

Phytoextracts as Crop Biostimulants and Natural Protective Agents

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Plants are the basic source of food, energy and dietary fibers for mankind. However, the production of cereal crops affected due to various biotic and abiotic factors due to anthropogenic activities. Fungal pathogens are responsible for plant diseases and cause high economic losses. Synthetic fungicides, which are toxic and harmful to the environment, are used to control plant diseases caused by fungal pathogens; the trend is shifting towards healthy, safe and sound ecofriendly control of fungal pathogens. Phytoextracts of *Beta vulgaris*, *Moringa oleifera*, *Citrus sinensis*, *Melia azedarach* and *Azadirachta indica* significantly inhibited the fungal growth and spore germination.

Keywords: abiotic stress ; biotic stress ; biostimulants ; phytoextracts

1. *Beta vulgaris*—Source of Glycinebetaine

Economically important cultivated beets such as fodder beets, sugar beets, garden beets (e.g., red beet) and leaf beets (e.g., Swiss chard) belong to the sub-species *Beta vulgaris* ^[1]. All beets originate from a halophytic plant, *Beta vulgaris* (sea beet or wild beet). Glycinebetaine (GB) is a quaternary ammonium compound naturally synthesized by various plant species. Involvement of GB in the protection of native protein from denaturation, cell membranes from oxidative damage and its contribution to cellular osmotic adjustments under water-limited environment make it a vital plant-osmolyte ^[2]. It is also involved in the regulation of various biochemical processes via systematic signaling pathways and studies also suggested its positive contribution to carbon, nitrogen reserves and reactive oxygen species neutralization ^[3]. Although several studies report different responses of *Beta vulgaris* to environmental stresses, research articles and reviews mostly focus on salt and drought response mechanisms in beets ^{[4][5]}. Therefore, we need breeding techniques and agronomic practices for better tolerance to biotic and abiotic stresses in *B. vulgaris* ^[6]. Thus, cultivated beets and their wild ancestor are important genetic sources for crop breeding programs and studying abiotic stress tolerance ^[5]. Sugar beet belongs to the family Chenopodiaceae, and beetroot also contains a significant fraction of antioxidants and other bioactive compounds such as betaine, betalain and ferulic acid ^[4]. Glycinebetaine was primarily discovered from sugar beet (*Beta vulgaris*), which accumulates GB up to 100 mM concentration ^[7]. These compounds can improve agricultural productivity through mitigation of adverse effects of environmental stresses on cultivated crops.

The exogenous application of GB improved plant growth and productivity under different stress conditions. Nowadays, a number of compounds including osmoprotectants such as proline and GB are used with exogenous application to plants to reduce the harmful effects of abiotic stresses including drought stress. GB, a quaternary ammonium substance, is an osmoprotectants that can effectively scavenge ROS in plant tissues ^{[8][9]}, and improves the photosynthetic rate by maintaining the Rubisco ultra-structure ^[10]. It is present in different amounts in plant parts including seed, stem, root and flowers ^[11]. During the early juvenile stage of plant, it is present in small amounts in the roots but later increases in leaves ^[12]. Different levels of GB can be observed in different plant species under different abiotic stresses depending on plant species, genotype, development stage, application modes and different stress conditions ^[13]. GB plays an essential role to provide protection from high accumulation of ROS species in plants under water shortage ^[14] and increases the photosynthetic defensive mechanism ^[2]. Rapid change in cellular metabolism, inferior level of water potential and ABA recognition sites give rise to accumulation of GB under water stress ^[10]. Furthermore, exogenously applied GB enhances yield and tolerance level by increasing chlorophyll contents, stimulating antioxidant defensive system, decreasing ROS and stabilizing the photosynthesis ability of photosystem II under drought stress ^[9]. The application of sugar beet extract also resulted in improvement in drought stress tolerance in okra plants through maintenance of ionic homeostasis which contributed to the better photosynthetic activity and yield attributes ^[5]. Similarly, improvement in growth and biochemical parameters of drought-stressed pea plants was recorded in response to sugar beet extract application ^[6]. Interestingly, economically important cereal crops such as wheat, rice, barley and maize do not synthesize or retain GB naturally. As a

way forward, exogenous application of sugar beet extract can be tested on major cereal crops to study its effects in abiotic stress tolerance particularly osmotic stress [15]. Moreover, various transgenic plants over-expressing GB biosynthetic genes and enhanced retention also exhibited drought and salinity tolerance.

2. *Moringa oleifera*—Source of Vitamins and Nutrients

Moringa, belonging to Moringaceae, is known as the “miracle tree” that has versatile uses in both animals and plants. The extract from *Moringa oleifera* serves as a cheap, eco-friendly, novel biostimulator, and bioenhancer that increases sustainable agriculture and crop production [15]. *Moringa* contains several essential components such as mineral nutrients, phytohormones (e.g., auxins, gibberellins, and cytokinins), vitamins, flavanols, phenols, sterols, and tannins, as well as several phytochemicals that make it highly beneficial for plants. It induces seed germination, plant growth, photosynthesis, and yields traits at a low cost. It also increases flowering, improves floral traits, fruiting, post-harvesting, and product quality of the fruit, and decreases senescence [16]. Plants are a rich source of different vitamins (carotenoids, B vitamins, ascorbic acid, tocopherols and quinines) that regulate biochemical and physiological processes and contribute to plant development and determine productivity. The *M. oleifera* Lam. is a tree found worldwide and is considered as bioregulator as it is a rich source of ascorbic acid, K^+ , Ca^{2+} , Fe^{2+} , riboflavin, carotenoids, phenolics and hormones including zeatin [17].

Exogenous application of *M. oleifera* extract improved seed germination and seedling establishment under normal and stress conditions [18]. Improvement in chlorophyll, activities of antioxidant enzymes and recovery in yield attributes of salinity stressed wheat are reported in response to *M. oleifera* extract application. Salinity tolerance in bean plants was also improved in response to foliar-applied extract of *M. oleifera* [15]. Another study reported that seed priming with *M. oleifera* extract mediated improvement in the germination and growth attributes of rangeland grasses such as *Cenchrus ciliaris*, *Echinochloa crus-galli* and *Panicum antidotale* [19]. The foliar application of *M. oleifera* extract mitigated cadmium toxicity in bean plants [20] and *Saccharomyces cerevisiae* [21]. Field trials are lacking which should be focus on future studies as *M. oleifera* extract could serve as a natural, cheap and green source of nutrients and vitamins that can be exploited to modulate crop growth and stress responses. *M. oleifera* roots, leaves, flowers, fruit, pods, and seeds have high nutrient values because it is rich in essential phytochemicals, e.g., minerals, vitamins, nicotinic acid, riboflavin, pyridoxine, β -carotene, flavonoids, glycosylates, phenolic acids, terpenoids, sterols, alkaloids, and fatty acids [20]. Therefore, it is used as herbal medicine and is known as a panacea. *Moringa* leaf extract has high nutrient and antioxidant value and is used as a therapeutic agent [21]. It serves as a potent antioxidant, as well as anti-inflammatory, anticancer, antimicrobial, antitumor, antitrypanosomal (control sleeping sickness), antiviral, antileishmanial, antidiabetic, antihypertension, and antispasmodic bioactive compounds [19]. Recently, *Moringa* seeds have been significantly characterized as having seed oil potential. *Moringa* seed extract is used against dyspepsia, heart disease, and eye diseases. *Moringa* seeds have strong antifungal activity against a zoophilic dermatophyte [22]. *M. oleifera* seeds contained active coagulant and antimicrobial agents, and this could be utilized for water purification as a viable replacement of proprietary chemicals such as alum sulfate [16]. Only in a few cases has an in vitro culture technique been used to promote the production of antioxidant compounds in moringa cells. Indeed, in recent decades, in vitro growth has been widely proposed as a means for inducing plant secondary metabolism, especially under stimulation by elicitors and stress conditions [15][16].

3. *Citrus sinensis*—Source of Ascorbic Acid

Ascorbic acid (AsA), also referred to as vitamin C, is a major nonenzymatic antioxidant in plants and plays an important role in alleviating certain oxidative stresses caused by biotic and abiotic stress [23][24]. AsA can enhance the growth of a plant and boost its capacity to withstand stress [25][26][27]. Moreover, AsA is the first line of plant defense against oxidative stress by removing a number of free radicals, such as $O_2^{\bullet-}$, HO^{\bullet} , and H_2O_2 , mostly as a substrate of APX, an essential enzyme of the ascorbate–glutathione pathway [10][28][29]. Ascorbate is a cofactor for several cellular enzymes, such as violaxanthin de-epoxidase, which is essential for photoprotection by xanthophyll cycle and other enzymes and is directly involved in the removal of ROS, and the addition of exogenous AsA will inhibit lipid peroxidation and decrease malondialdehyde (MDA) content in plant tissues, thus improving the antioxidant ability of plant tissues [24][30][31][32]. The effect of ascorbic acid on improving the salinity tolerance of potatoes was studied by Sajid and Aftab [33]. They noted that activity of most antioxidant enzymes, such as SOD, POD, CAT and APX, increased significantly under NaCl stress conditions after exogenous application of ascorbic acid, thereby improving plant survival under environmental stresses. Younis et al. [34] also stated that a marked and statistically significant increase in the percentage resistance to salt stress and growth of *Vicia faba* seedlings was caused by the exogenous addition of 4 mM ascorbic acid with NaCl to the stressful media during experimentation (12 days). Aly et al. [35] observed that addition of 1 mM of ascorbic acid to

Egyptian clover (*Trifolium alexandrinum* L.) seedlings grown in NaCl medium significantly increased seeds germination, carotenoids and chlorophyll and the dry mass of seedlings grown in NaCl medium.

Being a cofactor of various enzymes involved in phytohormone-dependent signaling cascades [36][37], it acts as a signaling molecule in various cellular and sub-cellular processes [38]. It can efficiently quench reactive oxygen species and thereby protect membrane structures and vital bio-molecules from oxidative stress [39]. The diverse involvement of ascorbic acid in the regulation of plant growth, physio-biochemical responses, flowering and most importantly stress sensing, signalling and regulation of ascorbate-glutathione cycle is well documented [40]. Sweet oranges are cultivated as the largest citrus fruit, and its global cultivation produces about 70% of total annual citrus yield [41]. The cultivation and production of oranges in Pakistan is ranked amongst the top suppliers. Sweet oranges are borne on a small flowering evergreen tree (7.5 to 15 m height) from the Rutaceae or citrus family and are rich source of vitamin C, and contain trace quantities of other vitamins and minerals including Ca, K, Mg, folate, thiamin and niacin [42]. Its juice is a good source of vitamin C, folate and polyphenols. The exogenous application of vitamin C improves stress tolerance among plants via regulation of cell expansion, ion transport, phytohormone signaling and reactive free radicals [30][43]. The use of *Citrus sinensis* extracts could potentially be an eco-friendly approach to induce multi-stress tolerance in plants and future studies should investigate its involvement and efficacy to regulate crop responses.

4. *Melia azedarach*—Source of Terpenoids

Melia azedarach is a deciduous tree of the *Melia* genus, which also commonly known as the purple flower tree, forest tree, and golden Lingzi. It is a fast-growing and high-quality timber tree; it is also a good nectar plant and a vital plant pesticide [44]. The timber, which resembles mahogany, is used to manufacture agricultural implements, furniture, plywood, etc. *Melia azedarach* is also of value for the health care and pharmaceutical industries, an effective composition due to its analgesic, anticancer, antiviral, antimalarial, antibacterial, antifeedant, and antifertility activity [45]. Furthermore, it is an important afforestation tree species, as are the surrounding greening tree species. *Melia azedarach* is widely distributed. It is native to tropical Asia and has been introduced to the Philippines, United States of America, Brazil, Argentina, African and Arab countries [44]. In China, it is concentrated in the south and southwest, with a relatively concentrated distribution in the east and central regions, and a marginal distribution area in the north, southwest, and southern Shanxi and Gansu [46]. For this reason, *Melia azedarach*, as a tree native to China, has diverse provenances [47].

Various naturally occurring secondary metabolites including terpenoids play developmental and regulatory roles among plants. Terpenoids are derived from isoprene units and such compounds serve as pigment molecules, vitamins, hormones and non-enzymatic antioxidants [48]. The diverse involvement of terpenoids in plant physio-biochemical functioning and regulation of stress tolerance is documented. The *M. azedarach* (Persian lilac or Chinaberry) is a deciduous tree from Meliaceae family is rich in terpenoids [44]. Different plant parts including fruit, root, bark, stem and leaf contain diverse chemical compounds such as azedarachins, trichillins, limonoids and meliacarpns. It is widely distributed in sub-continent countries including Pakistan, Nepal, Bangladesh, Sri Lanka and exhibit excellent medicinal properties [47]. Certain phenolic compounds also contribute to higher antioxidant activity of *Melia* [49]. Extracts of *M. azedarach* fruit were effective in controlling chickpea blight. Similarly, a pathogenic fungus, *Sclerotium rolfsii* was found to be controlled by the application of *Melia* extract [50]. Antifungal and antibacterial properties of the *M. azedarach* extract on pathogenic fungal species including *Fusarium oxysporum*, *Fusarium solani*, *Fusarium sambucinum*, *Fusarium oxysporum*, *Alternaria alternate*, *Botrytis cinerea* and bacteria including *Enterococcus faecalis*, *Escherichia coli* and *Bacillus subtilis* were prominent [51]. The application of *M. azedarach* leaf extract was reported to enhance salinity tolerance of pea plants [52]. The inhibitory effects of *M. azedarach* extracts were also recorded on germination and biochemical traits of radish [51] and future studies should investigate the crop-specific effect of *M. azedarach* extracts to potentiate its applications at larger agricultural scale.

5. *Azadirachta indica*—Source of Secondary Metabolites

About 135 compounds have been isolated from different parts of the *Azadirachta indica* (neem tree), and several reviews are available on the chemistry and structural diversity of these compounds [50]. As an ecologically friendly option, the formulation of biopesticides derived from the *A. indica* has been gaining interest. The main secondary metabolites responsible for the pesticide or antifeedant effecting *A. indica* are limonoids, or tetranortriterpenoids, azadirachtin being the most active compound [53]. *A. indica* cell culture is seen as an interesting alternate for the production of these secondary metabolites. In particular, stirred-tank bioreactors have been used for this purpose, although other reactor systems have been employed [54]. Additionally, shake flasks play an important role in the preparation of inoculum. However, the hydrodynamic environment resulting from the agitation speed and the bioreactor configuration affects the plant cell growth and the metabolite yield in stirred-tank bioreactors [50]. Therefore, it is important to establish the

relationship between the operating conditions of the bioreactor and culture response under hydrodynamic stress. The compounds have been categorized into two major classes such as isoprenoids and non-isoprenoids and exhibit incredible antifungal [55], antiviral [56], anticancer [57], antibacterial [58] and antioxidant properties [59]. Due to the presence of diverse secondary metabolites, neem extract application could induce biotic stress tolerance among plants against multiple pathogenic species.

Control of black scurf fungal disease in potato through exogenous neem extract is reported [60]. Neem extract mediated induction of biotic stress tolerance in pea plants against powdery mildew was linked with increased phenylalanine ammonia-lyase activity [54]. A recent study linked application of neem fruit extracts induced systemic acquired resistance in tomato plants against *Pseudomonas syringae* through increased activity of polyphenol oxidase enzyme [61]. Consistent with earlier reports, the application of neem and tulsi extracts reduced the severity of early blight of tomato through improvement in chlorophyll contents and increased antioxidant enzyme activities [62]. The use of neem extract suggested for management aphid attack on wheat [63] and corm-rot disease of *Gladiolus* [64] to prevent crop loss in Pakistan. Other than biotic stress, application of neem aqueous extracts improved growth and pigments which contributed improved photosynthesis in algae, *Nostoc muscorum* [65]. It is reported that neem extract reduced MDA contents and mitigated oxidative stress [65][66]. Based on the available literature, the application of neem extracts to crops can promote stress tolerance especially in response to pathogenic attack.

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