/- and 2400.00/-, respectively). Similar results have also been confirming by Kumar et al. (2016).However, in the case of protected conditions, the cost of cultivation had calculated in Rs. 128789.00. The variable cost structure shows that the highest proportion of 39,000/- was spent on hand pollination, followed by fertilizer and manure, harvesting, planting and transplanting, tamping preparation, weeding. Plant protection, plotting, and irrigation with expenses of 35,729.00/-, 10,000.00/-, 9640.00/-, 9000.00/-, 6550.00/-, 5520.00/-, 5400.00/- and 1850.00/-, respectively. Similar results have been confirming by Kumar et al., (2016), Kumar et al., (2017), and Sreedhara et al., (2013).

The outcomes of the experimentation as mentioned in Table 1. revealed that the total cost experienced on bitter gourd in the open field and under-protected conditions

were 65265.93/- and 268662.00/-per hectare, respectively. There were some major factors due to which the total cost of cultivation for protected cultivation was higher due to the high price of seeds, land rent, hand pollination, staking, and a large number of labor and maintenance of crops. Bitter gourd is a cross-pollinated crop under-protected condition hand pollination is an essential operation for fruit set, particularly in monoecious varieties/ hybrid.

Table 1: Economic investigation of bitter gourd cultivation under protected and open condition (Rs. /ha)

S.No.	Particular	Open Condition	Protected Condition
Cost structure			
1	**Field preparation**	4400	6100
2	**Ridging preparation**	2500	9000
3	**Seed sowing & transplanting**	3575	9640
4	**Fertilizer and manure**	9229.93	35729
5	**Stalking**	4350	5400
6	**Irrigation**	2150	1850
7	**Plant protection**	6425	5520
8	**Weed control**	9500	6550
9	**Hand Pollination**	0	39000
10	**Harvesting**	2400	10000
Variable cost		**44529.93**	**128789**
11	**Marketing cost**	2570	8540
12	**Management charge**	6500	48000
13	**Rental value of land**	11666	83333
Fixed cost (11 to 13)		**20736**	**139873**
Total cost (variable cost + fixed cost)		**65265.93**	**268662**

Net Field Benefits (NFB)

Producers are much concerned with the inconsistency of remuneration than with yields, so the net field benefits werecomputedbasedon the variable costs.A calculation disclosed that the highest net field benefit has been achieving with genotype V8 (Polo-71) in both open conditions (table 1.) and protected conditions (table 1.) of Rs. 340870.82and Rs 701061.00, respectively.

Economic analysis

Based on economic analysis for each genotype as mention in Table (1) was indicated that the highest benefit-cost ratio in open (4.12) and protected conditions (1.91) had achieved with genotype Polo-71, followed by Prachi (3.72) and Preeti (2.16)

in open condition, whereas under protected condition genotype Preeti (0.75). Fromthe current experimentation, it was elucidated that for variable cost the maximum difference had been observed for hand pollination, manures and fertilizers, and harvesting as compared to open cultivation of bitter gourd. Rental value and management cost of protected cultivation was higher, while in open cultivation expenditure on weed control and plant protection was maximum than the protected condition. The highest difference between open and protected conditions cultivation had found due to the high cost of hand pollination and manure and fertilizers viz., Rs 39000.00 and Rs 26499.07, respectively.

Conclusion

Based on economic analysis of each genotype result revealed that the highest benefit-cost ratio in open and protected conditions had been confirmed with genotype Polo-71, followed by Prachi and Preeti in open conditions. However, under the protected condition with genotype Preeti. The total cost of Bitter gourd was almost four times higher in comparison to open field conditions. According to economic analysis, the conclusion made that for the cultivation of bitter gourd, open cultivation is more beneficial to the farmers when compared to protected cultivation. Under Protected conditions, off-season cultivation is possible, if hand pollination is done. The gynoecious lines have also been used in the respective study.

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Table 4.14: Effect of net field benefits, net returns and benefit cost ratio of bitter gourd genotypes in open condition

Genotypes	Seed cost / ha	yield (q/ha)	Gross income (Rs/ha)	Variable cost (Rs/ha)	Total cost (Rs/ha)	Net field benefits	Net returns (Rs/ha)	Benefit cost ratio
V1	4125.00	44.16	110412.25	48654.93	69390.93	61757.32	41021.32	0.59
V2	7500.00	137.49	343736.25	52029.93	72765.93	291706.32	270970.32	3.72
V3	2600.00	28.33	70830.50	47129.93	67865.93	23700.57	2964.57	0.04
V4	4050.00	29.17	72913.75	48579.93	69315.93	24333.82	3597.82	0.05
V5	6000.00	56.66	141661.00	50529.93	71265.93	91131.07	70395.07	0.99
V6	9150.00	94.16	235407.25	53679.93	74415.93	181727.32	160991.32	2.16
V7	5600.00	61.66	154160.50	50129.93	70865.93	104030.57	83294.57	1.18
V8	12500.00	159.16	397900.75	57029.93	77765.93	340870.82	320134.82	4.12

Table 4.15: Effect of net field benefits, net returns and benefit cost ratio of bitter gourd genotypes in protected condition

Genotypes	Seed cost / ha	yield (q/ha)	Gross income (Rs/ha)	Variable cost (Rs/ha)	Total cost (Rs/ha)	Net field benefits	Net returns (Rs/ha)	Benefit cost ratio
V1	8250.00	101.80	305409.00	137039.00	276912.00	168370.00	28497.00	0.10
V2	15000.00	132.68	398049.00	143789.00	283662.00	254260.00	114387.00	0.40
V3	5200.00	112.77	338310.00	133989.00	273862.00	204321.00	64448.00	0.24
V4	8100.00	97.30	291900.00	120689.00	260562.00	171211.00	31338.00	0.12
V5	12000.00	140.40	421191.00	140789.00	280662.00	280402.00	140529.00	0.50
V6	18300.00	167.30	501891.00	147089.00	286962.00	354802.00	214929.00	0.75
V7	11200.00	126.55	379641.00	139989.00	279862.00	239652.00	99779.00	0.36
V8	25000.00	284.95	854850.00	153789.00	293662.00	701061.00	561188.00	1.91