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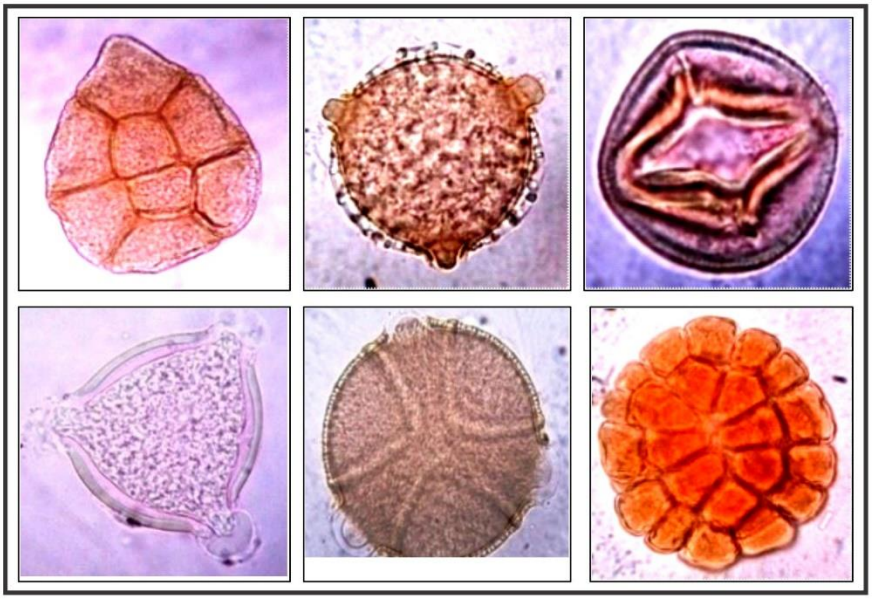

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Cover Photo: LM images of the pollen of some member of Fabaceae

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Taxonomic Investigation of Some Members of Fabaceae (Subfamily-Caesalpinioidae) With Special Reference to Pollen Morphology

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Abstract

Palynology, the study of pollen, had a big role in taxonomic identification, paleontology and forensics. Pollens from different plants had different morphology, such as in Fabaceae. This study aimed to determine the pollen grains morphology in Fabaceae (Subfamily Caesalpinioidae). Pollen morphology of 19 plant species under 7 genera Fabaceae (Subfamily Caesalpinioidae) were examined. Fresh pollen samples were collected from 4 plant species from the North 24 Pgns, 2 plant species from South 24 Pgns, 8 plant species from Kolkata, 2 plant species from Nadia and 3 plant species from Howrah. Pollens were acetolysed following standard method and observed under Compound microscope. Pollen shapes were recorded based on the P/E ratio. In general, all these pollens were small, medium and large size. Parameters measured in this study were the types of pollen sizes, pollen shape, aperture characteristic, and ornamentation type of exine and the most common aperture type was tricolporate. The most important characters included exine ornamentation (exine ornamentation type) and Apocolpium Index.

Keywords: Caesalpinioidae, morphology, palynology, pollen grains, acetolysis.

Introduction

Fabaceae a large heterogeneous family of flowering plants, occurs naturally in the tropics around the world. The Fabaceae **Adanson** or Fabaceae **Lindley** is a very large family of herb, shrub and trees with a great variety of habitat, including aquatics, xerophytes and climbers. The flowers of Fabaceae are extremely variable in size, form, colouration and pollinators. Many species are of economic importance to man. This family comprises 650 genera and 18,000 species (Dickison, 1981) with cosmopolitan distribution in tropical and temperate zones. Pollen morphology is a muchinteresting subject; it requires exact knowledge to describe pollen. This includes shape, polarity, symmetry, aperture, ornamentation, exine stratification and size of the pollen. Among the bases of angiosperm phylogeny, pollen morphology is unique in that through no other study can one obtain as great an amount of information from so little material within a short time (Walker and Doyle 1975). Palynology is the subject of modern science that deals with the study of pollen, spore and other palynomorphs (Agashe & Caulton 2007). Palynology has been recognized for years for its importance and application in different fields of sciences (Ajipe & Adebayo 2018). The pollen morphology is particularly useful in solving taxonomic problem, a specific character of pollen

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morphology could be designated to a certain family and sometimes even up to species level of plants (El-Amier 2015; Rabia et al. 2012). The sporopollenin layer on the pollen wall renders its inertness against acid and bacterial attack (Williams et al. 2018). Pollen grains have a unique biological characteristic, contain a large amount of genetic information, and exhibit strong genetic conservation, so they used for species identification (Almeida et al. 2018).

Materials & Methods

Pollen Morphology

All the plants were collected from South 24 Pgns, North 2 Pgns, Kolkata, Howrah and different blocks of Salt Lake City, mainly the portion of branch with inflorescence were taken. During the field trips five to ten individuals of mature plants of each species were collected and they were attached with numbered tags. The specimens were photographed, worked out, described and preserved in the form of herbarium sheets following the standard and modern herbarium techniques (Jain and Rao, 1977). The portions of plants were placed in between stacks of newspapers or blotting papers for proper dying. As a result of that, dried specimens are obtained which were mounted on herbarium sheets and properly labeled. Each species was identified in consultation with standard literature (Hooker, 1875; Prain, 1903; Anonymous, 1997; Hazra et al, 2000; Paria and Chattopadhyay, 2000). The search for literature including recent ones for determining the correct name of taxa was followed after The Plant List (web add.) and other literature including (Bennet 1987).

Parameters measured in this study were the types of pollen shape, pollen sizes, aperture characteristic, and ornamentation type of exine. The pollen shape classes are based on the ratio between the length of the polar axis (P) and equatorial diameter (E). P and E are measured from the equatorial view of a pollen grain and spore (Simpson 2010).

Table 1. The common shapes of the pollen grain

No.	Shape	Ration of P to E
1	Peroblate	< 0.50
2	Oblate	$0.50 \leq x < 0.75$
3	Subspheroidal	$0.75 \leq x < 1.33$
	- Suboblate	$0.75 \leq x < 0.88$
	- Oblate spheroidal	$0.88 \leq x < 1.00$
	- Prolate spheroidal	$1.00 \leq x < 1.14$
	- Subprolate	$1.14 \leq x < 1.33$
4	Prolate	$1.33 \leq x < 2$
5	Perprolate	> 2

The common shapes are presented in table 1. While the aperture morphology is an opening or thinning of the exine, physiologically it is a germination zone.

The exine ornamentation has two different types, the structure or texture and the sculpturing. The structure comprises of all the internal (infratectal) baculae of various form and arrangements. All the ektexine (including sexine and nexine) characters belong to the structural features, while the sculpturing comprises external (supratectal) geometric features without reference to their internal construction (G. (Gunner) Erdtman 1986).

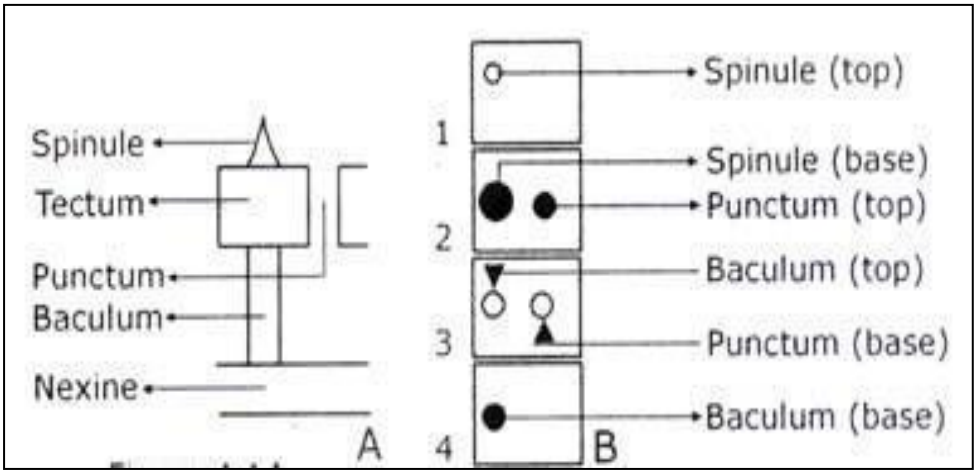

Fig. Illustrating diagram of the exine ornamentation structure. (A) Layers of exine. (B) Light and dark islands that appear from the high adjustment to low adjustment of the light microscope. 1, 2, 3, & 4 represent the first, second, third and fourth focus of microscope from the high to low adjustment.

After collection of polliniferous materials, the acetolysed methods for these material was followed as suggested by Erdtman (1952, 1969), Chanda (1966), and Bose *et al*.(2012) with slight modifications. The flowers or anthers containing pollen were fixed in glacial acetic acid, then transferred directly to the brass sieve, crushed and washed down to the centrifuge tube with 70% alcohol. The brass sieve was put into flame after each treatment until it became red hot to avoid contamination. Acetolysis mixture was prepared slowly by adding one part of concentrated sulphuric acid to nine parts of acetic acid anhydride (as alternative 5% - 10% NaOH/ KOH) in a measuring cylinder. The powdered material collected in the test tube was then suspended in the acetolysis mixture (ca. 10 ml of the mixture was added to each tube) and stirred carefully by glass rod. After thoroughly stirring, the mixture was heated to about 100˚C in a water bath, until the mixture attained light brown colour. After heating, the mixture was allowed to stand for a few minutes, then centrifuged and decanted. The process was repeated once and foam was removed by adding a few drops of 95% alcohol or acetone. The mixture was then filtered through a fine sieve and centrifuged. After decanting, distilled water was added to the sediment, half of which was kept for chlorination and 50% glycerine was added to the other half.

For chlorination 5ml of glacial acetic acid, 1-2 drops of concentrated sodium chlorate solution (as alternative H_2O_2) and a few drops of concentrated hydrochloric acid were added and the

mixture was stirred by a glass rod. The mixture was then centrifuged. After centrifuging and decanting 50% glycerine was added to it. Then the two parts (non-chlorinated and chlorinated) were mixed-together, centrifuged and decanted. The tubes containing acetolysed polliniferous sediments were kept in inverted condition on a filter paper for a couple of hours. For mounting on slides, a minute piece of glycerin jelly (prepared by Kisser's method) was taken on tip of a clear platinum needle (after making red hot in the flame and subsequently cooling), touched carefully the pollen precipitate in the test tube and then placed on a clean slide. Then the slide was heated gently and the after the jelly had spread evenly, by the platinum needle around the cover glass was placed on the material and sealed off with paraffin wax (melting point 60 ^{0}C to 62 ^{0}C).

The light microscopical work was done with a student microscope. Measurement was taken using high power and oil immersion objectives. The factors for measurements in high power and oil immersion objectives were 3.13µm and 1.22µm for 1 occular micro division respectively.

For taking measurements, etc. the methods of Chanda (1962, 1966) among others have been followed. In all cases, measurements and other observations were based on acetolysed grains unless otherwise mentioned. The measurements quoted in the pollen descriptions are generally based on an average of ten reading randomly choosen. In case of scanty occurrence, however, fewer grains were measured. The relevant data, measurements and other information are represented in Table 2.

Discussion

The pollen morphology of the Subfamily Caesalpinioidae of family Fabaceae representing 19 species under 7 genera have been investigated (Table 2). The pollen grains in the genera of the family are isopolar, radially symmetrical.

Pollen Size:

The size of a pollen grain are helpful for identification of species. The size of the pollen grains varies between 36.4 µm.-72.8 µm. in case of shape-class spheroidal grains and PA/ED 10.4 µm. / 13.0 µm. to 65.0 µm. / 59.8 µm.in case of other shape. The smallest grain is found in *Cassia kleinii* (PA/ED 15.6 µm. / 10.4 µm.) and the largest grains found in *Bauhinia purpurea* (PA/ED 65.0 µm. / 72.8 µm.).

Pollen Shape:

The shape of a pollen grains are usually specific to species. Shape is determined by the ratio between the length of polar axis and the equatorial axis (P/E ratio). The pollen grains are mostly Prolate. However, other shapes such as Sub-oblate, Oblate-spheroidal, Spheroidal, and Prolate-spheroidal also encountered.

Exine Ornamentation:

It is the evident from Table 2 that the exine of the investigated taxa have variable thickness. The thickest exine is noticed in *Bauhinia purpurea (*5.72 µm.), whereas, the thinnest exine are found in several taxa *viz. Cassia alata, Cassia javanica, Cassia kleinii, Cassia occidentalis, Cassia tora, Crotalaria pallida, Crotalaria verrucosa, Desmodiumgangeticum, Melilotus alba, Melletiaovalifolia,Tephrosiapurpurea* (1.30 µm.).

Table 2. Pollen morphological data

Sl. No.	Taxa	Aperture	Shape	Equitorial outline	PA	ED	PA/ ED	Colpus L/B	Pore diam.(L/B)	Exine	Sexine	Nexine	Apocolpium/ Apoporium	Apocolpium Index	Mesocolpium/ Mesoporium	Exine Ornamentation
1	***Bauhinia acuminata***	atreme	spheroidal	globose	59.8 - 65.0	59.8 - 65.0	1	–	–	3.90	1.30	2.60	–	–	–	clavate
2	***Bauhinia purpurea***	tricolporate	sub-oblate	triangular	33.8 - 39.0 59.8 - 65.0	41.6 - 44.2 67.6 - 72.8	0.81 0.88	26.0/ 10.4	20.8 /13.0	5.72	1.30	4.42	18.2 - 26.0	0.4	19.5- 26.0	striate
3	***Bauhinia tomentosa***	penta-colpate	spheroidal	globose	70.2 - 72.8	70.2 - 72.8	1	23.4/ 5.20	–	3.12	2.08	1.04	–	–	–	clavate
4	***Brownia coccinea***	tricolporate	prolate	globose	36.4 - 39.0	26.0 - 28.6	1.14	28.6/ 10.4	2.60 /2.60	5.20	1.30	3.90	7.80	0.3	–	reticulate

Table 2. Contd….

SL No	Taxa	Aperture	Shape	Equitorial outline	PA	ED	PA/ ED	ColpusL/B	Pore diam.(L/B)	Exine	Sexine	Nexine	Apocolpium/ Apoporium	Apocolpium Index	Mesocolpium/ Mesoporium	Exine Ornamentation
5	***Caesalpinia pulcherrima(RED)***	trisyn-colporate	sub-oblate	triangular	41.6-46.8	36.4-39.0	1.14	33.8/10.4	3.90/2.60	3.12	1.30	1.82	5.20	0.14	20.8-26.0	reticulate
6	***Caesalpinia pulcherrima(YELLOW)***	trisyn-colporate	sub-oblate	triangular	41.6-46.8	33.8-39.0	1.20	33.8/10.4	5.20/3.90	3.90	1.30	2.60	7.80	0.23	26.0-31.20	reticulate
7	***Cassia alata***	tricolporate	prolate	triangular	20.8-23.4	10.4-13.0	2	18.2/5.20	3.90/3.90	1.30	0.65	0.65	7.80-10.40	0.44	10.4-13.0	reticulate
8	***Cassia fistula***	trisyn-colporate	prolate	triangular	18.2-20.8	15.6-16.9	1.66	18.2/6.50	1.30/1.30	2.60	1.30	1.30	3.90-5.20	0.25	10.4-13.0	rugulate
9	***Cassia javanica***	trisyn-colporate	prolate	triangular	18.2-20.8	13.0-15.6	1.38	15.6/5.20	1.30/1.30	1.30	0.65	0.65	2.60	0.2	13.0	rugulate

Table 2. Contd….

Sl. No.	Taxa	Aperture	Shape	Equitorial outline	PA	ED	PA/ ED	Colpus L/B	Pore diam.(L/B)	Exine	Sexine	Nexine	Apocolpium/ Apoporium	Apocolpium Index	Mesocolpium/ Mesoporium	Exine Ornamentation
10	***Cassia kleinii***	trisyn-colporate	prolate	triangular	15.6-18.2	10.4 - 13.0	1.50	15.6 /5.20	3.90 /2.60	1.30	0.65	0.65	2.60	0.25	7.80-9.10	psilate
11	***Cassia laevigata***	tricolporate	prolate	triangular	20.8-23.4	10.4 - 13.0	2.00	13.0 /5.20	3.90 /2.60	2.60	0.78	1.82	3.90 - 5.20	0.37	10.4-13.0	reticulate
12	***Cassia occidentalis***	tricolporate	sub-prolate	triangular	23.4-26.0	18.2 - 20.8	1.28	23.4 /7.80	3.90 /3.38	1.30	0.65	0.65	13.0 - 15.6	0.71	13.0-15.6	regulate-fossulate
13	***Cassia siamea***	tricolporate	prolate	triangular	26.0-28.6	18.2 - 20.8	1.42	26.0 /7.80	3.90 /2.60	2.60	1.30	1.30	5.20 - 6.50	0.28	13.0-15.6	reticulate
14	***Cassia sophera***	tricolporate	sub-prolate	triangular	36.4-39.0	28.6 - 31.2	1.27	26.0 /10.4	3.90 /2.60	2.60	0.78	1.82	3.90 - 5.20	0.13	–	reticulate

Table 2. Contd….

SL No	Taxa	Aperture	Shape	Equitorial outline	PA	ED	PA/ ED	ColpusL/B	Pore diam.(L/B)	Exine	Sexine	Nexine	Apocolpium/ Apoporium	Apocolpium Index	Mesocolpium/ Mesoporium	Exine Ornamentation
15	***Cassia tora***	trisyn-colporate	prolate	trian gular	18.2 - 20.8	10.4 - 13.0	1.75	26.0 /10. 4	3.90 /2.6 0	1.3 0	0.6 5	0.6 5	2.60 - 3.90	0.2 5	7.8-10.4	reticulate
17	***Delonix regia***	tricolporate	prolate-spheroidal	trian gular	36.4 - 39.0	31.2 - 36.4	1.16	28.6 /7.8 0	7.80 /6.5 0	5.2 0	3.1 2	2.0 8	23.4 - 26.0	0.7 5	18.2-20.8	reticulate
18	***Peltophorum pterocarpum***	tricolporate	oblate-spheroidal	trian gular	39.0 - 41.6	36.4 - 40.3	0.98	28.6 /10. 4	3.90 /3.9 0	3.9 0	1.3 0	2.6 0	23.4 - 26.0	0.7 1	20.8-23.4	reticulate
19	***Saraca asoca***	trisyn-colporate	prolate	trian gular	36.4 - 39.0	20.8 - 23.4	1.75	26.0 /7.8 0	3.12 /2.6 0	2.6 0	1.3 0	1.3 0	2.60 - 3.90	0.1 1	10.4-13.0	reticulate

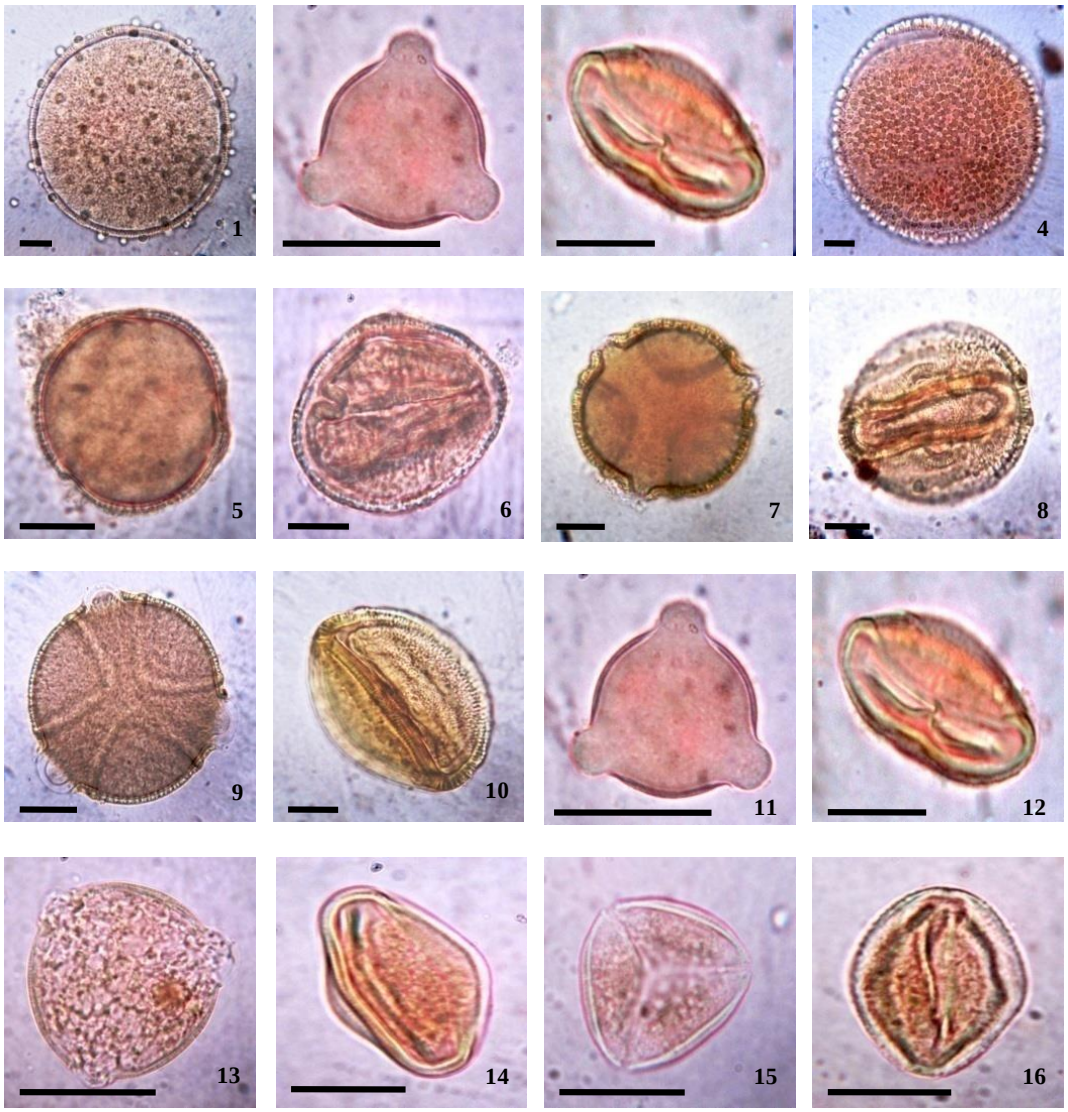

PLATE I. Pollen morphology of investigated taxa (Scale Bar -10µm.).

Fig: 1. Pollen of *Bauhinia acuminata*; 2. Polar view of *Bauhinia purpurea*; 3. Equatorial view of *Bauhinia purpurea*; 4. Pollen of *Bauhinia tomentosa*; 5. Polar view of *Brownia coccinia*; 6. Equatorial view of *Brownia coccinia*; 7. Polar view of *Caesalpinia pulcherrima*; 8. Equatorial view of *Caesalpinia pulcherrima*; 9. Polar view of *Caesalpinia pulcherrima* var. *flava*; 10. Equatorial view of *Caesalpinia pulcherrima* var. *flava*; 11. Polar view of *Cassia alata*; 12. Equatorial view of *Cassia alata*; 13. Polar view of *Cassia fistula*; 14. Equatorial view of *Cassia fistula*; 15. Polar view of *Cassia javanica*; 16. Equatorial view of *Cassia javanica*;

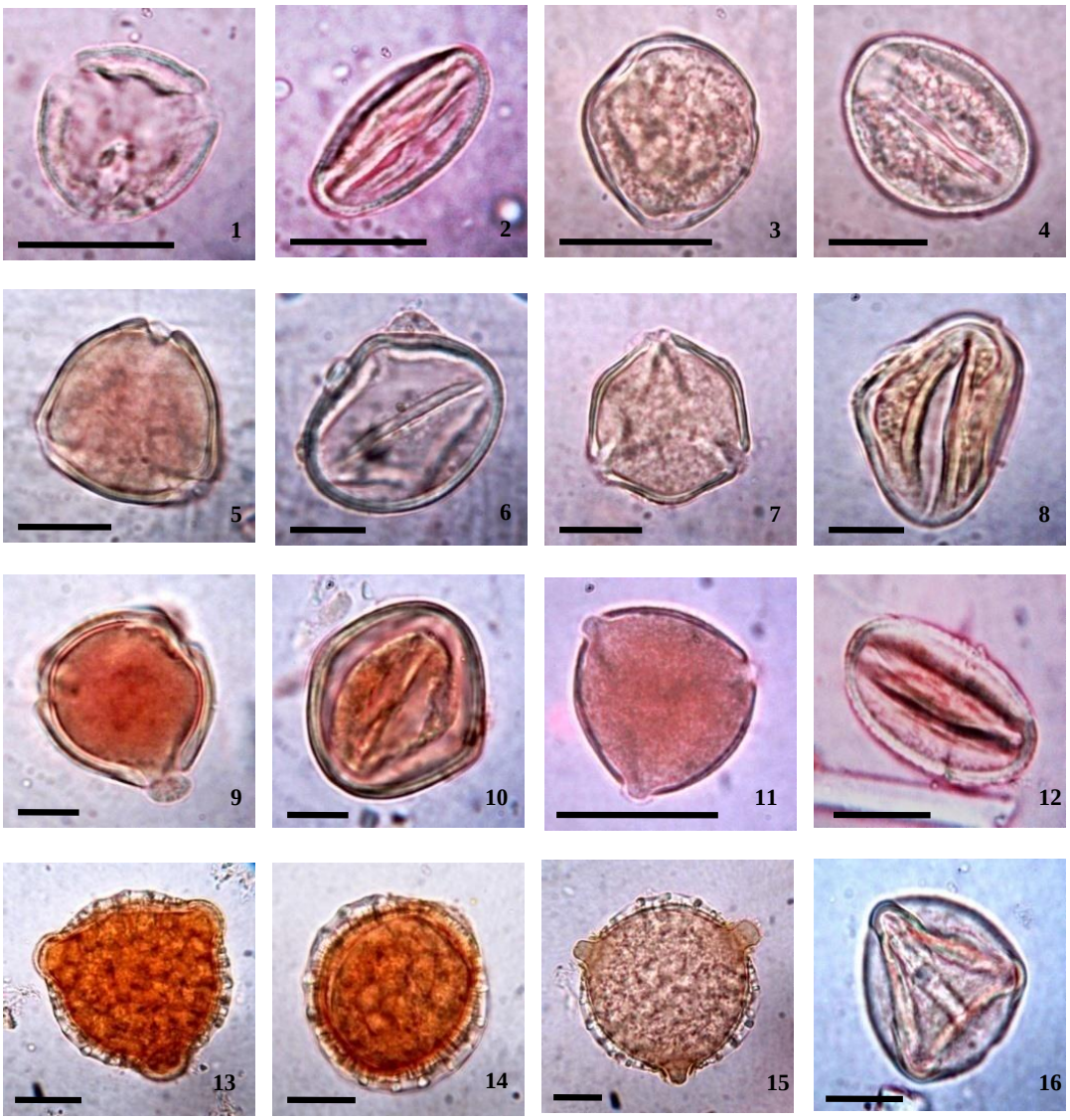

PLATE II. Pollen morphology of investigated taxa (Scale Bar -10µm.).

Fig: 1. Polar view of *Cassia kleinii*; 2. Equatorial view of *Cassia kleinii*; 3. Polar view of *Cassia laevigata*; 4. Equatorial view of *Cassia laevigata*; 5. Polar view of *Cassia occidentalis*; 6. Equatorial view of *Cassia occidentalis*; 7. Polar view of *Cassia siamea*; 8. Equatorial view of *Cassia siamea*; 9. Polar view of *Cassia sophera*; 10. Equatorial view of *Cassia sophera*; 11. Polar view of *Cassia tora*; 12. Equatorial view of *Cassia tora*; 13. Polar view of *Delonix regia*; 14. Equatorial view of *Delonix regia*; 15. Polar view of of *Peltophorum pterocarpum*; 16. Polar view of *Saraca asoca*;

Exine ornamentation of investigated pollen grains include psilate, verrulate, reticulate, reticulate-fossulate, regulate, clavate, striate (following Traverse 1988). Ornamentation like psilate and reticulate are common among the taxa studied (Table 2)

Apertures

Apertures are simple having colpus or pore only as well as compound consisting of an ectocolpus and an endocolpus, which make the pollen grains as colporate. The number of aperture are 3 in most of the taxa investigated. The colpus are long and narrow.

Apocolpium, mesocolpium and apocolpium index are some of the quantitative measurements of pollen grains, which sometimes help to distinguish closely related species. In the present investigation, apocolpium ranges from 2.60 µm.- 26.0 µm. among the investigated taxa. The mesocolpium varies from 7.80 µm.- 31.2 µm. among the investigated taxa. Apocolpium index ranges from 0.11 µm.- 0.80 µm. and may be used in distinguishing some taxa (Table 2).

Based on number, position and type of apertures of pollen grains, six pollen types can be recognized among the investigated taxa in order to summarize the pollen morphological features (Table 2). These pollen types include in the following sequence: trizonicolporate type, trisyncolporate type, penta-colpate type, atreme type, based on the numerical strength of taxa.

Conclusion

The morphological study of pollen of Subfamily Caesalpinioidae (Fabaceae) have revealed variation in pollen size, shape, aperture and exine ornamentation. The pollen observed were small, medium and large size, Prolate, Sub-oblate, Oblate-spheroidal, Spheroidal, in shape. In Caesalpinioideae tricolporate, trisyncolporate pollen grains are found More works need to be carried out to investigate the effects of climate change on pollen production and its morphology as these factors ultimately lead to success of pollination and more works need to get a successful result on pollen allergies. The results obtained indicate the need to continue palynological investigations on the Subfamily Caesalpinioidae of Fabaceae family.

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Survey and documentation of the Weed Flora in NBU Garden of Medicinal Plants

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Abstract

Weeds are the plants with generally undesirable properties. They spread rapidly and competitively. When it grows in garden it reduces air flow in garden, keeping plants wetter and more prone to pathogens. They are unattractive and can cause health problems such as Hay fever, skin rashes, etc. It also affects quality of product and income of grower. Traditionally, weed control in India has been largely dependent on manual weeding. Understand weed ecology, biology and using information technology should be part of developing and disseminating effective, economical and ecologically advantageous in India. Some weeds release nitrogen from root nodules into soil which automatically add fertilizer into the soil. A survey was conducted in NBU Garden of Medicinal Plants in West Bengal to identify most common and prevalent weeds associated with medicinal plants. A total of 86 different weed species belong to 25 families were identified of which 53 annual and 32 perennial. Among the most abundant weed species are *Axonopus compressus*, *Eleusine indica*, *Cyperus rotandus*, *Cyperus haspan*, *Kylinga brevifolia*, *Melastoma malabathricum*, *Osbeckia nepalensis*, *Nicotiana plumbaginifolia*, *Persicaria orientalis*.

***Keywords**:* Weeds, Soil erosion, Medicinal Plants, MPCA.

Introduction

A weed is a plant considered as undesirable in a particular situation, "a plant in the wrong places". Taxonomically, the term weed has no botanical significance, because a plant that is a weed in one context is not a weed when growing in a situation where it is in fact wanted and where one species of plant is a valuable crop plant, another species in the same genus might be a serious weed such as a wild bramble growing among cultivated loganberries. Weeds not only reduce yield by competing for available nutrients but harbor the pathogen which is harmful to the crops. They harbor rodents, insects, pests' disease and provide ideal conditions for their shelter and proliferation.

There is general agreement about the necessity to remove weeds from cultivated stands of Medicinal plants, and almost all technical papers providing indications for cultivation clearly state that Medicinal plant fields must be kept weed free as much, and as long, as possible. DE la Fuente et al. (2003) demonstrated

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that on biomass and seeds yield of coriander, especially under poor soil conditions, weeding had a greater effect than did fertilization. Furthermore, the absence of weeds from herbal products is mandatory in order to declare their high quality, irrespective whether they come from cultivation or wild collecting (FAH 2003). Yet, a surprisingly reduced number of works have been expressly addressed to the evaluation of the effects that weeds may exert on Medicinal Plants. Studies about this topic are scattered in the world literature, and very rarely the argument is treated in detail. Medicinal plants have the distinguishing property to be graded by the market according to their content in active components, i.e., the special metabolites that confer to them their medicinal properties. In many cases, these metabolites are synthesized by plants under environmental stress conditions; as competition with weeds is a special and often severe cause of stress, it should be argued that the best conditions for producing medicinal metabolites would be under weedy conditions.

The campus of NBU is quite rich itself with the record of over 700 plants. One *ex-situ* conservatory was established in the North Bengal University (NBU) campus in the year 1998. The garden was earlier named as "Garden of Medicinal Plants, NBU" and presently it is renamed as "*Centre for Aromatic and Medicinal Plant Garden*". The Garden has been developed with this basic floristic background and that is why a good number of medicinal and aromatic plants are growing here naturally. The garden of medicinal and aromatic plants housing several species of plants that are brought from various parts like MPCAs of West Bengal, Western Ghats, Eastern Ghats, central and Gangetic plains, North East India and various attitudinal ranges of entire Himalaya and successfully introduced time to time. It is spread over an area of 5 acre and is with well boundary. Different species of weeds which are growing in the garden along with the medicinal plant cause harmful effect to not only the garden plants but also to human health as well as cattle. Therefore, the present study was undertaken to investigate the distribution, severity and to understand the importance of the weed flora prevailing in the medicinal plant garden of University of North Bengal.

Materials and Methodology

Study area

The University of North Bengal was established by Act of the Legislature of West Bengal in 1962 and University Act was revised under West Bengal Act of XXV of 1981 and it came into force with effect from September 16, 1981 The campus occupies an area of about 330 acres 9 km outside Siliguri and Bagdogra Airport in the Terai region of Darjeeling district. The garden lies in 26°42′39″ N latitude and 88°21′18″ S longitude within Darjeeling District. A small river, Magurmari, is flowing through the campus and it divides campus in two halves. 10 small artificial ponds are also present in the Magurmari river valley. Another small river, Lachka on the Western border of the campus makes the main drainage system for the NBU campus. These two rivers are rain-fed and remain almost dry during the dry seasons. The NBU Campus has mixed deciduous type of forest, dominated by Sal, Litsea, Jarul, Sisso, Teak, Palash, Sirish etc. Sal Kunja is a largest and

natural left over forest patch inside the campus, apart from this several small patches of Rubber plantation, tea plantation and several more patches of plantation areas and/or social forestry makes the campus quiet green and clean. The central part of the campus area is covered with savanna type of grassland vegetation, dominated with two species of tall grasses, namely *Cymbopogon pendulus* and *Saccurum spontaneum*. The river valleys are covered with many species of grasses, ferns and other herbaceous flora.

Methodology

Regular surveys were carried out to determine the present status of the weed flora of the garden. Photographs of the plants in their vegetative and reproductive conditions were taken. The specimens were identified with the help of various literatures (Hooker 1872 - 1897; Prain, 1903; Hara, 1966; Ohashi, 1975; Hara et al., 1978, 1979, 1982; Grierson & Long, 1983, 1984, 1987, 1991, 1999, 2001; Noltie, 1994, 2000). For correct nomenclature and family delimitation reliable websites (www.theplantlist.org and www.ipni.org) were principally consulted. For RET (Rare, Endangered and Threatened) status elements Red Data Book for Indian Flora (Nayar and Sastry, 1987, 1990) and the IUCN red list (version 14; 2019) was followed.

Result and Discussion

A total of 86 different weed species representing 53 annuals and 32 perennials, comprising 8 grasses, 4 sedges and 4 broadleaved weeds were identified (Table 1). The annual species was greater in number than perennial species and overall annual grasses were more prevalent than perennial grasses due to lack of satisfactory control measure either cultural or herbicide application. The weed species represented 25 families from surveyed area. Among which Asteraceae family had the highest number of weed species (11), followed by Poaceae (8) Commelinaceae (6), Scrophulariaceae (6), Amaranthaceae (5), Euphorbiaceae (5), Polygonaceae (5), Acanthaceae (4), Cyperaceae (4), Solanaceae (4), Fabaceae (4), Lamiaceae (3), Lythraceae (2), Urticaceae (2), Onagraceae (2). Rests of the families were represented by one species each (Table 1).

Table 1. List of weeds of the NBU Garden of Medicinal Plants. [C= Common, R= Rare, VC= Very Common]

Species	Family	Flowering & fruiting	Distribution	Abundance	Uses
Hygrophila phlomiodes Nees	Acanthaceae	October	SW Asia	-	-
Hygrophila polysperma (Roxb.) T.	Acanthaceae	May - December	India	-	-

Anderson					
Hygrophila ringens (L.) R. Br. ex Spreng.	Acanthaceae	May - December	SW Asia	-	-
Phaulopsis imbricata (Forssk.) Sweet	Acanthaceae	November - March	Tropics	-	-
Alternanthera sessilis (L.) R.Br. ex DC.	Amaranthaceae	September - February	Asia	-	as vegetables
Alternanthera paronychioides A.St.-Hil.	Amaranthaceae	January - December	Native to tropical America	R	-
Amaranthus blitum subsp. *oleraceus* (L.) Costea	Amaranthaceae	June - December	Pantropical	VC	as vegetables
Amaranthus spinosus L.	Amaranthaceae	May - December	Pantropical	C	as vegetables
Amaranthus viridis L.	Amaranthaceae	April - June	Pantropical	VC	as vegetables
Centella asiatica (L.) Urb.	Apiaceae	April - August	-	C	taken locally to cure Dysentery
Acmella calva (DC.) R.K.Jansen	Asteraceae	May - December	SW Asia	C	-
Ageratum houstonianum Mill.	Asteraceae	Throughout the year	Naturalized in India	C	-
Ageratum conyzoides (L.)L.	Asteraceae	Throughout the year	Naturalized in India	C	-
Bidens pilosa L.	Asteraceae	Throughout the year	SW Asia	C	-

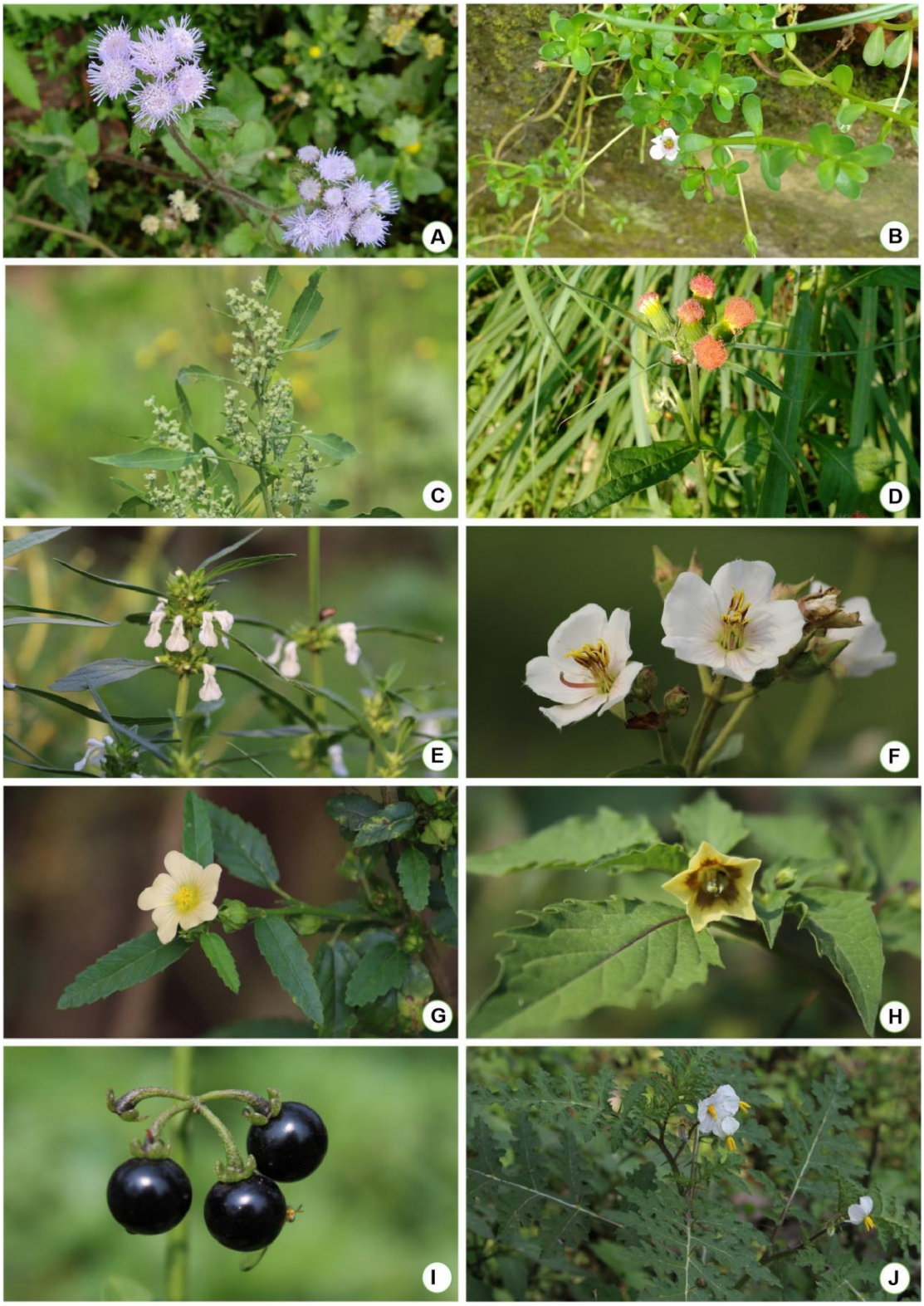
Crassocephalum crepidioides (Benth.) S.Moore	Asteraceae	July - December	SW Asia	C	-
Chromolaena odorata(L.) R.M.King & H.Rob.	Asteraceae	April - December	Naturalized in India	C	-
Emilia sonchifolia(L.) DC. ex DC.	Asteraceae	Throughout the year	SW Asia	C	-
Mikania micrantha Kunth	Asteraceae	Throughout the year	Naturalized in India	VC	-
Cyanthillium cinereum (L.) H.Rob.	Asteraceae	Throughout the year	SW Asia	C	-
Tridax procumbens L.	Asteraceae	November - March	Pantropical	C	-
Youngia japonica (L.) DC.	Asteraceae	April - October	SW Asia	C	-
Diplazium esculentum(Retz.) Sw.	Athyriaceae	July – February	Subtropics	C	as vegetables
*Cleome rutidosperma*DC.	Caparridaceae	July - February	Asia, Africa, America, Australia	-	-
Cleome viscosa L.	Caparridaceae	July - February	Asia, Africa, America, Australia	-	-
Chenopodium album L.	Chenopodiaceae	October - February	Cosmopolitan	-	as vegetables
Amischotolype hookeri(Hassk.) H.Hara	Commelinaceae	June - July	SW Asia	-	-
Commelina diffusa N. L. Burman	Commelinaceae	May - November	Tropics	C	-
Commelina suffruticosa	Commelinaceae	May - December	SW Asia	-	-

Blume					
Commelina benghalensis L.	Commelinaceae	May - December	Tropics	C	-
Cyanotis vaga(Lour.) Schult. & Schult.f.	Commelinaceae	July - October	SW Asia	-	-
Murdannia nudiflora (L.) Brenan	Commelinaceae	January - October	SW Asia	-	-
Bulbostylis densa (Wall.) Hand.-Mazz.	Cyperaceae	April - December	Tropics & sub-tropics	C	-
Cyperus rotundus L.	Cyperaceae	Throughout the year	Cosmopolitan	C	-
Cyperus haspan L.	Cyperaceae	June - September	Cosmopolitan	C	-
Kylinga brevifolia Rottb.	Cyperaceae	Throughout the year	Tropics	C	-
Croton bonplandianus Baill.	Euphorbiaceae	Throughout the year	Pantropical	VC	-
Euphorbia hirta L.	Euphorbiaceae	July - September	Pantropical	Sparce	-
Phyllanthus urinaria L.	Euphorbiaceae	April - November	Pantropical	C	-
*Phyllanthus fraterons*G.L.Webster	Euphorbiaceae	June - September	Pantropical	C	-
Ricinus communis L.	Euphorbiaceae	June - September	Pantropical	C	-
Sauropus quadrangularis (Willd.) Müll.Arg.	Euphorbiaceae	Throughout the year	Pantropical	R	-
Desmodium gangeticum (L.) DC.	Fabaceae	April - November	Asia	-	-
Desmodium trifolium (L.)	Fabaceae	Junuary - February	Asia	-	-

DC.					
Mimosa pudica L.	Fabaceae	March - November	Naturalized in India	-	-
Mimosa diplotricha Wright ex Sauvalle	Fabaceae	May - December	Native to tropical America	-	-
Anisomeles indica(L.) Kuntze	Lamiaceae	August - November	Asia	-	-
Hyptis brevipes Poit.	Lamiaceae	May - December	Tropics	-	-
Leucas zeylanica (L.) W.T.Aiton	Lamiaceae	August - February	SW Asia	-	-
Ammannia baccifera L.	Lythraceae	August - December	Asia, Africa	-	-
Rotala rotundifolia(Buch.-Ham. ex Roxb.) Koehne	Lythraceae	November - January	Asia	-	-
Sida acuta Burm.*f.*	Malvaceae	July - April	Asia	-	-
*Sida rhombifolia*L.	Malvaceae	July - April	Asia	-	-
Marsilea minuta L.	Marsileaceae	January - April	Asia, Africa	C	as vegetables
*Osbeckia nepalensis*Hook.f.	Melastomataceae	August - December	Asia	-	-
Melastoma malabathricum L.	Melastomataceae	Throughout the year	Tropics	-	-
Glinus oppositifolius(L.) Aug. DC.	Molluginaceae	March - July	Africa, Asia, Australia	C	-
Ludwigia octovalvis(Jacq.) P.H.Raven	Onagraceae	May - December	India	-	-

Ludwigia perennis L.	Onagraceae	July - November	Asia	-	-
Axonopus compressus (Sw.) P.Beauv.	Poaceae	August - December	Native to tropical America	VC	-
Chrysopogon aciculatus (Retz.) Trin.	Poaceae	June - October	Asia to Pacific	C	-
Cynodon dactylon (L.) Persoon	Poaceae	July - November	Tropics	VC	-
Dactyloctenium aegyptium (L.) Willd.	Poaceae	May - October	Tropics & sub-tropics	C	-
Eleusine indica (L.) Gaertn.	Poaceae	June - October	Tropics & sub-tropics	C	-
Imperata cylindrica (L.) P.Beauv.	Poaceae	April - August	Tropics	C	-
Panicum humile Steud.	Poaceae	August - December	Tropics & sub-tropics	C	-
Panicum repens L.	Poaceae	January - December	Tropics & sub-tropics	C	-
Persicaria hydropiper (L.) Spach	Polygonaceae	May - August	-	Sparce	-
Persicaria orientalis (L.) Assenov	Polygonaceae	July - September	Pantropical	C	-
Parsicaria tenella (Blume) Hara	Polygonaceae	May - September	Pantropical	C	-
Rumex dentatus L.	Polygonaceae	April - October	India	-	-
Rumex maritimus L.	Polygonaceae	April - October	India	-	-
Portulaca oleracea L.	Portulacaceae	January - December	Pantropical	C	-
Mitracarpus verticillatus(Schum. & Thorn.)	Rubiaceae	January - December	Tropics	C	-

Vatke					
Bacopa monnieri (L.) Pennell	Scrophulariaceae	August - November	SW Asia	-	-
Limnophila heterophylla (Roxb.) Buchanan & Hamilton	Scrophulariaceae	August - November	SW Asia	-	-
Torenia crustacea (L.) Cham. & Schltdl.	Scrophulariaceae	August - May	SW Asia	-	-
Bonnaya antipoda (L.) Druce	Scrophulariaceae	August - May	Tropics	-	-
Mazus pumilis (Burm.f.) Steenis	Scrophulariaceae	April - October	Asia	-	-
Scoparia dulsis L.	Scrophulariaceae	June - May	Asia	-	-
Nicotiana plumbaginifolia Viv.	Solanaceae	March - November	India	C	-
Physalis angulata L.	Solanaceae	April - January	SW Asia	-	-
Solanum nigrum L.	Solanaceae	November - March	SW Asia	-	-
Solanum sisymbrifolium Lam.	Solanaceae	January - December	Asia, Africa, America, Australia	-	-
Gonostegia triandra (Blume) Miq.	Urticaceae	January - September	Asia, Australia	C	-
Pouzolzia zeylanica (L.) Benn.	Urticaceae	September - April	Asia	C	-
Phyla nodiflora (L.) Greene	Verbenaceae	January - December	Pantropical	-	-



A. *Ageratum conyzoides* **B.** *Bacopa monnieri* **C.** *Chenopodium album* **D.** *Crassocephalum crepidioides* **E.** *Leucas indica* **F.** *Osbeckia nepalensis* **G.** *Sida acuta* **H.** *Physalis minima* **I.** *Solanum nigrum* **J.** *Solanum sisymbrifolium*

Conclusion

Weeds are unwanted to human controlled setting. While the weed is generally has a negative connection to the other plants. But most of the weeds are not dangerous, they gives economic and medicinal use also. Weeds are socially benefitted plants. They give beneficial properties and most of the collected species gives more medicinal used for curing diseases. Some beneficial aspects of weeds and are used as edible purpose such as their parts like leaves, roots, fruits, may be used for making medicine. Some weeds attract insects, which may protect other plants from harmful pests. Weeds may also act as 'living mulch' i.e. providing ground cover that reduces moisture loss and prevents erosion. Weeds may also improve soil fertility.

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Review on

Rice Germplasm: Source of Iron and Zinc for Nutritional Security

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Abstract

Rice is the most important food crop, more than half (½) of the world's population depends on it for their sustainable livelihood. Population growth is increasing day by day and it will reach more than > 9 billion by 2050, and to feed the overpopulation we need to produce nearly double amount of food grains to fulfil the demand. It was projected that Global rice yields and consumption rate will rise by 12% and 13% respectively by the year 2027 (FAO 2018). The Green Revolution has played a prime role in the 1960s -1970s to increase agricultural productivity worldwide to make many countries in food self-sufficiency leading to food secured world. The present situation is posing serious challenge for global food security in coming decades due to climate change, limited availability of arable land and water, more over other natural resources are continued to exhaustion. Rice is consumed as sole source of energy mainly in South and Southeast Asia, Africa, and Latin America which causes micronutrients deficiency leading to chronic malnutrition. Malnutrition due to inadequate intake of micronutrients mostly iron and zinc can lead to 'Hidden Hunger', which is responsible for many diseases. Important micronutrients Fe and Zn deficiencies in rice promoting the hidden hunger and causes anemia, stunted growth, poor cognitive development for iron deficiency and for zinc deficiency that causes reduced immunity, diarrhea, lesions on skin, mental lethargy. Approximately 2 billion people are suffering from malnutrition deficiencies for iron and zinc. Micronutrient elements Fe and Zn are available in various local rice varieties which ranged from 6.3-24.4 mg/kg Fe and 13.53- 58.4 mg/kg Zn. Biofortification of rice can assist to alleviate malnutrition associated diseases among the poor people those who are depended on rice as staple food for 40-70% daily caloric intake. Nutritional studies recommended that 24–28 mg/ kg Zn and 13 mg/ kg Fe concentration in polished grain is vital to attain the 30% of human estimated average requirement. Biofortification of cereal foods through conventional breeding can be a good opportunity to improve micronutrient deficiency in the diets. Wild rice accessions (*Oryza rufipogon, O.nivara, O. latifolia* and *O. officinalis*) may be used to improve the mineral nutrition in rice grain through breeding and conserve as important resources.

Key-Words: Rice germplasm, Nutritional security, iron zinc content, wild rice.

Introduction

Rice (*Oryza sativa* L.) is the most important staple food for livelihood of half of the world's population. Global rice production was 782 million tons (paddy rice) produced from an area of 167 million produced 172 Mt rice (paddy rice) from 44.5 Mh of cultivated land. Yield rate and total production must be increased to meet the future demand due to population explosion. India is the second largest producer of rice after China with a production of 112.76 million tons in kharih 2017-2018(USDA report 2019). Recently it has been reported that (irristat-2018), cultivated area of rice over 161 million

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hectares globally and produced 488.3 million tons milled rice in 2018 (Dixit et al 2019). Approximately 8–10 million tons rice is to be added more each year to meet future demands for sustainable global food and nutritional security (Trijatmiko et al. 2016; Tripathy et al. 2017). It is estimated that more than 820 million people in the world are in starvation and under nourishment which resulted in poor health and disease susceptible (FAO 2019). The United Nations Organization has fixed target to achieve the Zero Hunger by 2030 under SDGs agenda. Rice (*Oryza sativa* L.) is a most important staple food crop and more than half of the World population are dependent on it (Wang and Li 2005). Based on genetic analysis rice (*O. sativa*) is classified into two subspecies, japonica and indica (Kato 1928), but belongs to five groups such as indica, aus, aromatic, temperate japonica and tropical japonica (Garris et al. 2005). Cultivated rice was domesticated around 10,000 years ago from common Asian wild rice O. rufipogon and O. nivara (Kovach et al. 2007; Chen et al. 2019). Study showed that two subspecies japonica and indica have undergone considerable amount of phenotypic changes compared to the wild rice progenitor *O. rufipogon* and *O. nivara*. The *O. rufipogon* is considered as proto-japonica and *O. nivara* as proto-indica (Fuller et al. 2010).

It was estimated that at least two billion people are micronutrient deficient (Trijatmiko et al. 2016; Tripathy et al. 2017). Micronutrients mainly zinc (Zn) and iron (Fe) is essential element for the normal growth and development. Iron is necessary for the synthesis of oxygen-transporting proteins like hemoglobin and myoglobin and maintenance of immune functions (Stoltzfus 2001; Bollinedi et al. 2020). Iron deficiency in humans causes many diseases like anemia, premature births, impaired cognitive and motor normal growth and improvement. Other mineral element zinc is necessary for biological functions such as cell division, reproduction and immunity maintenance (Brown et al. 2004) and deficiency causes immune system dysfunction, anorexia, delayed wound healing, cognitive disorder, hypogonadism (Salgueiro et al. 2000; Bollinedi et al. 2020).

Rice Germplasm for Zinc and Iron Content

Different rice germplasm were tested and quantified for iron and zinc content in the polished rice, about 2-8 μg/g iron (Fe) and 16 μg/g zinc (Zn) was detected in rice (Gregorio et al. 2000; Graham 2003). Initiative to augment iron (Fe) in the rice grain through conventional breeding is inhibited by the limited natural variation of iron in diverse rice germplasm. But recommended dose for human intake of Fe and Zn is 15 mg per day (Calayugan et al. 2020). Therefore, breeding program has been initiated by Harvestplus to biofortify rice at the rate 13 μg/g Fe and 28 μg/g Zn to meet approximately 30% of the estimated average requirement (EAR) for mineral nutrients. Rice germplasm are the good source of iron and zinc and these resources can be utilized for the improvement of mineral content in the rice grain through breeding (Figure 1).

Inadequate intake of micronutrients mostly iron and zinc can lead to 'Hidden Hunger', which is responsible for many diseases affecting about 2 billion people worldwide. Iron (Fe) deficiency anemia (IDA) may hamper physical development, and reduce immunity mainly in the women and children (Ludwig and Slamet-Loedin 2019). Harvest Plus program has estimated minimum Fe 13 μg/g in polished rice to fulfil the 30% estimated average requirement (EAR) (Bouis et al. 2011). Therefore, biofortification for iron and zinc element (micronutrients) is needed to

combat the mineral deficiency related human diseases. Bioavailability of micronutrients (Fe and Zn) in the edible parts of staple food rice can be enhanced by many ways- conventional breeding program, genetic engineering technology and agronomic practices (Bouis and Saltzman 2017). Biofortification of rice can assist to alleviate malnutrition associated diseases among the poor people those who are depended on rice as staple food for 40-70% daily caloric intake (Bouis and Saltzman 2017). Zinc deficiency is the most important factor for illness and diseases in developing countries according to the report of World Health Organization causes diarrhea and respiratory diseases, impaired immune response, leading to 400,000 deaths annually across the world. The zinc content in brown rice of the different germplasm of India including landraces, breeding lines, ranged from 7.3 to 52.7 mg/kg (Rao et al. 2020) mean value ranged from 15.9 to 27.3 mg/kg. In some cases it has reported that grain zinc content ranges 13.5 to 58.4 mg/kg. The zinc content in polished rice ranged from 4.8 to 40.9 mg/kg in rice germplasm of India. The zinc content in the Farmers' rice varieties of India ranged from 5 - 25 mg/kg in brown rice (Babu et al. 2014; Anandan et al. 2011), in polished rice zinc content varies 12-14 mg/kg in popular varieties of India. Other report showed that zinc content in polished rice ranged from 4.8 - 40.9 mg/kg (Rao et al. 2020). Harvest Plus program has estimated the threshold value of zinc content in polished rice is 28 mg/kg. It was also observed that overall mean percentage of loss of zinc content is around 19.0% during polishing, which means 1.9 mg/kg loss of zinc and 10 mg/kg of brown rice at the time of polishing. Zinc percentage loss ranged from 5 to 30% in different germplasm during polishing, the variation may be due to the presence of diverse magnitude of thickness in the aleurone layer which also depends on rice varieties' genetic makeup (genotypes) (Gregorio et al. 2000; Sellappan et al. 2009). They showed wide range of iron from 6.9 to 22.3 mg/kg, and zinc ranged from 14.5 to 35.3 mg/kg in brown rice (Maganti et al. 2019). Loss of iron during polishing is more (16 to 97%) compared to zinc ranged from 1 to 45 % (Maganti et al. 2019). Rice germplasm can be considered as promising donors of zinc if they content ≥35 mg/kg in brown rice (Rao et al. 2020). Nutritive value mainly zinc and iron cab be improved through technological advancement in polished rice to alleviate malnutrition related to micronutrients deficiencies because per-capita consumption ranges from 62kg/year to 190 kg/year in rice consuming Asian and African countries (Dixit et al 2019), suffer from chronic micronutrient malnutrition even called as 'hidden hunger' (Muthayya et al. 2013). Fe and Zn deficiencies are in principal widespread micronutrient deficiencies in humans, affecting two billion people and 0.8 million deaths occurs annually due to malnutrition (WHO 2011). World food production was increased in 1996s significantly through the application of advancement of science and technology in agriculture leading to make many countries food self-sufficiency and globally referred to as 'Green Revolution' (Ortiz 2011) which also helped to prevent occurrence of frequent famines and reduced socio-economic disturbances (Khush 1999) in the developing countries. Green Revolution produced at least 20% more food grains in the countries of Asia and Latin America and kept food prices at least 19 % less. Although it is significantly

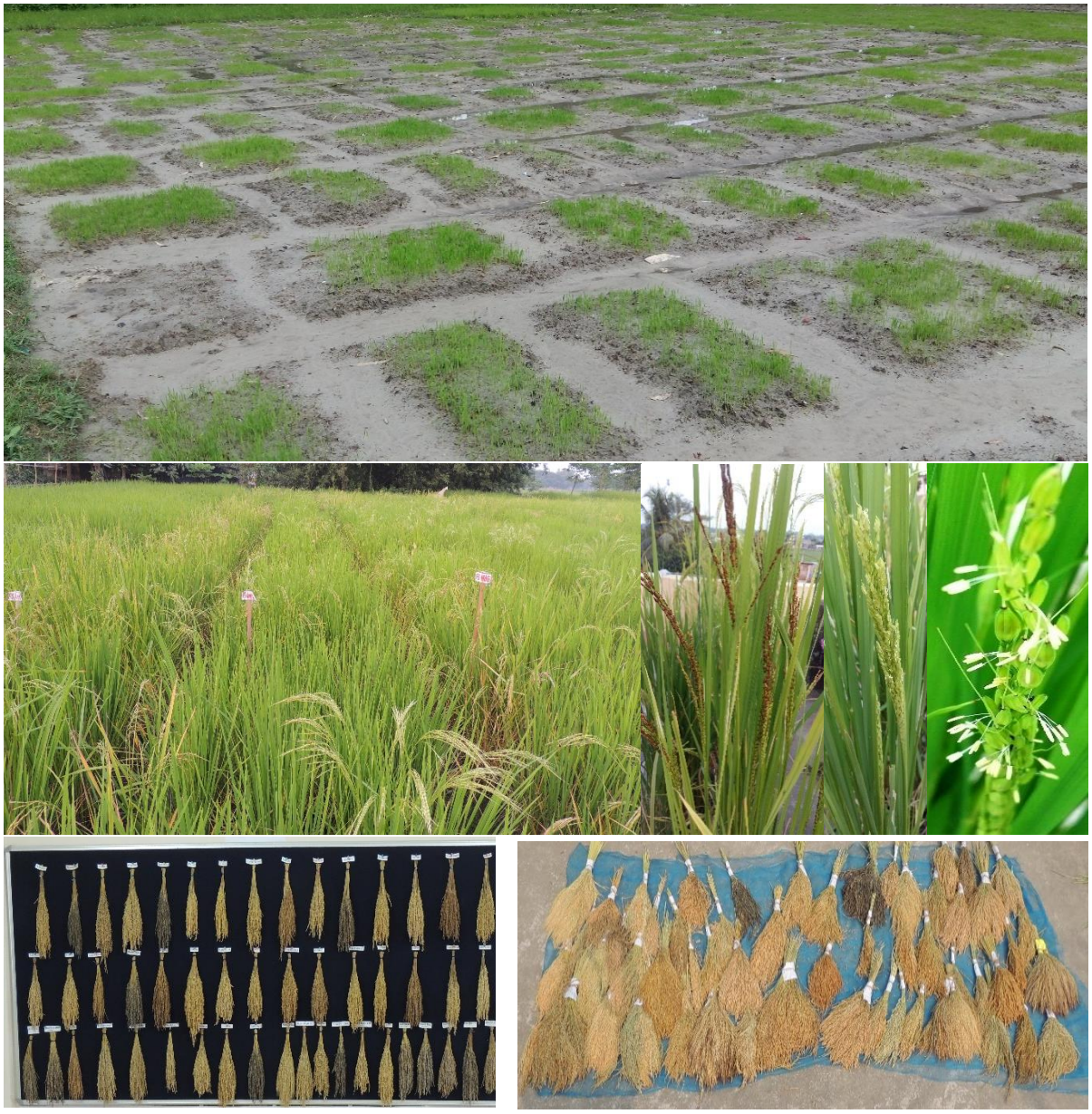

Figure 1. Local rice varieties are conserved through *in situ* on-farm conditions (more than 65 rice varieties conserved) and evaluated for the quantification of minerals like zinc and iron.

reduced the proportion of under nourished people worldwide, still the problem of malnutrition existed due to lack of quality grain (micronutrients) which is also associated with the socio-economic saddle (Pingali 2012) of the poor people. At least 2 billion people affected due to micronutrient deficiencies in staple food (called as hidden hunger) mainly in South Asia, Latin America and sub-Saharan Africa (FAO 2015), and urgently demand for solution of this chronic problem of hidden hunger. Biofortification of cereal foods through conventional breeding can be a good opportunity to improve micronutrient deficiency in the diets (Bouis and Salzman 2017). Successful breeding program depends on some preconditions such as availability of genetic variation within the gene pool of specific traits. Breeders generally target the additive genetic effects, transgressive segregation pattern, or heterosis principles of the target traits for their improvement in

the breeding lines; it is possible if numerous genetic variations exist in the germplasm of the crop (Garcia-Oliveira et al. 2018). In rice, polishing of grain removes up to 50% iron from the raw brown rice (Gregorio et al. 2000). Micronutrient zinc (Zn) concentration is two-fold higher in *indica* rice type compared to japonica rice type, but contains less amount of iron (Fe) (Yang et al. 1998). Modern rice varieties are with less amount of Fe and Zn compared to farmers' varieties and landraces (Anandan et al. 2011) because breeders have not given prime importance to the enrichment of micronutrients, instead yield and other traits.

Zinc and iron availability in the soil

Level of these nutrients in cereal grains depends on the availability of these nutrients elements in the soil conditions and other environmental factors, infertile soils and obviously the germplasm types (Velu et al. 2014). Wild rice accessions may be used to improve the mineral nutrition in rice grain through breeding and conserve as important resources (*Oryza rufipogon, O.nivara,O. latifolia* and *O. officinalis*) (Anuradha et al. 2012).

Detection methods of mineral elements zinc and iron

Screening and evaluation of micronutrients contents in the available germplasm is necessary for the possibility of introgression of favorable gene through recombination in breeding lines for high zinc and iron content without compromising yield and grain quality. Biofortified rice varieties with high zinc and high iron can help to achieve nutritional security in the country. The ICP-AES technique is used to quantify mineral elements zinc and iron content in rice grain. Fully quantitative methods are employed in the detection of microelements in the rice grains such as inductively coupled plasma-optical emission spectrometry (ICP-OES), ICP-mass spectrometry, the energy-dispersive X-ray fluorescence spectrophotometry (Pfeiffer and McClafferty 2007).

Genetic Technology for Biofortification of Iron and Zinc in Rice

Transgenic rice overexpressing the nicotianamine synthase genes of rice (OsNAS1) and barley nicotianamine aminotransferase gene (HvNAAT) can increase the accumulation of Fe in the endosperm (up to 55 μg/g) (Diaz-Benito et al. 2018). Overexpression (OE) of SoyFerH1 gene has also increased the Fe (up to 38 μg/g) in the rice endosperm (Swarna, BR29, IR64) using endosperm specific promoters such as OsGluB1, OsGtbl, OsG1b) (Slamet-Loedin et al. 2015). Overexpression of the the rice ferritin gene (OsFer2) increased the Fe concentrations in T3 rice grain (up to ∼15.9 μg/g) compared to the control cultivar Pusa-Sugandh II with approximately 7 μg/g (Paul et al 2012). Iron uptake and translocation can be increased by OE the OsYSL15, which is accountable for the uptake of Fe (III)–DMA (Lee et al. 2009a) and OsYSL2 for the uptake of Fe (II)–NA from the rhizosphere (Ishimura et al. 2010) resulting in higher Fe content up to 7.5 μg/g in polished rice compared to control cultivar (∼1.8 μg/g) (Senoura et al. 2017). YS1 (Yellow Stripe) gene functions as a proton-coupled symporter for phytosiderophore (PS)-chelated metals which has high affinity Fe3+-MA transporter. Better results were attained in the OsNAS2 plants with a Fe level-up to 19 μg/g in polished rice (Johnson et al. 2011) compared to wild type (4.5 μg/g).

Multigene cassette can be inserted in to the plant genome for the overexpression of the genes for desired enhancement of minerals in the grain endosperm. Storage gene PvFER, chelator gene AtNAS1, and iron storage gene AtNRAMP3 has been expressed in rice resulting in enriched grain with 13.65 μg/g iron in greenhouse condition (Wu et al. 2019). Other result showed little higher amount of Fe 15 μg/g concentration in polished grain (Trijatmiko et al. 2016).

Agronomic Biofortification

Agronomic biofortification can be opted to enrich iron in the rice grain through soil agronomic practices using fertilizer-based procedure that is easy and cost effective (Cakmak and Kutman 2017; (Ludwing and Slamet-Loedin 2019), because rice mainly cultivated in lowland irrigated areas where Fe is accessible in high amount. It can be an alternative of genetic biofortification methods. Main obstacle is that the translocation of the mineral from the vegetative part to the grain (Slamet-Loedin et al 2015). The Zn fertilizers may be used in the rice fields deficient in available Zn levels to enrich grain with high Zn content (Johnson-Beebout et al. 2009). Additionally a combination of genetic and agronomic approaches can be required to enhance grain Zn concentration. Prevalence of zinc deficiency throughout the world has been assessed to be ~20% in soils (Hotz and Brown 2004).

Conventional Breeding as an Effective Tool for Zn Biofortification

During Green revolution 1960s developed HYV rice varsities but grains of HYVs contain lesser amounts of nutrients; and polishing further diminishes the nutrients concentration mainly iron and zinc (Rao et al. 2020). Biofortification has been initiated by CGIAR through the launching of Harvest Plus Challenge Program in 2003 (Harvest Plus 2003) to enrich rice grain with iron and zinc using breeding system. The achievement of biofortification depends on the existence of diversity for the desired trait available in the germplasm, to be used in breeding program for successful recombination of the trait (iron and zinc content) with yield stability. To phenotype zinc content in the rice grain X-ray fluorescence spectroscopy (XRF) or ICP-AES can be used. Conventional breeding principles have been used to develop one improved breeding line with enriched iron in brown rice (21 mg/kg) by crossing IR72 (HYV) with traditional variety (ZawaBonday) (Gregorio et al. 2000). Protein content in milled rice is approximately 7% w/w (8.5% in unpolished brown rice) which is low compare to other cereal foods (wheat, barley and millets). Major protein is glutelin about 60-80% and prolamine consisting only 20-30% of the total amount (Xu and Messing 2009). Rice supplies around 40% of the total protein consumed by peoples in developing countries and protein quality is high, because the protein has essential amino acid lysine richness (3.8%) (Shobharani et al. 2006). Anti-nutritional factor as like as phytic acid make complex structure with seed proteins and essential minerals mainly Zn, Fe, and Ca, ultimately reduced the bioavailability of these micronutrients. Nutritional studies recommended that 24–28 mg/ kg Zn and 13 mg/ kg Fe concentration in polished grain is vital to attain the 30% of human estimated average requirement (Bouis et al. 2011).

Micronutrients uptake depends on the plant age, tissue specific demand and root system but overall mechanism accountable on genotype constitution of the rice varieties (Fageria 2013).

Environmental factors are also responsible for Zn accumulation in rice grains such as Zn availability in the soil, temperature and atmospheric carbon dioxide level (Welch and Graham 2002; Fernando et al. 2014b). During Green Revolution prime focus was to develop high yielding varieties to increase yield without considering quality (Graham and Welch 1996). At present, emphasis has been given to develop high grain quality rice varieties specially zinc and iron to alleviate hidden hunger related diseases (Kant et al. 2012; Myers et al. 2014). Germplasm screening is the most excellent choice before begin the genetic approach (breeding program) to enrich Zn concentration in the grain. Through which we will know the availability of unique genetic variation existed in the gene pool of local landraces, traditional varieties and even in wild species to accomplish breeding targets of high zinc in rice endosperm. Enormous germplasm collection of IRRI has been analyzed for Zn concentration in brown rice, which showed considerable genetic variation in zinc content (13.5–58.4 mg/Kg), with an average of 25.4 mg/Kg (Boonchuay et al. 2013). Range of zinc varies from 7.3 to 52.7 mg/kg in some Indian rice germplasm (Rao et al. 2020). BRRI dhan-62 has been developed and released by Bangladesh (2013) containing zinc concentration 20-22 mg/kg in brown rice; it is the world's first rice variety with enriched Zn. Harvest Plus program has suggested 30 mg/kg Zn concentration in brown rice, BRRI dhan-62 contains Zn amount is below the target value (Shahzad et al. 2014). The Zn biofortification can be enhanced by using the under-utilized genetic resources of rice germplasm (genetic factors) including environmental aspects, and field management policies (agronomic factors). Therefore, it is to understand completely that what roles played by these factors in Zn accumulation in the rice endosperm. These factors may be the vital bottleneck for Zn absorption and remobilization in the different plant tissues and ultimately to grain leading to biofortification of Zn by enriching concentration (Nakandalage et al 2016).

The amount of micronutrients (zinc and iron) in rice grain is a major factor for determining its nutritional value (Anuradha et al. 2012). Rice grain (brown) is consisting of endosperm (90 % w/w), bran (6-7 % w/w) and embryo (2-3 % w/w) (Chen et al. 1998). Bran layer is the key storehouse for dietary fiber, proteins, vitamins, minerals, and lipids compared to the central endosperm layer (Shahzad et al. 2014). Rice grain comprises of 80% starch, 7.5% protein, 0.5% ash, and 12% water. On average 300 g rice is ingested per day by adult people of China and India and about 62-190 kg annual consumption (Lu et al. 2008). The daily Zn requirement is 15 mg for adult (Lu et al. 2008; Lu et al. 2013) and children above 4 years old.

The Zn concentration is 3 times more in the bran layer than that of hulls and endosperm (Lu et al. 2013). Polished rice delivers only one fifth amounts of daily Zn requirements, because during polishing bran layer is removed which depletes zinc from rice grain leading to zinc deficient rice grain (Sharma et al. 2013). Therefore, Zn deficiency is a global health problem specifically in Asia and Africa where rice has take as staple diets (Impa and Johnson-Beebout 2012; Myers et al. 2015; 2015). More than 2 billion people in Asia and 400 million in Sub-Saharan Africa are at risk of Zn deficiency associated diseases.

It is important to increase the Zn concentration in the rice endosperm to combat the zinc deficiency related

diseases. That can be accomplished by understanding the genetic mechanism of Zn uptake by root system, its transport and remobilization in the different plant tissues and interactions to the environmental factors to raise Zn concentration in rice endosperm.

Conclusion

New improved breeding lines can be developed using the promising local germplasm (>35 mg/kg Zn) as donor through recombination breeding and selection to fulfil the goal of HarvestPlus to alleviate zinc deficiency related diseases. It has been observed that approximately > 70% of micronutrients is removed during polishing of rice grain. External application of zinc in the soil during cultivation can be a crucial to maintain the nutrient performance potential of a biofortified rice variety. Recommended daily allowance (RDA) of zinc is 12 mg/kg for male and 10 mg/kg for female. Biofortified rice intake with enriched zinc content can meet 38 to 47% of the RDA for male and 46 to 57% of the RDA for female to keep healthy us. In order to meet the RDA value of zinc, as sole source of diet, it must have 54.5 to 68.2 mg/kg zinc in polished rice. Available rice germplasm does not content such amount of zinc in the grain. Advanced transgenic technology has been utilized to enrich rice grain with high quantity of zinc 34.9 to 55.5 mg/kg by inserting soybean ferritin gene. Transgenic IR64 rice lines with nicotianamine synthase (OsNAS2) and soybean ferritin (SferH-1) genes inserted has increased zinc content to 45.7 mg/kg in polished rice without changing any other important traits such as yield and quality.

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Copper toxicity in plants: a review and a case study on tea

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Abstract

Copper in trace amounts is essential for various metabolic processes in the plant such as photosynthesis, carbohydrate distribution, and protein metabolism but at high concentration it causes physiological stress through generation of free radicals that induce the production of reactive oxygen species (ROS) via Haber-Weiss and Fenton reactions. Copper-induced generation of hydrogen peroxide, hydroxyl radicals, or other reactive oxygen species has been directly correlated with the damage to protein and lipids that may lead to reduced growth and even death. Tea (*Camellia sinensis* L. (O.) Kuntze) is an economically important plantation crop in India with round the year productivity. Copper based fungicides are cheap and effective in controlling fungal diseases and are used consistently throughout the year to combat different fungal diseases that pose a major threat to tea production. Excess Cu^{2+} has been found to alter several physiochemical parameters in the tea plants. A more detailed study on mechanisms of Cu^{2+} toxicity at the gene level is warranted.

Key words: Copper, stress, tea, reactive oxygen species, antioxidative enzymes.

Introduction

The role of copper in plants depends greatly on its concentration. Copper in trace amounts is an essential micronutrient for algae and higher plants for its role as a cofactor for metabolic processes like photosynthesis, respiration, carbohydrate distribution, nitrogen fixation, protein metabolism, ethylene perception, oxidative stress reduction, cell expansion and cell-wall lignification. At higher concentrations, copper can induce several negative effects including generation of reactive oxygen species, exchange of essential metal ions from the active sites and visible symptoms such as chlorosis, necrosis and growth inhibition (Marschner 1995; Prasad, 2004; Rehman et al. 2019). A well coordinated procedure of uptake, buffering, translocation and storage processes is necessary to uphold essential concentrations of the metal in various tissues and compartments within the narrow physiological limits (Clemens et al. 2002). Copper is transported into the plant cell by COPT family of transporters on the plasma membrane which has been described as a group of highly hydrophobic proteins; all its members contain 3 trans-membrane domains and specific Cu^{2+} binding site rich in methionine and histidine residues at the amino terminus (Kampfenkel et al. 1995; Sancenon et al. 2003; Andres-Colas et al. 2006). Copper homeostasis is maintained inside the cell by copper chaperones which sequester copper to a non-reactive form and also interact with other transport proteins for delivering copper to its necessary destinations (Himelblau and Amasino 2000; Company and Gonzalez-Bosch 2003; Chu et al. 2005). Two P-type ATPases, PAA1 and PAA2, are required for efficient copper delivery across the plastid envelope and the thylakoid membrane, respectively, in *Arabidopsis*

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(Shikanai et al. 2003; Abdel-Ghany et al. 2005). Inside the root, Cu^{2+} is said to be strongly accumulated in the cortex and the concentration decreases sharply from the outer to the inner cell layers (Adruini et al. 1996; Ducic and Polle 2005). Copper is poorly translocated by xylem and thus uptake by shoots is very low (Liao et al. 2000).

The aim of this review is to summarize the toxic effects of Cu^{2+} and focus on the recent developments on the various underlying metabolic changes that bring about such toxic effects. We also focus on tea, which is the most popular drink in the world after water. Tea (*Camella sinensis* L. O. Kuntze) is a perennial evergreen plantation crop with productivity round the year. The harvest includes tender shoots that are plucked normally at one to three weeks interval. This induces further vegetative growth and ensures continuous supply of green flushes (Burgess and Carr 1997; Karmakar and Banerjee 2005). Fungal pathogens such as *Exobasidium vexans* are capable of infecting the pluckable tender leaves thereby warranting a regular spraying of copper fungicides in heavy doses especially during the six month long monsoon period (May-October) when fungal infections assume massive proportions. This causes a buildup of Cu^{2+} in the soil over the years and the concentration of Cu^{2+} can easily overcome the threshold limit for toxicity.

Copper in plants

One of the major sites of copper accumulation in plants is the chloroplast. This metal is directly involved as a component of plastocyanin (PC) in the photosynthetic electron transport chain. PC is one of the most abundant proteins of thylakoid lumen (Kieselbach et al. 1998) and is essential for electron transfer between the cytochrome b6f complex and photosystem 1 (Weigel et al. 2003). The metal has a distinct regulatory role in electron transport between the photosystems as the constituent of PC (Maksymiec 1997). In the chloroplast stroma, Cu/Zn superoxide dismutase (SOD) requires Cu^{2+}, along with Zn, as cofactors to catalyze the dismutation of superoxide radicals (O_2^-) threby forming H_2O_2 and O_2^-. In *Arabidopsis thaliana*, out of seven identified SOD genes, the most active CSD1 and CSD2 genes both encode a Cu/Zn SOD with CSD1 activity in the cytosol and CSD2 activity in the stroma (Kliebenstein et al. 1998). Polyphenol oxidase is another Cu^{2+} protein found in the thylakoids of some plants, such as spinach (Kieselbach et al. 1998), but not in other species such as *A. thaliana* (Schubert et al. 2002). The enzyme has been proposed to be involved in the photoreduction of O_2^- by PS1 (Vaughn et al. 1988). Cu^{2+} mediates the activity of several other enzymes such as ascorbate oxidase which catalyses the reduction of O_2^- to water. The enzyme contains 8 Cu^{2+} ions which participate in the transfer of electrons in presence of ascorbate, the reducing substrate (Maksymiec 1997). Other important Cu containing proteins within plant cells include the mitochondrial cytochrome-C oxidase enzyme, the ethylene receptors in the endomembrane system and various apoplastic oxidases (Cohu and Pilon 2007). Copper is also necessary for amine oxidase function where it catalyses oxidative deamination of polyamines with the simultaneous formation of aldehyde, ammonia and H_2O_2 (Maksymiec 1997).

Copper as a toxic element

Inspite of the indispensability of copper in plant metabolism, excess copper has strong toxic effects. Copper can be limiting to plant productivity in crops when below 5 μg g^{-1} dry weight (DW),

whereas toxicity is reported above 30 μg g^{-1}DW (Marschner 1995). The most common feature of copper toxicity is the decrease in mass of roots. Copper toxicity can be damaging to plant roots, with symptoms ranging from disruption of the root cuticle and reduced root hair proliferation, to severe deformation of root structure (Sheldon and Menzies 2005; Lequex et al. 2010, Rehman et al. 2019). Cu^{2+} is toxic to plant cell which lead to plant retardation and leaf chlorosis (Rhoads et al. 1989; Yadav 2010). High Cu^{2+} concentrations predisposes photosystem II to photoinhibition (Patsikka et al. 2002), causes reduction in chlorophyll content arising from partial destruction of grana and modification of the protein-lipid composition of thylakoid membranes (Lidon ane Henriques 1991; Maksymiec 1997). Copper toxicity can also results in significant alteration in the concentration of minerals such as Fe, Mg, Ca, Zn, K and Na in both root and shoot (Lidon and Henriques 1993; Lequex et al. 2010).

Copper is relatively abundant in the earth's crust and better soluble, therefore more mobile than other heavy metals in the surface environment (Flemming and Trevors 1989). Copper concentration in non-polluted soils range from 10 to 80 ppm Cu^{2+} but soils located near mining areas or metal-processing industries may be contaminated by very large amounts of Cu^{2+} (Hagemeyer 2004). The bioavailability is determined by the form taken by the metal (ionic, complex or precipitated) which depends on environmental factors and therefore, varies widely, giving rise to possible conditions of toxicity (Flemming and Trevors 1989 Greger 2004). The level of bioavailable copper is increased by human activities which either increases the abundance or causes changes in soil chemistry thus affecting the solubility (Rhoads et al. 1989; Flemming and Trevors 1989). In the soil, copper remains immobilized onto the organic materials such as fulvic and humic acids and to clay and mineral surfaces. The bioavailibility in soil is strongly dependent on factors such as pH, cation exchange capacity (CEC), clay content, water hardness and organic matter content (Flemming and Trevors 1989; Greger 2004; Rooney et al. 2006, Rehman et al. 2019). Low pH increases the metal availability since the hydrogen ion has a higher affinity for negative charges on the colloids, thus competing with the metal ions of these sites, therefore releasing metals (Greger 2004). Rhoads et al. (1989) found that growth of tomato plants was reduced at soil pH below 6.5 with soil-copper levels above 150 mg. Thus soil properties have a significant impact in the expression of toxicity of copper in plants.

Agricultural soil in many parts of the world is contaminated by heavy metals (Brun et al.2001; Ballabio et al. 2018). The use of Bordeaux mixture for almost one century against vine downy mildew has caused severe copper contamination of soil in many wine-producing regions (Van-Zwieten et al. 2004). Copper contamination also caused serious problems in cereals such as rice (Lidon and Henriquesa 1993), wheat (Lanaras et al. 1993) and barley (Vassilev et al. 2003). Graham et al. (1986) found that excess fungicidal copper reduced seedling growth in citrus and also inhibited colonization of the roots by mycorrhizal fungus. In citrus orchards, stunted tress were produced with less mycorrhizal colonization under higher Cu concentrations and low pH (<5) conditions of the soil. In India, the major tea cultivation area comprises the eastern sub-Himalayan region where the soil is mainly acidic in nature (pH 4.2-5.8) (Singh and Singh 2006). While this is good for tea cultivation (Sarkar 1994), but it increases the possibility of Cu^{2+} ions

accumulated in the tea garden soils to become more available for absorption by plants which may lead to toxicity.

Copper in tea gardens

An example of an industry in India which depends primarily on copper fungicides is the tea industry. India is second only to China in tea production and the largest consumer of tea in the world. Currently, India produces 23% of total world production. It is the second largest industry in terms of employment and generally drives the economies of the regions where the tea gardens are concentrated, for example Assam and sub-Himalayan West Bengal (Selvakumar and Jeyaselvam 2012). Tea plants are cultivated extensively as large plantations where it is often allowed to grow under variant soil and climatic condition thereby making them prone to attacks by fungal pathogens. Major diseases include blister blight, brown blight, grey blight and black rot in leaves, and branch canker, thorny blight and pink disease in stems. To control the diseases, copper-based fungicides are used excessively in tea gardens of North East India including Assam and sub-Himalayan West Bengal (Barua 1988). The fungicides that are used most commonly include basic copper sulphate, Bordeaux mixture (a combination of hydrated lime and copper sulphate), Bicoxy (a new formulation of copper oxychloride 50% WP) and various customized formulations of copper sulphate and copper oxychloride (Worthing 1983; Singh 2005). A survey covering several tea gardens of the Darjeeling and adjoining Jalpaiguri district of sub-Himalayan West Bengal conducted by the authors has revealed that copper-fungicides are extensively used in the tea gardens of the Dooars and Terai region and also in the hilly regions of West Bengal. Copper based fungicides are used in large scale because they have multisite activity with a low risk of pathogens developing resistance (Van-Zwieten et al. 2004) and are relatively less phytotoxic than Ni based fungicides. In fact, copper based fungicides are highly recommended in literature and are often regarded as the most efficacious and economic fungicide for controlling the foliar diseases of tea (Singh 2005).

Mechanisms of Cu^{2+} toxicity

Copper is a redox active metal with an electrochemical potential of -260V. The redox nature of Cu^{2+} ions makes it very useful as a cofactor in electron transfer reactions (Ducic and Polle 2005). However, the reversible oxidation–reduction property of Cu^{2+} could also result in oxidative stress if Cu^{2+} would be present as a free ion. Heavy metals in general have been recognised as a major toxicant in plant cells due to their capability of generating reactive oxygen species (ROS) such as hydroxyl radical ($OH^{\bullet}$) superoxide (O_2^-) and hydrogen peroxide (H_2O_2), which can damage the biomolecules such as membrane lipids, proteins and nucleic acids. During the reduction of oxygen to water, ROS may be produced by a chain of reactions which initially needs energy input but subsequently occur spontaneously. O_2^- is a short-lived and moderately reactive ROS which reduces quinines and transition metal complexes of Fe^{3+} and Cu^{2+} thereby affecting the metal containing transporters and enzymes. O_2^- can additionally combine with protons in aqueous medium and form hydroperoxyl radicals ($HO_2^{\bullet}$) which can induce lipid auto-oxidation in membranes (Shaw et al. 2004). H_2O_2 is relatively long-lived and moderately reactive which oxidises the thiol groups of some enzymes (e.g. enzymes of the Calvin cycle and Cu-Zn SOD) and inactivates them (Vranova et al. 2002). However, the

most reactive of all the ROS is the hydroxyl radical ($OH^{\bullet}$) which can potentially react with all types of biomolecules and in excess can cause cell death because cells do not have any enzymatic antioxidant system to quench it. The radical is formed from H_2O_2 by the Haber Weiss and Fenton reactions and Cu^{2+} being a redox active metal catalyzes the formation of this most harmful active radical (Arora et al. 2002; Vranova et al. 2002) as summarized below:

$$H_2O_2 + O_2^{-} \xrightarrow{Cu+,\ Cu2+} OH^{-} + OH^{\bullet} + O_2^{-}$$

Haber Weiss reaction

One of the richest sources of ROS in plants is the chloroplast. These can be formed due to the highly energetic electron transfer reactions triggered by chlorophyll excitation along with an excess supply of oxygen. Singlet oxygen ($^1O_2$) can formed during de-excitation of chlorophyll which causes major oxidative damage to biomolecules. High light intensity can cause over reduction of PS1 and generation of excessive NADPH which cannot be utilized by the CO_2 fixation process thereby reducing the $NADP^+$ pools. O_2^-which is abundant in the chloroplast can take up electrons from PS1 in such a situation, which leads to production of ROS through the Mehler reaction (Sharma et al. 2012). Under conditions of low CO_2 fixation such as cold temperature or low CO_2 availability, excess reduction of PS1and increase in ROS levels can occur even at moderate light intensities. As H_2O_2 or O_2^- are only moderately reactive, therefore, the main responsible factor for the intense biological damage is the metal ion which catalyzes the formation of the highly toxic hydroxyl free radical ($OH^{\bullet}$) from H_2O_2 (Maksymiec 1997). Thus ROS may be generated in the plant due to several abiotic as well as biotic causes but true damage is caused by the additional metal toxicity.

The hydroxyl radical ($OH^{\bullet}$) can either add onto the biological molecules or eliminate hydrogen from them by forming water. The hydroxylated biomolecules can in turn hydroxylate other molecules thereby initiating a chain of reaction or change to stable oxidised products. The activated hydroxylated molecules can also dismute themselves by forming intermolecular cross links (Shaw et al. 2004). Oxidised Cu^{2+} ions can be actively involved in electron transfer during formation of stable oxidized products. In reactions where the $OH^{\bullet}$radical eliminates H from biomolecules, it leaves an unpaired electron in the organic molecule thereby forming a reactive organic radical which can then react with oxygen to form peroxy radical ($ROO^{\bullet}$). The peroxy radical is again a reactive species and can eliminate hydrogen from other biomolecules and change them into organic radical products thereby creating a chain of reactions. The peroxidation reaction is evident in lipid peroxidation reactions that take place in cell membranes to form lipid peroxides (ROOH) (Shaw et al. 2004; Arora et al. 2002). However, in presence of reduced Cu^{2+} ions which can participate in Fenton reaction (shown below), the highly reactive alkoxy radical (RO^{-}) is formed from the ROOH which is as damaging as the hydroxyl radical thus opening up another cascade of immensely damaging oxidative reactions.

$$ROOH + Cu^{+} \longrightarrow OH^{-} + Cu^{2+} + RO^{-}$$

A study on the toxicity mechanisms suggest that the generation of reactive oxygen species is a natural phenomenon but is increased to alarming proportions due to presence of stress factors. Presence of Cu^{2+} ions above the threshold limit is

immensely stressful to plants due to its redox nature as it can catalyze and enhance the formation of all types of ROS by participating actively in several types of oxidative reactions.

Plant response to Copper toxicity

Plants have developed a wide range of protective mechanisms for mitigating copper toxicity. Primary defence mechanisms prevent metal to enter into the cell via exclusion, or binding of metal to cell wall and other ligands, organic acids, amino acids, glutathione (GSH) or phytochelatins (PCs) to render them harmless (Antosiewicz and Wierzbicka 1999; Rehman et al. 2019). Antioxidative mechanisms that control the level of ROS and shield the system before the sensitive parts of the cellular machinery gets damaged are mediated by molecules which have been broadly divided into two types, the high molecular weight enzymatic catalysts and the low molecular weight antioxidants (Pinto et al. 2003). The enzymes involved in scavenging ROS include SOD, catalase (CAT), peroxidases (POD) and glutathione peroxidase and those involved in detoxifying lipid peroxidation products include glutathione-S-transferases (GST), phospholipid-hydroperoxide glutathione peroxidase and ascorbate peroxidase (APX). Table 1 enlists the different enzymes which have been studied in relation to copper toxicity. The low molecular weight compounds that act as cellular antioxidants are ascorbate, glutathione, phenolics, flavonoids, carotenoids and tocopherols. Besides these, a whole array of enzymes is needed for the regeneration of active forms of the antioxidants such as monohydroascorbate reductase and glutathione reductase (Blokhina et al. 2003; Pinto et al. 2003).

Binding of copper and its sequestration

Plant adapt to heavy metal stress by acquiring several strategies, the most prominent being the synthesis of phytochelatins and metallothioneins which contribute to metal detoxification by chelation of the metal ions. Phytochelatins are simple thiol rich metal binding peptides containing glutamate, cystein and glycine in ratios of 2:2:1 to 11:11:1 (Grill et al. 1985; Prasad 2004). These peptides are synthesized non-translationally from glutathione in the presence of heavy metals by the enzyme phytochelatin synthase (Grill et al. 1989). Apart from being a precursor to phytochelatins, glutathione is also an important antioxidant molecule, which plays a predominant role in protection against free radicals (Alscher 1989). Copper induced metallothioneins are low molecularincrease in phytochelatin synthesis results in oxidative stress through the depletion of the antioxidant glutathione. De Vos et al. (1992) showed that copper tolerance in the plant species*Silene cucubalus* does not depend on the production of phytochelatins but is related to the ability of this plant to prevent glutathione depletion resulting from copper-induced phytochelatin production weight proteins with high cystein content, which bind metal ions to form metal thiolates and metal thiolate clusters. Class III metallothioneins are found in plants and is reported to be induced by the presence of a variety of metals including Cd, Cu, Zn, Pb, Hg and Ag (Hamer 1986; Prasad 2004). However, phytochelatins rather than metallothioneins are mainly responsible for detoxification of toxic heavy metals (Yadav 2010). Moreover, metal binding ability is higher in phytochelatins than in metallothioneins on a per-cysteine basis (Mehra andMulchandani 1995).

Table 1 Enzymes/Metabolites whose levels have been studied after copper exposure

Enzyme/Metabolite	Plant	Location	Reference
Peroxidase	*Zinnia elegans* and *Cosmos sulfureus*	shoots and roots	Tsay et al. 1995
	Zea mays L.	leaves and roots	Mocquot 1996
	Helianthus annuus	leaves and roots	Garcia et al. 1999
	Oryza sativa	leaves	Fang and Kao, 2000
	Capsicum annum	seedlings	Diaz et al. 2001
	Phaseolus vulgaris	leaves and roots	Cuypers et al. 2002
	Allium sativum	leaves and roots	Meng et al. 2007
	Erica andevalensis	leaves, roots	Oliva et al. 2010
	Zea mays	roots	Zhao et al 2010
	Vigna mungo	seedlings	Solanki et al. 2011
	Beta vulgaris L.	leaves	Morales et al. 2012
	Camellia sinensis	leaves	Saha et al. 2012
Catalase	*Avena sativa*	leaves	Luna et al. 1994
	Lycopersicon esculentum	leaves, stem and roots	Mazhoudi et al. 1997
	Oryza sativa	seedlings	Chen et al. 2000
	Camellia sinensis	root	Ghanati et al. 2005
	Prunuscerasifera	seedlings	Lombardi and Sebastiani, 2005
	Zea mays	roots and shoots	Pourakbar et al. 2007
		leaves and roots	Moravcová et al, 2018
	Vigna mungo	seedlings	Solanki et al. 2011
	Atriplex halimus	leaves	Brahim and Muhamed, 2011
	Cucumi sativus	roots	Iseri et al. 2011
	Lens culinaris	shoots	Hossain et al. 2020
Superoxide dismutase	*Nicotiana*	leaves	Pitcher et al. 1991
	tabacumGlycine max	root	Chongpraditnun et al. 1992
		leaves	Sen Gupta et al. 1993
	Nicotiana tabacum and *Pisum sativum*	root	Hartley-Whitaker et al. 2001
	Holcus lanatus	roots	Wang et al. 2004
	Brassica juncea	root	Ghanati et al. 2005
	Camellia sinensis	root and shoot	Lombardi and Sebastiani, 2005
	Prunuscerasifera	root, stem and	Peng et al. 2006
	Elsholtzia	leaves	Ke et al. 2007
	splendens	roots and leaves	Meng et al. 2007

	Daucus carota	leaves and roots	Zhang et al. 2008
	Allium sativum	root	Gao et al. 2008
	Elsholtzia haichowensis	root, stem and leaves	Nie et al. 2012
	Jatropha curcas	leaves	Moravcová et al, 2018
	Zea mays	leaves and roots	Azooz et al. 2012
	Triticum aestivum cv. Hasaawi	seedlings	
Ascorbate peroxidase	*Avena*	leaves	Luna et al. 1994
	sativaLycopersicon esculentum	leaves, stem and roots	Mazhoudi et al. 1997
	Phaseolus vulgaris	leaves and roots	Weckx and Clijsters, 1996
	Oryza sativa	root	Chen et al. 2000
	Camellia sinensis	root	Ghanati et al. 2005
	Morus rubra	leaves	Tewari et al. 2006
	Oryza sativa	root and shoot	Thounaojam et al. 2012
	Camellia sinensis	root and shoot	Hajiboland and Bastani, 2012
	Camellia sinensis	leaves	Saha et al. 2012
	Lens culinaris	shoots	Hossain et al. 2020
γ-glutamylcysteinyl synthetase	*Camellia sinensis*	leaves	Yadav and Mohanpuria, 2009
	Triticum aestivum	leaves	Shan et al. 2012
Glutathione reductase	*Silene cucubalus*	root	De Vos et al. 1992
	Panax ginseng	roots	Ali et al. 2006
	Morus rubra	leaves	Tewari et al. 2006
	Zea mays	roots and leaves	Pourakbar et al. 2007
	Oryza sativa	root and shoot	Thounaojam et al. 2012
	Triticum aestivum	leaves	Shan et al. 2012
	Zea mays	roots	Wang et al. 2011
	Zea mays	leaves	Nie et al. 2012
	Lens culinaris	shoots	Hossain et al. 2020
Dehydroascorbate reductase	*Cucumis sativus*	roots and leaves	Arora et al. 2002
	Panax ginseng	roots	Ali et al. 2006
	Triticum aestivum	leaves	Shan et al. 2012
	Lens culinaris	shoots	Hossain et al. 2020
Phenylalanine ammonia lyase	*Phyllanthus tenellus*	leaves	Santiago et al. 2000 Basak et al. 2001
	Camellia sinensis	leaves	Chakraborty et al. 2002

	Camellia sinensis	leaves	Kovacik and
	Matricaria recutita	root and leaves	Backor, 2007
	Glycine max	roots	Chmielowska et al. 2008
	Jatropha curcas	root, stem and leaves	Gao et al. 2008
Polyphenol oxidase	*Camellia sinensis*	leaves	Basak et al. 2001
	Jatropha curcas	root, stem and leaves	Gao et al. 2008

In addition, phytochelatins possess the ability to scavenge ROS and thereby aid in mitigating oxidative stress (Tsuji et al. 2002).

Accumulation of amino acids like proline has been observed in response to several biotic and abiotic stresses in plants. Content of free proline has been found to be related to Cu^{2+} tolerance in plants (Backor et al. 2003; Chen et al. 2004). Excess Cu^{2+} has been found to result in inadequate proline (Thomas et al. 1998) and lead to the malfunctioning of copper exclusion machinery (Chen et al. 2004). Copper complexes with amino acids such as proline, histidine or nicotinamine play important role in xylem sap transport (Liao et al. 2000).

Antioxidant response

Plants possess well developed defence system against ROS which restricts its formation and maneuver its removal. Inside the plant cell, superoxide dismutases (SOD) provide the first line of defence against ROS. The enzyme is located in different cell compartments including mitochondria, chloroplast, glyoxisomes, peroxisomes, microsomes, apoplast and cytosol (Alscher et al. 2002) and catalyzes the disproportionation of O_2^- to H_2O_2 and molecular oxygen (Scandalios 1993). SOD enzymes are classified based on the metal cofactors: the Cu-Zn SOD, the Mn-SOD and Fe-SOD (Bowler et al. 1994). Although each type of SOD predominates in specific cell compartments, their occurrences are not restricted, and all types can be detected in most of the cellular locations (Arora et al. 2002). An increased level of SOD has been correlated to enhanced oxidative stress protection in plants (Sen Gupta et al. 1993). Increase in SOD activity has been reported against copper induced stress in tolerant plants such as *Prunuscerasifera* (Lombardi and Sebastiani 2005); *Elsholtzia haichowensis* (Zhang et al. 2008); *Elsholtzia splendens* (Peng et al. 2006); *Jatropha curcas* (Gao et al. 2008); *Holcus lanatus* (Hartley-Whitaker et al. 2001); *Daucus carota* (Ke et al. 2007); *Ceratophyllum demersum* (Rama Devi and Prasad 1998); *Brassica juncea* (Wang et al. 2004); *Hydrilla verticillata* (Srivastava et al. 2006); *Zea mays* (Nie et al. 2012), *Triticum aestivum* cv. Hasaawi (Azooz et al. 2012), *Allium sativum* (Meng et al. 2007) etc. However, Weckx and Clijsters (1996) observed that SOD was not involved in the defence mechanism against copper induced oxidative stress in primary leaves of *Phaseolus vulgaris*. Contradictory results have also been recorded regarding the response of catalase (CAT) against copper stress. Both CAT and peroxidase (POD) are involved in the removal of H_2O_2 that accumulates due to dismutation of O_2^- by SOD. Catalase activity did not increase in Cu^{2+} stressed roots of rice seedlings (Chen et al. 2000) or in black gram (Vigna mungo) seedlings (Solanki et al. 2011) and decreased in *Lens culinaris* seedlings (Hossain et al. 2020). On the other hand,

CAT activity was reported to increase in *A. halimus* leaves (Brahim and Muhamed 2011) *Prunuscerasifera* (Lombardi and Sebastiani 2005), *C. sativus* roots (Iseri et al. 2011) and in maize roots, shoots and leaves (Pourakbar et al. 2007, Moravcová et al. 2018) in response to excess Cu^{2+} concentrations. The mobilization of POD in response to Cu^{2+}induced oxidative stress in plants is well accepted (Fang and Kao 2000; Diaz et al. 2001; Cuypers et al. 2002; Meng et al. 2007; Solanki et al. 2011). Apart from POD and CAT, the enzymes and metabolites of the ascorbate-glutathione cycle are also involved in the removal of H_2O_2. The majority of these enzymes [ascorbate peroxidase (APX), glutathione reductase (GR), and dehydroascorbate reductase (DHAR)] have been found in chloroplasts, cytosol, mitochondria, and peroxisomes (Dat et al. 2000). Glutathione and ascorbate accumulate in these cellular compartments and their redox state is maintained through glutathione reductase (GR), monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR).

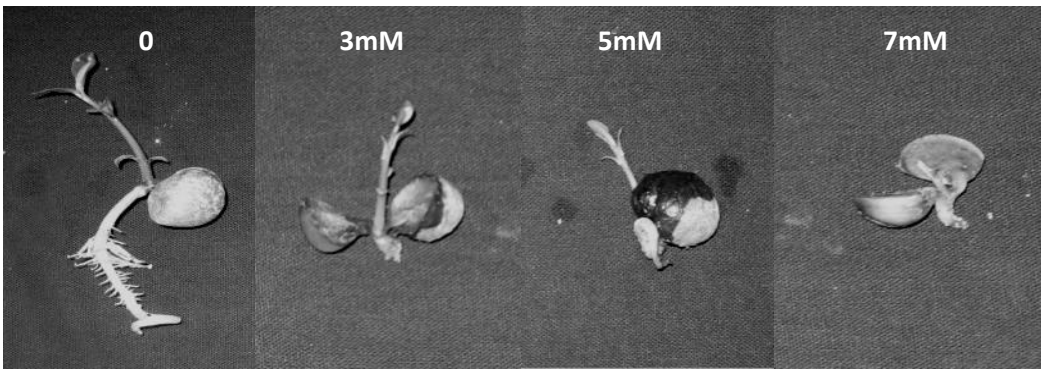

Fig. 1 Effect of excess copper on germination of tea seeds: reduction in root and shoot lengths of germinated tea seeds of TS 462 variety on exposure to different concentrations of $CuSO_4$ (indicated in the figure) photographed after 27 days of treatment

All these enzymes along with ascorbate and glutathione have a pivotal role in defence against ROS induced oxidative damage (Arora et al. 2002; Yruela 2005; Sharma and Dietz 2008; Shan et al. 2012). De Vos et al. (1992) observed that glutathione depletion is the major cause of Cu^{2+} induced oxidative damage in Cu^{2+} sensitive *SiIene cucubalus* plants. It has been shown that tolerance to a copper-enriched environment, and the accompanying oxidative stress in *Enteromorpha compressa* occurs through the accumulation of copper, activation of ascorbate peroxidase, synthesis of ascorbate (accumulated as dehydroascorbate) and consumption of glutathione and water-soluble phenolic compounds (Ratkevicius et al. 2003).

Stress in tea

A literature survey revealed that several studies have been conducted on different types of abiotic stresses in tea. Plants of different cultivars of tea have been grouped into the tolerance classes: susceptible and resistant, in response to drought stress (Chakraborty et al. 2002; Damayanti et al. 2010), cold stress (Upadhyay 2012) and heavy metal stress (Yadav and Mohanpuria 2009). Several parameters have been identified such as rates of photosynthesis and transpiration, relative water content, stomatal

conductance and leaf total soluble sugar content (Damayanti et al. 2010), root and shoot extension (Burgess and Carr 1997), levels of proline and antioxidative enzymes (Chakraborty et al. 2002; Upadhyay and Panda 2004; Upadhyay et al. 2008), morphological characters (Waheed et al. 2012) etc. in order to screen tea cultivars for drought tolerance. Additionally, studies on alterations in bioconstituents that determined quality of tea in the tea clones under soil moisture revealed a decrease in PAL activity in both tolerant and susceptible clones which correlated with a lower flavonol content and quality deterioration (Jeyaramaja et al. 2003).

Tea plants exposed to excess heavy metals have shown several alterations in physiological and biochemical parameters. Increased level of lipid peroxidation and a reduction in photosynthetic rate, transpiration rate, chlorophyll and protein content and biomass production were found in plants exposed to excess Cd (Mohanpuria et al. 2007; Shi et al. 2008). Oxidative stress was evident as the transcript levels of glutathione biosynthetic genes showed up-regulation while glutathione-S-transferase (GST), the enzyme which help in sequestration of high levels of metal ions to vacuole, did not show any change on Cd exposure (Mohanpuria et al. 2007). Hajiboland and Bastani (2012) observed that CO_2 assimilation and dry matter production decreased while antioxidant enzyme activity and proline content increased significantly in tea plants under Boron deficiency and water stress. Mukhopadhyay et al. (2013) observed that both deficiency and excess in zinc caused a considerable decrease in shoot and root fresh and dry masses. Zinc stress decreased net photosynthetic rate, transpiration rate, stomatal conductance, and content of chlorophylls *a* and *b* and increased the content of superoxide anion, malondialdehyde, hydrogen peroxide, and phenols. Although the activities of ascorbate peroxidase, catalase, superoxide dismutase, and peroxidase as well as expression of respective genes were up-regulated, the authors concluded that the overall antioxidant system did not afford sufficient protection against oxidative damage (Mukhopadhyay et al. 2013). Treatment of tea plants with excess heavy metals such as mercury (II) and nickel (II) decreased the chlorophyll content of the leaves, along with a significant reduction in Hill activity (Basak et al. 2001). The activities of antioxidative enzymes viz. Superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) was increased by Aluminium in the roots of cultured tea cells and also in intact plants (Ghanati et al. 2005). Aluminum (Al) inhibited tea pollen tube growth but the effect was found to be alleviated by fluorine (Konishi and Miyamoto 1983) which is accumulated by tea plants normally in high excess (Ruan et al. 2004). Tea plants tolerated fluorine at concentrations < 0.32 mM (Li et al. 2011). Fresh and dry mass, chlorophyll content and net photosynthetic rate decreased while proline, malondialdehyde and hydrogen peroxide contents increased with increasing fluorine concentrations. Activity of antioxidant enzymes also showed significant alterations thereby suggesting that antioxidant defence system of leaves did not sufficiently scavenge excessive reactive oxygen species generated due to excess fluorine (Li et al. 2011).

Cu^{2+} stress in tea

Although copper-based fungicides are being used in tea gardens for several decades (Sarmah 1960), we know little about the role of excess Cu^{2+} on tea plants and at what concentrations it may be considered as a pervasive threat (Saha et al. 2012). Only a few studies have focused

on Cu^{2+} toxicity in tea (Basak et al. 2001; Yadav and Mohanpuria 2009; Saha et al. 2012; Dey et al. 2014, 2015) and these have revealed that number physiochemical parameters are altered on exposure to excess copper. For example, the chlorophyll and protein contents were found to decrease in Cu^{2+} treated plants (Basak et al. 2001; Yadav and Mohanpuria 2009; Saha et al. 2012). Germination of tea seeds were also affected in presence of excess copper. Substantial reduction in the length and biomass of root and shoot (Fig.1) was observed (Mandal et al, 2013). Excess Cu^{2+} caused an increase in lipid peroxidation, phenolics and antioxidative enzyme levels such as POD, SOD and APX in multiple cultivars of tea (Saha et al. 2012; Dey et al. 2015). A significant difference among cultivars was noted where the more sensitive cultivar seemed to lose its antioxidative capacity at Cu^{2+} concentrations higher than 400 µM while the more tolerant cultivar was able to withstand a maximum of 600 µM of Cu^{2+} ions. Two new isozymes were also found to be induced in the leaves of tea exposed to high concentration of Cu^{2+} (Saha et al. 2012). Yadav and Mohanpuria (2009) observed that expression of the enzymes γ-glutamylcysteinyl synthetase, glutathione synthetase and phytochelatin synthase was elevated more in the tolerant tea cultivar than the susceptible one when exposed to excess Copper and Aluminium.

Conclusion

Heavy metal stress is one of the major problems that limit agricultural productivity of plants. Plants show relative differences in their heavy metal tolerance capacity among the species and also among cultivars of the same species. Copper stress in general induces ROS and generates oxidative stress. It has been found that in addition to accumulated metal ions, high levels of ROS adversely affected the plants. Such ROS related damages have been observed in tea cultivars also. Although of the negative impact of excess Cu^{2+} in tea plants have been documented, the level of Cu^{2+} accumulation caused due to long term application of Cu^{2+}-based fungicides in tea gardens and its bioavailability under tea garden conditions are yet to be studied. Additionally, more detailed studies on mechanisms of Cu^{2+} toxicity in the tea plant, especially at the gene level are necessary. Identification of genetic determiners of tolerance may make the resistant cultivars a potential source for genetic manipulation of other important elite cultivars.

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Understanding the functional attributes of different microbial enzymes in bioremediation

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Abstract

Bioremediation uses biological organisms and their metabolic processes in order to degrade contaminants present in water, soil etc. Microbes have the vast potential are the major resource for bioprocess of using microbial enzymes reduces the toxicity of pollutants caused by the waste materials like pesticides, insecticides, plastics, other hydrocarbon-containing substances and obtain novel useful substances for mankind and the environment. Enzymes produced by bacteria, fungi, plants play a key role in the biodegradation of toxic organic compounds. The purpose of bioremediation processes that will an eco-friendly and cost-effective mechanism. The aim is to develop an advanced technique in bioprocesses that will help to minimize toxin risk and thereby acquire new, usable substances. Some of the bioremediation-related compounds like oxidoreductases hydrolases, dioxygenase, peroxidases, and laccase are most widely considered. The aim of the review is to express the role of microbial enzymes on the bioremediation of toxic, hazardous environmental pollutants.

Keywords: Bioremediation; Environment; Enzymes; Hydrocarbons; Pollutants;

Introduction

Microorganisms are widely distributed in the biosphere due to their metabolic activity is very impressive and they can grow in any nutritional condition. The enzyme plays a very important role in the sustainability of all life forms (Abatenh et al., 2017). Living organisms produce enzymes that act as a catalyst in chemical reactions. The term 'Enzyme' was first coined by Wilhelm Friedrich Kühne (Sheehan and Himmel, 1999). Nowadays human population, urbanization and industrialization are increasing that are associated with constantly elevating pollution levels (Kekkonen, 2017). One important issue is a huge amount of waste water generated from dairy, food industries, oil refinery, poultry house and wool processing factories (Kumar et al., 2019; Ara et al., 2019; Singh et al., 2019). Microorganisms act as a significant pollutant removal in soil, water, sediments (Bajaj and Singh, 2015). Enzymes are of great importance in the development of industrial bioprocesses as they play a crucial role as metabolic catalysts (Singh et al., 2019). The majority of currently used industrial enzymes are hydrolytic in action that is used for the degradation of various natural substances (Kirk et al., 2002). Other enzymes include carbohydrates, primarily amylases, proteases, lipases, proteases, xylenes and celluloses used in various industries such as starch, textile, detergent, baking and food industry (Gurung et al., 2013; Tripathi et al., 2020).

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Microbial enzymes have been extensively used for the biodegradation of pollutants including both organic and inorganic pollutants. This process is known as bioremediation that uses metabolic processes of biological organisms in order to degrade contaminants so that they remain no longer in harmful form (Canak et al., 2018). Instead of simply collecting the pollutant and storing it, bioremediation is a well-organized procedural activity that is applied to break down or transform to less toxic or non-toxic elemental and compound forms (Abatenh et al., 2017). Bioremediation involves utilization of biological agents to clear the contaminated or polluted sites (Sharma, 2019). A study showed that bacteria, fungi and archaea are the prime organisms that are used as a biochemical agent in the bioremediation process (Abatenh et al., 2019). Both bacteria and fungi rely on the participation of different extracellular and intracellular enzymes respectively for the process of bioremediation (Karigar and Rao, 2011). Utilizing microbial enzymes is an eco-friendly, least harmful and cheaper method to remove toxic products from the environment (Sharma et al., 2019). An attempt has been made in this review to highlight the different categories of microbial enzymes and their role in bioremediation.

Types of microbial enzymes

Enzymes are biocatalysts that carry out chemical reactions and depending upon the type of reaction these are classified into six different categories like oxidoreductase, transferase, hydrolases, lyases, ligases and isomerases. Each above of the following enzyme has been discussed in the following section.

Oxidoreductases

Oxidoreductase is a large family of enzymes that catalysed the biological oxidation-reduction processes. Various bacteria, fungi and higher plants carry out detoxification of toxic organic compounds through oxidative coupling with help of these oxidoreductases. As a result, heat or energy is generated and oxidoreductase will bring about degradation of pollutants. The heat is utilized by microorganisms for their metabolic activity (Medina et al., 2017). Microbes extract energy via energy-yielding biochemical reactions mediated by oxidoreductase and break the chemical bond that assists the transfer of electrons from a donor to acceptor. During the oxidation-reduction process the contaminations are finally oxidized into harmless compound. Oxidoreductase have been utilized in the bioremediation of numerous natural and anthropogenic pollutants. Oxidoreductase detoxifies various synthetic organics such as phenolic, azo rings, and aniline substances which are the essential for xenobiotics or soil environment.

A gram-positive bacteria *Bacillus safensis* CFA-06 produces oxidoreductase to degrade the petroleum compounds (Fonseca et al., 2015). After lignin degradation various harmful phenolic compounds are released in the environments which are degraded by oxidoreductases through polymerization and co-polymerization (Husain, 2006). Colour compounds produced from textile

industries are released into the environment and are degraded by various

Table 1. List of microbial enzymes with source and their usage in different bioremediation processes

S. No.	Enzyme	Source organism	Application	Reference
1	Oxidoreductase	*Bacillus safenis*	Used for bioremediation of contaminated soil, xenobiotics, decolorization and degradation	Husain, 2006; Bansal And Kanwar, 2013;
2	Monooxygenease	*Bacillus megaterium* BM3	Degrade hydrocarbons like substitute methanes, alkanes, haloalkanes and aromatic heterocyclic hydrocarbon	Roccatano, 2015
3	Diooxygenease	*Pseudomonas putida* F1	Degrade aromatic compounds into aliphatic products	Mukherjee and Roy, 2013
4	Laccase	*Rhizoctonia praticola, Trametes hispida, Bacillus vallismortis fmb103, Pleurotus ostreatus*	Depolymerization of lignin to an array of phenols and degradation of bisphenol A	Dodar et al., 2004; Rodriguez et al., 1999; Legerska et al., 2016; Strong and Claus, 2011
5	Peroxidase	*Escherichia coli Bacillus* sp, *Pseudomonas* sp, *Thanatephorus* sp, *Auricularia* sp, *Pleurotus ostreatus*	Degrade lignin and oxidises manganese, methoxybenzenes, and phenolic aromatic substrate	Bansal and Kanwar, 2013; Abdel-Hamid et al., 2013
6	Lignin peroxidase	*Phanerochaete chrysosporium, Trametes versicolor*	Oxidise various compounds and biodegrade plant cell wall constitute lignin.	Xuet al., 2014; Abdel-Hamid et al., 2013; Behbahani et al., 2016; Piontek et al., 2001
7	Manganese Peroxidase	*Trametes sp, Peniophora incarnata*	Oxidise lignin and other compounds	Zhanga et al., 2016; Lee et al., 2016
8	Versatile Peroxidase	*Pleurotus eryngii*	Oxidized both phenolic and non-phenolic lignin model dimers	Koch et al., 2017
9	Hydrolase	*Microbacterium* sp	Bioremediation of pesticides, insecticides	Karigar & Rao, 2011; Lei et al., 2017
10	Lipase	*Pseudomonas aeruginosa,*	Degrade cooking waste	Verma et al., 2012; Sharma et al., 2011

11	Cellulase	*Clostridium,Cellulomonas, Thermomonospora, Trichoderma, Aspergillus, Humicola*	Convert waste cellulosic material into food, paper and pulp industry	Kuhad et al., 2011; Hmad and Gargouri, 2017
12	Protease	*Aspergillus* sp, *Bacillus licheniformis, Bacillus* sp	Hydrolyze peptide bonds	Pandey et al., 2017; Tripathi et al., 2020

oxidoreductase enzyme (Novotny et al., 2004).

Oxygenases

Oxygenase belongs to the oxidoreductase group of enzymes. They play a key role in the aerobic degradation of aromatic compounds, catalyses the cleavage of the ring in aromatic compounds by adding one or two molecules of oxygen. On the basis of the number of oxygen atoms are used they are grouped into two categories the monooxygenase and dioxygenase. One of the bacterial microbe *Pseudomonas* sp. LBr produces glyphosate oxidase (GOX) which is involved in the bioremediation of pesticides. GOX converts glyphosate into amino methyl phosphonate (AMPA) and releases the keto acid glyoxylate (Scott et al., 2008). Some marine bacteria also produce oxygenase for the degradation of organic pollutants (Sivaperumal et al., 2017).

Monooxygenase

Monooxygenases incorporate one atom of the oxygen molecule into the substrate in the metabolic pathways. Monooxygenase are classified into two types based on the presence of cofactor: flavin dependent monooxygenase and P450 monooxygenase. Flavin dependent monooxygenase used for the degradation of chlorine containing pesticides like endosulfan (Bajaj et al., 2010). P450 monooxygenase isolated from the bacterium *Bacillus megaterium* BM3 has the capacity to degrade a variety of substrates such as fatty acid and aromatic compounds (Roccatano, 2015). Monooxygenase act as biocatalysts in bioremediation process due to their highly regioselectivity and stereoselectivity on wide range of substates. Monooxygenase carry out desulfurization, dehalogenation, denitrification, ammonification, hydroxylation, biotransformation and biodegradation of various aromatic and aliphatic compounds (Arora et al., 2010).

Dioxygenase

Dioxygenases are multicomponent enzymes systems that introduce molecular oxygen into their substrate. Dioxygenases are primarily oxidizing aromatic compounds. They are the key enzymes in pathways for the bacterial degradation of aromatic hydrocarbons. On the basis of their mode of action, they are classified into (1) aromatic ring hydroxylation dioxygenases (ARHDs) (2) aromatic ring cleavage dioxygenases (ARCDs) (Parales and Ju, 2011). Toluene dioxygenase (TOD) produced by *Pseudomonas putida* F1 catalyses the degradation of toluene (Mukherjee and Roy, 2013). The catechol dioxygenases are found in the soil bacteria causes biotransformation of aromatic and

aliphatic products (Muthukamalam et al., 2017). Various aromatic compounds released into the environment from different industries. Dioxygenase breaks down the aromatic ring at 1 and 2 position (Guzik et al., 2013). Naphthalene dioxygenase isolated from *Pseudomonas putida* involve the naphthalene degradation (Gennaro et al., 1997).

Laccases

Laccases are copper containing oxidases produced by certain plants, fungi, insects, and bacteria, catalyses the oxidation of a wide range of reduced phenolic and aromatic substrates followed by reduction of molecular oxygen to water. It is found in multiple isoforms and is found both inside and outside of the cell (Mai et al., 2000). Laccase isolated from fungus *Trametes hispida*is able to be decolorize azo dyes by oxidizing their bonds and transform into less harmful substances present in the environment (Rodriguez et al., 1999; Legerska et al., 2016). Laccase produced by *R. praticola* have the ability to degrade and biotransform phenolic compounds (Strong and Claus, 2011). Laccase isolated from fungus *Trametes versicolor* is a powerful enzyme for the bioremediation of a wide range of pollutants like phenolic and aromatic compounds (Chakroun et al., 2010).

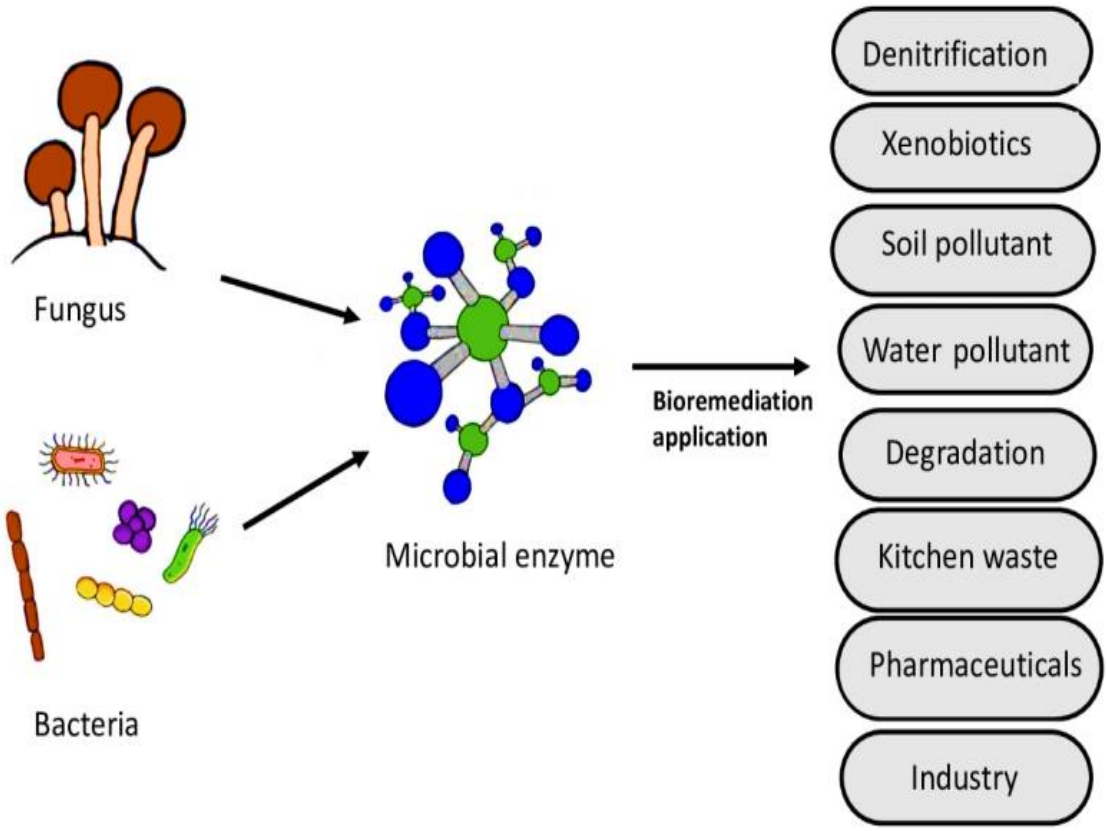

Fig 1 Microbial enzymes and their application in different aspects of bioremediation

Peroxidase

Peroxidase plays a key role in the degradation of lignin and other aromatic compounds by using hydrogen peroxide and a mediator produced by animals, plants, fungi and bacteria. They are ubiquitous in nature and can be heme or non-heme proteins (Bansal and Kanwar, 2013). The heme–containing peroxidases can be divided into two groups: one group found in animals and other group found in fungi, bacteria and plants. Peroxidases have the potential to decrease water pollution by bioremediation of phenols, cresol and chlorinated phenolic compounds in wastewater. Soybean peroxidase and chloroperoxidase have been examined for the degradation for the thiazole compounds (Sharma et al., 2018).

Among the bacterial strains, *Escherichia coli*, *Bacillus sp.*, *Pseudomonas sp.* are predominant peroxidase producers. In fungi, it is found in *Thanatephorus sp, Auricularia sp, Pleurotus ostreatus.* Among peroxidases, lignin peroxidase (LiP), manganese–dependent peroxidase (MnP) and versatile peroxidase (VP) due to their high potential to degrade toxic substances in nature (Abdel-Hamid et al., 2013).

Lignin peroxidase

Lignin peroxidase (LiP) are heme proteins secreted by fungi such as *Phanerochaete chrysosporium*, *Trametes versicolor* and bacteria (Xuet al., 2014). Lignin shows a great application for the treatment of water and in the field of bioremediation (Abdel-Hamid et al., 2013). LiP degrades lignin and other phenolic compounds. It also oxidizes halogenated phenolic compounds, polycyclic aromatic compounds, and other aromatic compounds followed by a series of nonenzymatic reaction. LiP plays a central role in the biodegradation of the plant cell wall constitute lignin. Lignin degradation by bacterial peroxidase is more efficient as compared to fungal peroxidases on the basis of specificity and thermostability (Behbahani et al., 2016). It is also able to oxidize aromatic compounds with higher redox potential (Piontek et al., 2001).

Manganese peroxidase

Manganese peroxidase (MnP) is a hydrogen peroxidase dependent enzyme, but it can only oxidize organics when in the presence of MN (II). It is generally found in basidiomycetes fungus. MnP oxidizes Mn (II) to Mn (III), which acts as an obligatory oxidation intermediate for the oxidation of various compounds. The Mn (II) ions migrate away from the enzyme and start the oxidation of the lignin and other compounds. These catalyses the degradation of several phenols, amine containing aromatic compounds, and dyes (Ten Have and Teunissen, 2001). MnPTra-48424 was identified and purified from white rot fungi *Trametes sp*. 48424. This enzyme has strong capability to decolorize different kinds of dyes such as indigo, anthraquinone, azo and triphenylmethane, while other dyes such as indigo carmine and methyl green combined with heavy metal ions and organic solvent (Zhanga et al., 2016) During the degradation of anthracene, gene (pimp1) encoding manganese-dependent peroxidase was found in *P. incarnata* KUC8836. This gene was further expressed in fungi *Saccharomyces cerevisiae* to enhance the bioremediation process (Lee et al., 2016). Immobilization of MnP was also done with chitosan beads activated by glutaraldehyde

show a greater potential for decolorization of dye effluent from the textile industry (Bilal and Asgher, 2016).

Versatile peroxidase

Versatile peroxidase (VP) enzymes are able to directly oxidize MN^{2+}, methoxybenzenes, phenolic aromatic substances like MnP and LiP. Versatile peroxidase has a significant broad substrate specificity and the tendency to oxidize substrates in the absence of manganese as compared to the other phenolic substances. It is able to oxidize both phenolic and nonphenolic lignin model dimers. Because of its high productivity it is often used for bioremediation (Pinto et al., 2020).

Hydrolytic enzymes

Hydrolytic enzymes are most commonly used for bioremediation of pesticides and insecticides to reduce their toxicity. In order to reduce toxicity, hydrolytic enzymes break chemical bonds between toxic molecules. The oil spill, organophosphate, and carbamate insecticides are easily degraded by this process. The degradation of toxic organic compound through bioremediation is safe and economical compared to physico-chemical process (Karigar & Rao, 2011). It also catalyzes condensation and alcoholysis. The main advantage of this enzyme is its availability, non-selectiveness and good tolerability. Extracellular hydrolytic enzymes including lipases, DNases, amylase, protease, xylanases have many applications in food industry, chemical industries biochemical sciences and feed additive. The hemicellulose, cellulase and glycosidase are especially active in biomass degradation (Porro et al., 2003). Hydrolyzing enzyme gene was isolated from *Microbacterium sp*. djl -6F which is then cloned into *Escherichia coli* BL21 (DE3). It was observed that these enzymes able to hydrolyze carbenazim a widely used fungicide (Lei et al., 2017).

Lipases

Lipases are ubiquitous in nature and have been extracted from bacteria, plant, actinomycetes, and animal cells. Microbial lipases are more versatile because of their potent application in industries. These enzymes catalyses various reactions such as hydrolysis, inter-esterification, esterification, alcoholysis and aminolysis (Prasad & Manjunath, 2011). It helps in the drastic reduction of organic pollutants present in the contaminated soil. Lipase hydrolyses the fatty acids into triglycerol, diglycerol, nonglycerol, and glycerol. (Ghafil et al., 2016). Lipase activity is responsible for the most useful indicator parameter for testing hydrocarbon degradation in soil. An oil degrading lipase have been isolated from fungus *Pseudomonas aeruginosa* SL-72 which then further used for the bioremediation of crude oils (Verma et al., 2012). Along with its usage in bioremediation, lipase has many potential applications in food, chemicals, detergent manufacturing, cosmetics and paper industry however, its production is costly (Sharma et al., 2011).

Cellulase

Cellulases are the most abundant biopolymer found on the Earth. Cellulase enzymes are capable of degrading crystalline cellulose to glucose (Sharma et al., 2017). Cellulases are inducible enzymes synthesized by a large diversity of microorganisms including both fungi

and bacteria during their growth on cellulosic materials (Ma et al., 2013; Quintanilla et al., 2015). Cellulases produced by microorganisms can be cell-bound, associated with cell envelope or extracellular (Yang et al., 2016). These microorganisms can be aerobic, anaerobic, mesophilic, or thermophilic. Among them, the genera of *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Trichoderma*, and *Aspergillus* are the most extensively studied cellulose producers (Kuhad et al., 2011). Cellulases are usually a mixture of several enzymes and three major groups of cellulases such as endoglucanase, exoglucanase or cellobiohydrolase and β-glucosidase are involved in the hydrolysis process. Some alkaline cellulases are produced by *Bacillus* strains and neutral and acidic cellulases by *Trichoderma* and *Humicola* fungi (Hmad and Gargouri, 2017). These cellulases have been employed for the bioremediation of ink in paper and pulp industry during recycling of paper (Karigar and Rao, 2011). Cellulases produced by *Humicola* species is highly adaptive for harsh environmental conditions such as high pH and temperature and can be used in detergents and washing powders industry for the breakdown of hydrogen bond (Imran et al., 2016)

Protease

Proteases constitute a very large and complex group of enzymes that hydrolyzes peptide bonds in aqueous environment and synthesize them in non-aqueous environment (Pandey et al., 2017). These are commonly used in the detergent and pharmaceutical industries, followed by the food industry (Tripathi et al., 2020). The major sources of protease enzymes are Animals, plants and microorganisms (both fungal and bacterial). Proteases are classified into two groups: endopeptidases and exopeptidases on the basis of pH, substrate specificity, similarity to well characterized enzymes, and the active site amino acid the site of action on polypeptide chains (Raveendran et al., 2018; Tavano, 2017). It has been reported that 29 *Bacillus* species and 17 fungal species produces alkaline protease (Jisha et al., 2013). Commercial producers of alkaline proteases include protein engineered *Bacillus licheniformis*, alkalophilic *Bacillus sp, and Aspergillus sp*. (Ellaiah et al., 2002).

Conclusion

Pollution of soil and water from agricultural chemicals and synthetic hydrocarbons is a major issue of concern in the World. Because of their widespread use, they have now been marked as serious environmental pollutants in a variety of marine and terrestrial ecosystems. To Bioremediation is eco-friendly for the clearance of all such harmful substances from the environment in a natural way. Enzymes present in microorganisms have proven to be the most efficient in this bioremediation process as they help nature to rejuvenate, utilizing the existing substances and manipulating it according to need. Another advantage of using microbial enzymes is that it does not create any hazardous by-products, which usually occurs while using non-biological systems. Therefore, isolation and identification of these microbial enzymes is great field of research for the biotechnologists all over the globe and will help in making the planet pollution free in a sustainable way.

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Review on

Bisphenol A toxicity in aquatic flora: Impacts and possible remediation

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Abstract

Bisphenol A (BPA), is one of the high volume produced chemical which is extensively used as raw material for polycarbonate and epoxy resin manufacturing. Being one of major used and disposed material from a wide source, traces of BPA have been diagnosed from everywhere. BPA has been identified as an endocrine disruptor compound (EDC) for most of animals, due to structural similarity with hormones, and hinders many physiological functions. This review work focuses on the status of BPA in water bodies of different parts of the world. The review also focuses on the impact of BPA on aquatic plants and its possible remediation. Sub-standardly imposed policies by several countries and failure of water resource governance are rapidly leading towards incautious release of plastics and other BPA associated waste products in environments. BPA pollution affects humans, animals and even plants. Among the aquatic flora, most affected plant groups are the algal groups and macrophytes. At lower BPA concentration, many beneficial bacterial strains also show sensitivity whereas some other strains are known to metabolize or remove BPA from the water bodies. In this connection, several aquatic macrophytes have also been reported to contribute in the removal of BPA from the aquatic ecosystem.

Keywords: Bisphenol A, Endocrine disruptor compound (EDC), Macrophyte, Polycarbonate plastics, Phytoremediation.

Introduction

Enormous rise in human population in current and previous centuries immensely impacted the climate and most of the environmental factors (Amoatey and Baawain, 2019). Among these factors, water pollution (both freshwater and marine waterbodies) directly leads to severe human health problems and deterioration in healthy aquatic ecosystems around the globe. Both the fresh water and marine ecosystems play an important role in climate preservation, purification of water, nutrient recycling, and also provides habitat for aquatic flora and fauna (Oertel and Salánki, 2003). Irresponsible human actions in industrial, agricultural and urban wastes management have led toward serious damages to the water bodies and aquatic ecosystems in last few decades (Meijide *et al*., 2018). Huge number of contaminants are introduced every year as agricultural and industrial wastes in water bodies (lake, rivers, oceans, groundwater etc.). These contaminants are released in the form of heavy metals (As, Cd, Cr, Cu, Hg, Ni and Pb), nutrients (N, K, P etc.), polycarbonates and organic pollutants (e.g., petrochemicals). Meanwhile, substandard imposition of environmental

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regulations resultsin the disruption of water quality in the environment (Hu and Cheng, 2013). These contaminants exceedingly influence the aquatic ecosystem and also show toxic effects on the aquatic flora and fauna (Sangai *et al*., 2016).

Polycarbonate contaminants are identified as one of the major water pollutant in the past few decades, which causesseveredamage to the aquatic ecosystem. Most used polycarbonates and epoxy resins are manufactured from an organic compound -Bisphenol A (BPA). Release of plastics into the environment during the manufacture of chemicals, processing, product packaging, transportation and improper disposal without proper regulation, results in to the release of BPA in the environment (Staples *et al*., 1998). A wide range of products are manufactured from BPA e.g., food containers, electronic parts, protective envelops for electrical, adhesives, dyes, paints, housing materials, thermal paper, paper coating, compact disks, automobile parts etc., which also contributes to the release of BPA in the environment (Staples *et al*., 1998).

Due to such wide-ranging source and sub-standard enforcement of discharge regulations, BPA has been ubiquitously detected in environment including waterbodies, soil and landfill leachate (Staples *et al*., 1998). They are also found as floating and non-floating polycarbonate plastics dumped worldwide in fresh waterbodies as well as oceans (Duxbury, 1992). Rivers, lakes and seafloor majorly dumped with plastics debris which are non-floating in nature slowly and gradually releases BPA (Galgani *et al*., 1996; Gregory *et al*., 1997). Water surface covered with dumped plastics all over the world constitutes about 45% of the buoyant plastics (Duxbury, 1992).

Bisphenol-A is chemically 2,2-bis-(4-hydroxyphenyl) propane. It has been estimated that approximately 8.4 million tons of BPA have been used in 2018 globally for the production of epoxy resin and polycarbonate plastic (Staples *et al*., 1998; PRWeb report, 2013). This huge amount of plastic production and the rapid degradation of plastics releases BPA (3-7 day's half-life) into the waste water, which exhibits moderate toxicity and lower accumulation in aquatic organisms (Staples *et al*., 1998).

BPA has been identified as an endocrine disruptor compound (EDC) for most of animals, due to structural similarity with hormones, and a result it hampers many physiological functions (Fang *et al*., 2020). Accumulation of BPA in phytoplankton causes acute toxicity and trophic transfer to zooplanktons (Radix *et al*, 2002). Toxic effect of BPA includes reduction in cell number, size and chlorophyll, observed in both freshwater and marine microalgae e.g., *Chlorella sorokiniana*, *Monoraphidium braunii*, *Chlorella pyrenoidosa*, *Nannochloropsis* sp. etc. (Gattullo *et al*, 2012; Eio *et al*., 2015; Guo *et al*., 2017; Ishihara and Nakajima, 2003). Effect of BPA exposure can also be observed in few other aquatic plants e.g., *Ipomoea aquatica*, water hyacinth, *Azolla filiculoides*, etc. (Noureddin *et al*., 2004; Kang and Kondo, 2006; Zazouli *et al*., 2014).

Traditional methods used in wastewater treatments employs physical and chemical methods, which are costly and hostile for environment health (Zhou *et al*., 2004). Phytoremediation is one of emerging biological methods for BPA

removal from waste waterbodies. In the treatment of wastewater, appointment of algal-bacterial system to tree species have shown very promising results e.g., *Chlorella sorokiniana* (Microalga), *Azolla filiculoides* (Aquatic macrophyte), *Portulaca oleracea* (Herb), *Bruguiera gymnorhiza* (Mangrove), *Eucalyptus perriniana* (Tree) help in BPA elimination from waste water (Hamada *et al*., 2002; Imai *et al*., 2007; Saiyood *et al*., 2013; Zazouli *et al*., 2014; Eio *et al*., 2015).

Plenty of studies have been already reported for aquatic invertebrates, fishes, amphibians, birds and mammals subjected to BPA exposure, but there is lack of information for aquatic flora. The objective of this review work is primarily understanding BPA toxicity and its impact on the aquatic plant groups, and to also provide information about possible remediation by these aquatic plant groups.

Bisphenol A and its analogues

BPA has been recognized as one of world's highest produced chemicals and quite well known as endocrine disrupting chemical (EDC). It is mainly used in manufacturing of polycarbonates, epoxy resins, medical equipment, electronic components, pipes, thermal paper, most of water and food containers along with many other household and electronic products (Staples *et al*., 1998). At ambient condition BPA shows solid crystals or flake like physical properties along with low volatility, higher melting point (150-155º C) and moderate solubility in aqueous solution (Staples *et al*., 1998).

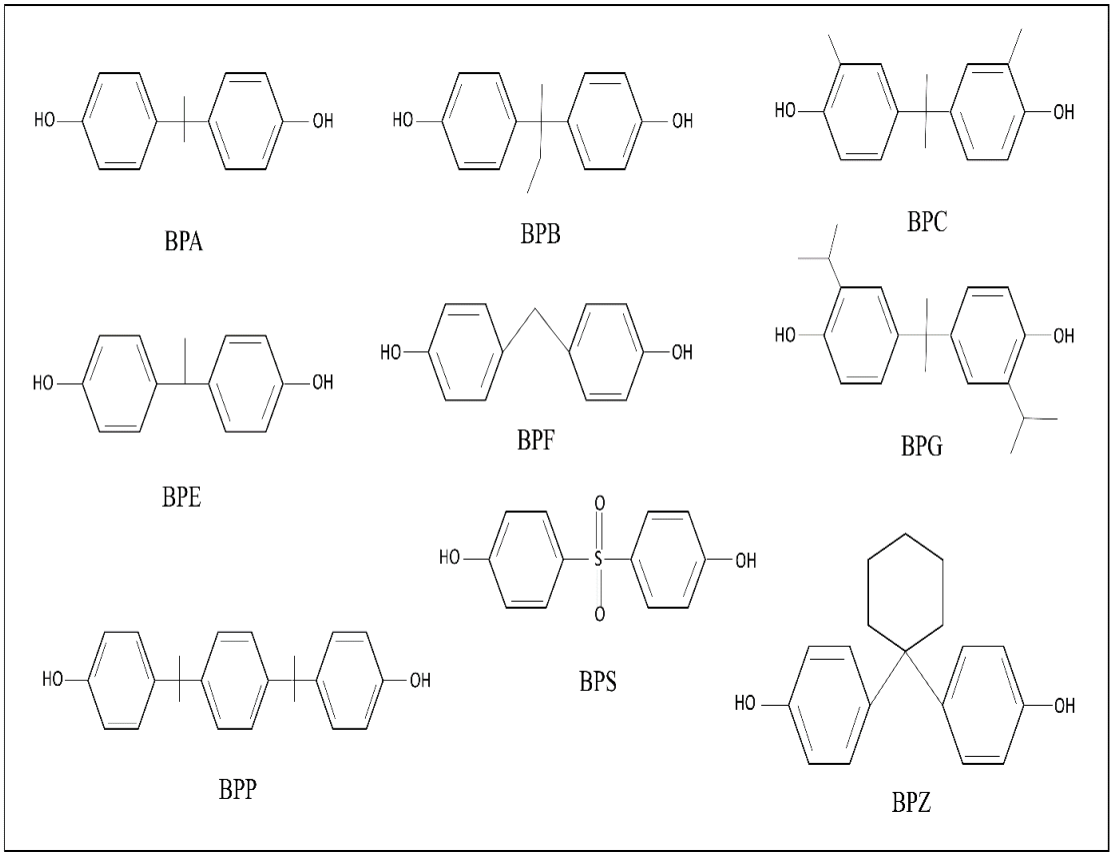

Figure 1 Chemical structures of Bisphenol A and its few common analogues.

A large number of chemicals are known to have structural similarity with BPA. In this connection, the chemical structures of few commonly studied BPA analogues have been presented in Figure 1. Among the analogues, Bisphenol F and Bisphenol S have also been widely used in polycarbonate manufacture. Several studies suggested most of these analogues also imparts EDC like functions (estrogenic, anti-estrogenic, androgenic, and anti-androgenic) with similar physiological effects like BPA (Rochester and Bolden, 2015). Few studies have claimed that some of the analogues has potential to replace BPA in the industrial sector.

Environmental distribution of BPA

Being one of the major disposed waste materials from a wide range of source, traces of BPA is present all over the environment. Environmental distribution of BPA is as follows -

BPA in air

Air is a geochemical reservoir for most of organic and inorganic particles (Sangai *et al*., 2016). BPA has lower volatility, although it can easily mix up with the atmosphere through industrial wastes (in particle form), which has been estimated to be approximately 100 tons per year (Sangai *et al*., 2016). BPA is also released via combustions of polycarbonate and epoxy resin products, domestic plastic wastes, paint spraying, electronic wastes etc. (Owens *et al*., 2007).

BPA in soil

Although BPA shows moderate affinity, slower biodegradation and lower half-life (1-10 days), the availability in soil and sediments, makes it notable pollutant (Sangai *et al*., 2016; Loffredo and Senesi, 2006). Land applied biosoilds or sewage sludge, mismanaged disposal of plastic wastes from industry, urban cites are among the primary source for BPA in soil (Lemos *et al*., 2010). With each passing year, record increase in the concentrations of BPA in the soil is occurring which poses serious concerns for many organisms. In a study, BPA has also been found to be interfere with the symbiotic association between *Sinorhizobium meliloti* and leguminous plant roots, indicating its prowess as a soil contaminant (Sangai *et al*., 2016).

BPA in water

Disposal of waste water, solid wastes from industry and households contains many water pollutants along with BPA and its analogues (Lee *et al*., 2015; Yu *et al*., 2015). Detection of BPA in different waterbodies has been reported which includes lakes, rivers, ground water, seawater and even present in drinking water (Sakai *et al*., 2007; Wan *et al*., 2018; Yang *et al*., 2014). Several studies have already determined BPA concentrations in various water bodies, including fresh and marine water, a list of which is presented in **Table 1**.

The concentrations of BPA vary according location, type of waterbodies or amount disposed waste materials. Observed BPA concentrations in Indian River waters ranges 0.006 µg/L to 14.8 µg/L (Lalwani *et al*., 2020; Yamazaki *et al*., 2015). Yamuna river reported most polluted and contains highest BPA concentrations (0.079 – 14.8 µg/L) among studied rivers in India (Lalwani *et al*., 2020). BPA levels in Indian municipal sewage industrial wastewater and drinking waters are 0.019 to 1.95 µg/L, 0.036 to

Table 1 Worldwide Bisphenol A concentrations in water surfaces (µg/L) and sediments (µg/g d.w.)

Study Locations	Source of Samples	BPA concentrations		Reference
		Values	Units	
Asia				
India	**River water**			
	Yamuna River (Near Delhi)	0.079 -14.8	µg/L	Lalwani *et al*., 2020
	Mula Mutha River (Maharastra)	0.1-0.15	µg/L	Lalwani *et al*., 2020
	Indus River (Jammu & Kashmir)	ND		Lalwani *et al*., 2020
	Hooghly River (Kolkata)	0.016-0.089	µg/L	Lalwani *et al*., 2020
	Cooum River (Chennai)	0.2-14.2	µg/L	Lalwani *et al*., 2020
	Kaveri River (Chennai)	0.006-0.13	µg/L	Yamazaki *et al*., 2015
	Adyar River (Chennai	0.05-0.51	µg/L	Yamazaki *et al*., 2015
	Lake water			
	Puzhal Lake	ND		Yamazaki *et al*., 2015
	Industrial and Municipal wastewater			
	Nationwide waste water	0.036-8.99	µg/L	Lalwani *et al*., 2020
	Sewage water (Patna)	0.019-0.022	µg/L	Karthikraj and Kannan, 2017
	Raw sewage water (Chennai)	0.054-0.077	µg/L	Karthikraj and Kannan, 2017
	Buckingham canal (Chennai)	0.835-1.95	µg/L	Yamazaki *et al*., 2015
	Drinking water			
	Nationwide sources	0.005-0.26	µg/L	Arnold *et al.*, 2013
China	**River water**			
	Pearl River (Guangdong)	ND-0.097	µg/L	Yamazaki *et al*., 2015
	West River (Guangdong)	ND-0.043	µg/L	Yamazaki *et al*., 2015
	Liaohe River (Liaoning)	0.006-0.14	µg/L	Jin and Zhu, 2016
	Liuxi River (Guangzhou)	0.075-7.48	µg/L	Huang *et al.*,2018
	Lake water			
	Taihu Lake	0.028-0.56	µg/L	Jin and Zhu, 2016
	Lake Basin (Eastern China)	0.001-5.35	µg/L	Zhang *et al.*, 2014
	Drinking waters			
	Nationwide sources	0.073-0.678	µg/L	Si *et al.*,2019
	Coastal area of Shenzhen	0.0011-0.776	µg/L	Liu *et al.*, 2010
Japan	**River waters**			
	Edogawa River	0.006-0.021	µg/L	Yamazaki *et al.*, 2015

	Tamagawa River	0.011-0.110	µg/L	Yamazaki *et al.*, 2015
	Arakawa River	0.003-0.057	µg/L	Yamazaki *et al.*, 2015
	Tokyo Bay	ND-0.431	µg/L	Yamazaki *et al.*, 2015
North	Fresh water surface	ND- 0.30	µg/L	Staples *et al.*,2018
America	Marine water surface	0.0011-0.024	µg/L	Staples *et al.*,2018
	Freshwater sediments	0.007-0.039	µg/g d.w.	Staples *et al.*,2018
	Marine sediments	0.001-0.1	µg/g d.w.	Staples *et al.*,2018
	River waters			
USA	Chateauguay River	ND-0.005	µg/L	Goeury *et al.*, 2019
Canada	Richelieu River	ND-0.004	µg/L	Goeury *et al.*, 2019
	Des Prairies River	0.005-0.017	µg/L	Goeury *et al.*, 2019
	Mille Iles River	ND- 0.007	µg/L	Goeury *et al.*, 2019
Europe	Fresh waters	ND-0.30	µg/L	Staples *et al.*,2018
	Marine waters	0.007-0.15	µg/L	Staples *et al.*,2018
	Freshwater sediments	0.007-0.177	µg/g d.w.	Staples *et al.*,2018
	Marine sediments	0.0003-0.063	µg/g d.w.	Staples *et al.*,2018
	River waters			
Portugal	Ave River	0.029-0.098	µg/L	Rocha *et al.*, 2013
	Cavado River	0.030-0.041	µg/L	Rocha *et al.*, 2013
	Douro River	ND-0.050	µg/L	Rocha *et al.*, 2013
Greece	Loudias River	ND- 0.138	µg/L	Arditsoglou and Voutsa, 2008
Spain	Garonne River	ND-0.060	µg/L	Gallart-Ayala *et al.*, 2010
	Waste water	0.115-0.183	µg/L	Gallart-Ayala *et al.*, 2010
Africa	**River waters**			
South	Bloukrans River	0.0173-0.477	µg/L	Farounbi and Ngqwala, 2020
Africa	Swartkops River	0.0067-0.341	µg/L	Farounbi and Ngqwala, 2020
	Tyhume River	0.017-0.117	µg/L	Farounbi and Ngqwala, 2020
	Waste waters	0.017-1.468	µg/L	Farounbi and Ngqwala, 2020
Polar region	Remote sites	11-17.3	pg/L	Fu and Kawamura, 2010

* ND- No detection, * d.w. - dry weight

8.99 µg/L and 0.005 to 0.026 µg/L respectively (Lalwani *et al*., 2020; Yamazaki *et al*., 2015; Arnold *et al*., 2013; Karthikraj and Kannan, 2017). Detection of BPA in major Chinese river and lakes also reported, ranges no detection to 7.48 µg/L (Yamazaki *et al*., 2015; Huang *et al*., 2018; Jin and Zhu, 2016; Zhang *et al*., 2014a). BPA in nationwide drinking water sources and coastal waters in China are 0.073 to 0.678 µg/L and 0.0011 to 0.776 µg/L (Liu *et al*., 2010; Si *et al*., 2019). Yamazaki *et al*. (2015) reported BPA levels in surface waters of Japanese rivers

detection to 0.431 µg/L in Tokyo Bay.

Rivers in USA and Canada contains very low concentrations of BPA (no detection to 0.017 µg/L) (Goeury *et al*., 2019). Major fresh water surface and sediments contains up to 0.30 µg/L and 0.007 to 0.039 µg/g dry weight (d.w.) BPA respectively (Staples *et al*., 2018). Similarly in European fresh water surface and sediments hold BPA up to 0.30 µg/L and 0.007 to 0.177 µg/g d.w. respectively (Staples *et al*., 2018). Recent reports have shown that the American and European marine surface water contains 0.011 to 0.024 µg/L and 0.007 to 0.015 µg/L BPA respectively (Staples *et al*., 2018). Detection of BPA was also reported in few European rivers e.g., Ave river (Portugal), Douro river (Greece), Loudias river (Spain) etc. (Arditsoglou and Voutsa, 2008; Gallart-Ayala *et al*., 2010; Rocha *et al*., 2013). Few rivers of South Africa also contain significantly higher levels of BPA e.g., Bloukrans River (0.0173 to 0.477 µg/L), Swartkops river (0.006 to 0.341 µg/L), Tyhume river (0.017 to 0.117 µg/L) etc. (Farounbi and Ngqwala, 2020). Trace of BPA was also reported in few remote sites (11 to 17.3 pg/L) of both poles (Ji *et al*., 2014).

Concentrations of BPA has been measured in multiple studies from water bodies and sediments, and great variations have been reported. From Table 1, we can conclude that the highest concentrations of BPA (i) in river water can go up to 14.8 µg/L, (ii) in marine water 0.431 µg/L, (iii) in sediments 177 ng/L, and (iv) highest environmental concentration of BPA (17.2 mg/L) found in landfill leachate.

BPA Toxicity on aquatic flora

Frequent studies suggested existence of BPA in almost every ecosystem and impacts all life forms. Recent studies have reported several effects of BPA including cytotoxicity, reproductive toxicity, genotoxicity, dioxin like effects, endocrine disrupting effect etc. on both plants and animals. The aquatic flora greatly diverse, from single cell phytoplankton to complex multicellular macrophytes, are variably affected by BPA. Effect of BPA on aquatic plant groups are discussed below:

Algae

In the aquatic food chain, algae play a key role as primary producers and found relatively sensitive to many chemicals (Abdel-Hamid, 1996). BPA toxicity to the algae was reported in several studies e.g., *Chlorella sorokiniana*, *Monoraphidium braunii*, *Chlorella pyrenoidosa*, *Nannochloropsis* sp., *Stephanodiscus hantzschii*, *Scenedesmus obliquus* etc. (Ishihara and Nakajima, 2003; Li *et al*., 2009; Gattullo *et al*., 2012; Zhang *et al*., 2014b; Eio *et al*., 2015; Guo *et al*., 2017). The laboratory-based reports describing the toxic effects on these algae are presented in **Table 2**. BPA incurs significant decrease in growth, inhibition in synthesis of chlorophyll and carotenoids, reduction in the efficacy of photosystems, cytotoxicity by generation of ROS, reduction in activity of antioxidative enzymes etc.

Macrophytes and Mangroves

Among free floating macrophytes *Lemna* sp. is found to be much sensitive to BPA. At 20 mg/L concentration of BPA, significant decrease in growth was observed due to reduction in leaf density and biomass (Mihaich *et al*., 2009). In a study, another free floating macrophyte *Azolla filiculoides* has been found to have reduced growth on exposure to BPA,

possible explanations could be reduction of photosynthetic pigments and inhibitory effects on nitrogen fixation (Zazouli *et al*., 2014). Raj *et al*. (2015) reported at 20 mg/L of BPA concentration, *Pistia stratiotes* show chlorosis, shriveling and shrinkage. On exposure to BPA for longer duration, significant change was observed in physiological responses in submerged macrophyte *Ceratophyllum demersum* (Zhang *et al*., 2017). Two other Macrophytes e.g., *Hydrilla verticillata* and *Potamogeton illinoensis* exhibits significant reduction in biomass, slower growth rate and increase in root density under BPA exposure (Zhang *et al*., 2017; Trueman and Erber, 2013). Zhang *et al*. (2007) found multiple effects of BPA toxicity e.g. decrease in plant growth, reduced rate of antioxidative enzymes (POX and GSH) and also shown disturbance in the cell membrane. Under exposure to BPA (40 mg/L), *Bruguiera gymnorhiza* (Mangrove) responded mainly

Table 2 Bisphenol A toxicity on aquatic flora

Aquatic Flora	Toxic Effects	BPA conentrations	References
Algae			
Chlamydomonas maxicana	Significant reduction in growth, dry weight and photosynthesis rate	10 µg/L	Ji *et al.*, 2014
Chlorella vulgaris	Inhibited growth and reduced chlorophyll content.	10 µg/L	Ji *et al.*, 2014
Pseudokirchneriella subcapitata	Reduced cell count and inhibited growth.	10 mg/L	Nakajima *et al.*, 2007
Ditylum brightwellii (Diatom)	Highly sensitive, reduction in chlorophyll and cell count.	0.1 mg/L	Ebenezer and Ki, 2016
Prorocentrum minimum	Highly sensitive, reduction in chlorophyll and cell count.	0.1 mg/L	Ebenezer and Ki, 2016
Tetraselmis suecica (Marine)	Sharp decrease in cell number and chlorophyll content.	2.5 mg/L	Ebenezer and Ki, 2016
Floating macrophytes			
Azolla filiculoides	Growth inhibition, reduction of N_2 fixation, photosynthesis.	25 mg/L	Zazouli *et al.*, 2014
Lemna gibba	Significant sensitivity, reduction in biomass and frond density.	20 mg/L	Mihaich *et al.*, 2009
Pistia stratiotes	Shrivelling, chlorosis and shrinkage of leaves	20 mg/L	Raj *et al.*, 2015
Submerged macrophytes			
Ceratophyllum demersum	Reduction in physiological responses and increase in POX activity	10 mg/L	Zhang *et al.*, 2017
Elodea nuttallii	Growth inhibition, reduced POD and GSH activity, cellular phospholipids disturbance	20 mg/L	Zhang *et al.*, 2007
Hydrilla verticillata	Reduction in growth due to accumulation in roots and leaves	20 mg/L	Zhang *et al.*, 2017
Potamogeton illinoensis	Decrease in dry biomass and higher root density on exposure	15 µg/L	Trueman and Erber, 2013
Mangroves			
Bruguiera gymnorhiza	Leaf damaging effects like necrosis and wilting	40mg/L	Saiyood *et al.*, 2013

through exhibiting leaf damaging symptoms like chlorosis, necrosis and wilting, along with reduced growth rate (Saiyood *et al*., 2013).

Possible Remediation

Environmental reduction or elimination of a compound up to non-toxic level from the air, water and soil has been one of the primary concerns shown by numerous researchers in last few decades. In water quality management, phytoremediation has been welcomed as one of best technique available due to its cost effectivity and ecological sustainability. In last few years, phytoremediation technique is universally accepted by several scientists, government authorities and also by common public, because of its great potential. Using green plants to degrade, remove, transform and stabilize organic or inorganic pollutants from air, water and soil has been of great interest for researchers due to being environment friendly approach, sustainable, low cost and energy requiring process for treatment (Loffredo *et al*., 2010; Okuhata *et al*., 2010). There are plenty of studies reported to employment of microorganism to higher plants for BPA removal from waste water. Also, assessment of few reports, claiming bioaccumulation, bio-transfer, absorption and metabolizing BPA by plants has been reported (Saiyood *et al*., 2010).

Bacteria

In the aquatic environment, BPA contamination is increasing at an alarming rate and immediate remediation is recommended for the sake of ecosystem revival. Several studies have reported that, in BPA removal one of the efficient method is to use bacterial strains. There are few bacterial strains that show potential efficiency in biodegradation of BPA by both gram-positive and gram-negative strains (**Table 3**). Among aquatic microbial strains *Pseudomonas* sp. (TA3, KA$ and KA5) has shown degradation capability of 66%, 90% and 91% respectively in 10 days duration from the initials BPA concentrations of 1000 µg/L (Kang and Kondo, 2002).

There are also few reports that show the potential of BPA degrading bacterial strains from sludge e.g., *Achromobacter xylosoxidans* and *Cupriavidus brasilensis* have shown efficacy up to 90% in 5 days of exposure (Fischer *et al*., 2010; Li *et al*., 2012). Soil and sediment residing microbial strains also exhibits potential ability to remove or metabolize 1000 µg/L BPA within 2- 3 days e.g., *Bacillus* sp. (up to 59%), *Klebsiella* sp. (up to 57%), *Enterobacter* sp. (up to 68%), *Bordetella* sp. (up to 41%), *Sphingomonas* sp. (up to 38%) etc. (Matsumura *et al*., 2009).

Algae

Algal groups also play a very important role in the remediation of many toxic chemicals due their versatility in habitat. Few fresh water and marine algal species has been studied for their role in BPA degradation efficacy (**Table 4**). Hirooka *et al*. (2003) reported a blue-green alga *Anabaena variabilis* and a green alga *Chlorella fusca* have shown capability to remove 23% and 85%, respectively from initial concentration of 10 mg/L BPA in a time duration of 5 days. Recently, multiple reports suggested different groups of algae from both fresh water and marine has BPA removal capability e.g., *Selanastrum*

Table 3 BPA biodegrading bacteria and their efficiency

Bacterial strains	BPA degraded or removed (%)	BPA concentrations (mg/L)	Duration (Days)	References
Water				
Pseudomonas sp. strain KA4	90%	1	10	Kang and Kondo, 2002
Pseudomonas putida strain KA5	91%	1	10	Kang and Kondo, 2002
Pseudomonas sp. strain TA3	66%	1	10	Kang and Kondo, 2002
Streptomyces sp.	90%	1	10	Kang *et al.*, 2004
Sphingomonas sp. strain BP-7	100%	1	40	Sakai *et al.*, 2007
Leachate and sludge				
Achromobacter xylosoxidans strain B-16	90%	3	5	Zhang *et al.*, 2007
Cupriavidus basilensis strain JF1	90%	1	5	Fischer *et al.*, 2010
Sediments and soil				
Bacillus sp. strain GZB	51%	5	8	Li *et al.*, 2012
Pseudomonas sp. strain SU1	51 %	1	2.5	Matsumura *et al.*, 2009
Bacillus sp. strains NO13	56%	1	2.5	Matsumura *et al.*, 2009
Bacillus sp. strains NO15	59%	1	2.5	Matsumura *et al.*, 2009
Bacillus sp. strains YA27	32%	1	2.5	Matsumura *et al.*, 2009
Klebsiella sp. strains NE2	51%	1	2.5	Matsumura *et al.*, 2009
Klebsiella sp. strains SU3	26%	1	2.5	Matsumura *et al.*, 2009
Klebsiella sp. strains SU5	57%	1	2.5	Matsumura *et al.*, 2009
Enterobacter sp. strains HI9	60%	1	2.5	Matsumura *et al.*, 2009
Enterobacter sp. strains HA18	68%	1	2.5	Matsumura *et al.*, 2009
Bordetella sp. strain OS17	41 %	1	2.5	Matsumura *et al.*, 2009
Sphingomonas sp. strains SO11	38%	1	2.5	Matsumura *et al.*, 2009
Sphingomonas sp. strains SO1a	34%	1	2.5	Matsumura *et al.*, 2009
Sphingomonas sp. strains SO4a	35%	1	2.5	Matsumura *et al.*, 2009

capricornutum (90%), *Chlorella sorokiniana* (50%), *Spirogyra sp.* (upto 96%),*Monoraphidium braunii* (48%), *Scenedesmus acutus* (64%),*Stephanodiscus hantzschii* (48%), *Skeletonoma costatum* (90%)etc.(Dorn *et al*., 1987; Nakajima *et al*., 2007; Li *et al*., 2009; Gattullo *et al*., 2012; Eio *et al*., 2015; Gracia-Rodriguez *et al*., 2015).

Macrophytes

The aquatic ecosystems are dominated by macrophytes and presents great potential to remove most of water pollutants. The macrophytes are major biological tools for phytoremediation, and also provides substrates and surface to the microbes and algae, which also play a role in

Table 4 Phytoremediation by algae and their efficiency

Algae	BPA degradation 0r removed (%)	Duration (Days)	BPA Concentrations (mg/L)	Reference
Fresh water				
Spirogyra sp.	96%	8	2	Garcia-Rodriguez *et al.*,2015
Selanastrum capricornutum	90%	4	2.7	Dorn *et al.*, 1987
Chlorella sorokiniana	50%	7	10	Eio *et al., 2015*
Monoraphidium braunii	48%	4	4	Gattullo *et al.*, 2012
Scenedesmus acutus	64%	8	2	Nakajima *et al.*, 2007
Chlorella fusca	85%	5	10	Hirooka *et al.*, 2003
Anabaena variabilis	23%	5	10	Hirooka *et al.*, 2003
Marine				
Ste*phanodiscus hantzschii*	48%	16	1	Li *et al.*, 2009
Skeletonoma costatum	90 %	4	1	Dorn *et al.*, 1987

accumulation, removal and metabolism of pollutants. Macrophytes can be classified into emergent, floating and submerged, on the basis of their residence on aquatic ecosystem.

Among the floating macrophytes *Lemna* sp. has been reported to have the efficiency to remove 1 mg/L BPA and requires more than 100 days (Reis and Sakakibara, 2012). However, free floating macrophytes like *Eichhornia crassipes* and *Pistia stratiotes* has been reported to have much faster rates of metabolizing BPA into β-glycoside through roots and then transported in to shoot with 100% efficacy in 3 days (Kang and Kondo, 2006; Raj *et al*., 2015).

Another emergent aquatic macrophyte *Ipomoea aquatica* has shown promising results and metabolize 100% of BPA (5mg/L) in a period of 21 days (Noureddin *et al*., 2004). Among the submerged macrophytes *Ceratophyllum demersum* (95%), *Elodea nuttallii* (50%), *Hydrilla verticillata* (62%), *Potamogeton crispus* (70%), *Myriophyllum spicatum* (83%), *Egeria densa* (27%) etc. have also shown good potential to remove BPA from the aqueous medium in a short time span of time (Zhang *et al*., 2007; Reis and Sakakibara, 2012; Zhang *et al*., 2017).

Conclusion

Urbanization and industrialization lead to increase in pollution by disposing off several pollutants in the environment, which causes severe damages to the ecosystem. Available studies have revealed that BPA induces cellular toxicity

Table.5 Phytoremediation by Macrophytes and their efficiency

Aquatic Flora	BPA degraded or removed (%)	BPA concentration (mg/L)	Duration (Days)	Reference
Floating				
Riccia fluitans	96%	1	110	Reis and Sakakibara, 2012
Azolla filiculoides	90%	10	20	Zazouli *et al.*, 2014
Lemna sp.	96%	2	8	Garcia-Rodriguez *et al.*, 2015
Lemna aoukikusa	73%	1	110	Reis and Sakakibara, 2012
Spirodella polyrhiza	60%	5	20	Li *et al.*, 2014
Pistia stratiotes	88%	10	3	Raj *et al.*, 2015
Eichhornia crassipes	100%	10	1	Kang and Kondo, 2006
Ipomoea aquatica	100%	5	21	Noureddin *et al.*, 2004
Submerged				
Ceratophyllum demersum	95%	5	10	Zhang *et al.*, 2017
Elodea nuttallii	50%	10	15	Zhang *et al.*, 2007
Hydrilla verticillata	62%	5	12	Zhang *et al.*, 2017
Potamogeton crispus	70%	5	10	Zhang *et al.*, 2017
Myriophyllum spicatum	83%	5	10	Zhang *et al.*, 2017
Egeria densa	27%	1	110	Reis and Sakakibara, 2012
Mangrove				
Bruguiera gymnorhiza	100%	20	51	Saiyood *at al.*, 2013

disturbance in cellular constructs, growth inhibition, photosynthetic hindrance and many more consequences in most of the aquatic flora. Although, toxicity of BPA on aquatic flora requires more studies for understanding the mechanism and pathways involving the accumulation and degradation of BPA. This review work focuses on the presence of BPA in environment and its effect mainly on the aquatic ecosystem. Increasing level of BPA release in environment demands strict regulation, policies and development of technologies to lower or eliminate this

toxic pollutant. In this connection, many bacterial strains and macrophytic species can be used having capability to remove BPA from aquatic systems in a sustainable manner.

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