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Deleterious Rhizobacteria As A Potential Bioherbicide-A Review

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Abstract

Weed is a serious problem in crop production as it competes with the crop for essential growth factors and results in remarkable yield losses. Conventionally, many agronomic practices have been adopted for weed management, but they are less efficient, expensive, and laborious. Chemical herbicides are effective, but their long-term repeated use may cause weed resistance and serious environmental pollution. Considering all the secondary effects and environmental impact of herbicides, the future of weed management is to rely on alternative approaches such as the biological method of weed control. One such upcoming biological approach to control weed is the use of deleterious rhizobacteria (DRB). DRB is reported to suppress the weed dynamics providing scope for the crop to compete with the suppressed weeds for the essential growth requirements. This review focuses on the potentiality of DRB to be used as a bioherbicide.

Keywords: *bioherbicide, deleterious rhizobacteria, phytotoxin, Pseudomonas, weed*

I. INTRODUCTION

Weeds are a serious problem in crop production as it competes with the crop for light, water, CO_2, space, and nutrients. Among the pests, weeds account for a 45% reduction in yield as compared to 30% by insects, 20% by disease, and 5% by other pests [39]. If it is not effectively controlled in a cultivated area, weed may become a serious biotic factor affecting crop production. Looking into the ill-effects of weeds, many control measures have been taken up, such as cultural, manual, and mechanical methods, but these are less efficient, expensive, and laborious. Although the common and cheaper method of weed management, i.e., the use of herbicides, has been most favored due to its practicality and immediate results, it has harmful effects on our environment. Use of herbicide can cause pollution and ecological disturbance; it can leave poisonous herbicide residues and render a weed resistant to a particular herbicide. Reference [4] reported that sorghum crops grown in succession showed high sensibility to the residual activity of sulfentrazone, diclosulam, and imazethapyr. As reported by [11], weeds have evolved resistance to 21 out of the 25 known herbicide sites of action and to 152 different herbicides. The ALS inhibitors (126 resistant species) are most prone to resistance, followed by the triazines (69 species) and the ACCase inhibitors (42 species). Some weeds have developed multiple herbicide resistance. Considering all the secondary effects and environmental impact of herbicide, there is a demand to develop a more effective and environment-friendly weed management practice that minimizes the dependence on herbicide use. Hence, the biological method of weed control is becoming a more attractive option for weed management. One such upcoming biological approach to control weed is the use of deleterious rhizobacteria (DRB). The objective of this review is to focus on the use of DRB as a potential biological weed control agent.

The bacteria found in a different part of root-like rhizoplane, rhizosphere, and within the root, i.e., endorhizal bacteria or endophytes, are known as rhizobacteria [35]. Plant growth-promoting rhizobacteria (PGPR) are the non-infective beneficial organisms that enhance the growth of the plant through numerous mechanisms, *viz.*, plant growth regulator synthesis, assistance in nutrient uptake, induces disease resistance in host plant through the antagonistic effect on detrimental pathogens, and are also known as YIB (yield-increasing bacteria), biocontrol-PGPR or biofertilizers. On the other hand, deleterious rhizobacteria (DRB) are detrimental bacteria that negatively affect root growth and thus plant growth and yield. DRB is also popularly referred to as yield decline (YD) bacteria [22].

Although rhizobacteria are classified as PGPR or DRB, morphological and physiological characteristics do not help to differentiate them into a class or another [35]. The rhizobacteria can act as PGPR by having a positive influence on the growth and promotion of plants. Some ways by which rhizobacteria promote growth are nitrogen fixation, phosphorous solubilization, production of vitamins, growth hormones, and suppression of disease-causing pathogens. But if excess growth hormones are produced, then the growth of plants is suppressed, and the rhizobacteria are considered as DRB. DRB can suppress plant growth by some mechanisms like the production of hydrocyanic acid (HCN), phytotoxin, exopolysaccharide (EPS), and antibiotics, etc. There may be more mechanisms that are yet to be studied properly.

Advantages of using DRB as bioherbicides are environment-friendly, suppress weed growth, enhance selectivity, lower weed resistance and production cost [37]. Numerous studies revealed the novelty of DRB is a non-chemical approach to impair the growth and suppression of weed [18], [23]. The concept of weed management through DRB depends on the suppression of weed development before or coincident with the emergence of crop plants rather than the development of endemic disease on established weeds. Therefore, DRB suppresses the early growth of weeds so that the crop can compete with the weakened weed seedlings instead of the complete elimination of weeds [43], [23].

Some of the rhizobacterial strains possess a range of diverse properties that have different responses to plant species. Strains that only inhibited the growth of weed without negatively affecting crops can be considered as candidates for further tests as potential biological control agents [26]. [34] reported that *Azospirillum brasilense* strains L4 prevented the germination of the parasitic weed *Striga hermonthica* whereas it promotes the growth of sorghum [*Sorghum bicolor* (L.) Moench]. Wild radish growth was suppressed without any detrimental impact on the grapevine and subterranean clover by the strains of *Pseudomonas fluorescens* and *Alcaligenes xylosoxidans* isolated from that weed [6]. [32] informed that *Pseudomonas putida* FH160, *Stenotrophomonas maltophilia* FH131, and *Enterobacter taylorae* FH650 enhances the wheat biomass by diminishing the competitive ability of downy brome (*Bromus tectorum* L.) when wheat is infested by downy brome.

DRB is applied directly to the soil or vegetative residues for the suppression of weed growth through an attack of germinating seeds or emerging seedlings. Host specificity of rhizobacteria varies, with some isolates significantly suppressing the growth of host plants as well as non-host weed species and occasionally crop plants. For example, growth of green foxtail (*Setaria viridis* (L.) Beauv.) was suppressed by 57% of rhizobacteria isolated from several weed hosts, but morning-glory (*Ipomoea hederacea* L.) and barnyard grass (*Echinochloa crusgalli* (L.) Beauv.) growth was suppressed by only 32 and 37% of the rhizobacterial isolates, respectively [26]. Thus, DRB can be an innovative eco-friendly approach for weed management instead of applying hazardous chemical herbicides.

II. Mechanisms of DRB in weed suppression

Mechanisms by which rhizobacteria suppress plant growth are not fully understood. A few researchers reported that the ability of rhizospheric bacteria to produce various metabolites might be one mechanism that inhibits plant growth by disrupting various plant physiological processes. Overproduction of indole-acetic acid [40], [49], production of siderophores [30], extracellular polysaccharides [5], [16], and hydrogen cyanide [24], [36], [41] are some of the mechanisms of weed suppression by DRB. The presence of any one or all of these mechanisms may indicate potential growth-suppressive activity and may be useful in selecting DRB for use as a bioherbicide. The concentration of metabolites produced by DRB or its relative tolerance by plant species decides if a metabolite acts as a growth inhibitor or promoter [20].

A. Hydrogen cyanide (HCN)

Cyanide production is considered a trait of DRB because large amounts of HCN depress root respiration and indirectly impair nutrient uptake [41]. Inhibition of various processes like nitrate and CO_2 assimilation; disruption or reduction of oxygen in the electron transport chain in photosynthesis, and cytochrome respiratory chain are performed by HCN, which is a toxic gas that forms metal complexes with various enzymes' functional groups. The concentration of HCN produced by DRB determines the ability of specific isolates to be deleterious [24]. The availability of precursors such as methionine, glycine, and proline and the cyanogenic glucosides determine the rate of HCN produced [19]. The environmental factors, i.e., soil water potential, light intensity, nutrients, etc., affecting root exudation may also be another important aspect [42]. HCN has been reported as phytotoxic to *Lactuca sativa* and *Echinochloa crusgalli* [24], [52]. Pseudomonads like bacteria are a major group of potential DRB having the ability to produce cyanide. Reference [25] shown that weed suppression of *Amaranthus spinosus* and *Portulaca oleracea* in both laboratory and glasshouse trials were due to cyanide produced by *Pseudomonas aeruginosa*. However, wheat (*Triticum aestivum*) seedlings treated with *P. aeruginosa* exhibited less inhibitory effect than weed seedlings. Rhizobacterial isolates *Xanthomonas spp.* Having the ability to produce HCN as a secondary metabolite was tested to inhibit the growth of *Parthenium hysterophorus* by 32-53% and crop plants by 17-47% under laboratory conditions [37]. HCN produced by pseudomonads were also reported to significantly reduce the seedling growth of lettuce, barnyard grass, and green foxtail [24]. Therefore, cyanogenic DRB can be used as an alternate approach for weed management.

B. Plant growth regulating substances

Indole-3-acetic acid (IAA) is a plant growth regulating compound which is an active form of the auxin-type compound in plants. Some of the bacteria associated with plant release IAA, which promote plant growth, and simultaneously IAA enhances leakage of plant cell nutrients through cell wall loosening, which promotes the growth of nutrient-deficient bacteria[29], but the plant growth is retarded when there is over the release of IAA [38]. Excess ethylene concentration causes inhibition of root growth and elongation. When an excess amount of IAA is produced, it stimulates aminocyclopropane-1-carboxylate (ACC), which is a precursor of ethylene in plants. Growth suppressive effects appear to be related to root colonization because the density of bacteria on root surfaces is often proportional to IAA production[47]. Auxin is released by microorganisms in the presence of suitable precursor such as L-tryptophan (L-TRP). [40] reported that under laboratory conditions, root growth of field bindweed (*Convolvulus arvensis* L.) was inhibited up to 90.5% by *Enterobacter taylorae* having high auxin producing potential.

Ethylene is a volatile plant growth regulator which affects the physiology of the plant. A lower concentration of ethylene augments root growth and length, but a higher concentration of it suppresses the growth. High levels of ethylene produced by some rhizobacteria are used as germination biostimulants of the parasitic weed Striga sp. which is then used as a potential biological control strategy [1].

C. Phytotoxins

One of the mechanisms of action towards the suppression of plant growth is the production of phytotoxins by bacteria [8]. Less work has been initiated on the phytotoxins responsible for weed suppression. Some of the phytotoxins are reported to affect the plant metabolism through a decrease in cell membrane integrity and macromolecule synthesis[48], [35]. Root growth inhibition of downy brome (*Bromus tectorum*) by *Pseudomonas fluorescens* strain D7 [48] was due to complex of peptides and fatty acid esters in a lipopolysaccharide matrix [10]. Phenazine-1-carboxylic acid, 2-amino phenoxazone, and 2-amino phenol released by *Pseudomonas syringea* strain 3366 inhibited the growth of downy brome [8]. Disrupted plant cell membranes and walls of leafy spurge (*Euphorbia esula*) roots colonized by DRB as observed under electron microscope were due to the production of unidentified phytotoxins and/or enzymes [45]. *Pseudomonas fluorescens* isolate WH6 produces Germination Arrest Factor (GAF), which is associated with a particular ninhydrin-reactive compound, 4-formylaminooxyvinyl-glycine, a member of naturally occurring compounds known as oxyvinylglycines[33], which known to block reactions catalyzed by enzymes dependent upon pyridoxal phosphate as a co-factor. These enzymes include ACC synthase, which catalyzes a critical step in the biosynthesis of the plant hormone ethylene.

D. Exopolysaccharide (EPS)

Pseudomonas spp are considered as potential plant pathogens which have the ability to produce a diverse range of EPS. EPS causes wilting of plants through the facilitation of systemic colonization of plant tissue with bacteria which interferes with the movement of water through xylem vessels [13]. Survival of plants is hampered when EPS are produced by bacteria under adverse environmental conditions such as moisture stress and high temperatures [5]. Reductions in leafy spurge (*Euphorbia esulavirgata*) callus growth were frequently associated with rhizobacterial isolates expressing both EPS (exopolysaccharide) and HCN activity [22]. *Xanthomonas campestris pv poae* (strain JT-P482) has been used as bioherbicide, causing significant wilting in annual bluegrass without detrimental effect on other turf grasses via the production of a polysaccharide substance that prevents water transport [14], [7]. This bacterium multiplies in the vascular system and causes wilting and wounds in the stem and leaf tissues and, finally, death of the annual bluegrass without affecting desirable turfgrass species.

E. Effects of combined mechanisms

Often a single DRB is reported to exhibit several mechanisms for plant growth suppression. In a study, leafy spurge suppression by a DRB was due to several mechanisms, viz., HCN, IAA, and EPS production [22]. Thus, DRB may function through multiple mechanisms of action. [50] and [3] reported that DRB strains with single mechanisms of action that coexist in the rhizosphere might function synergistically to suppress plant growth.

III. Factors affecting the efficiency of DRB

The rhizosphere population dynamics may be governed by many interacting processes influencing their population size. These processes may include bacterial growth, survival, death, emigration, and immigration as influenced by the chemical, biological and physical environment of the rhizosphere [31]. The rhizobacterial colonization in the roots may be sensitive to soil factors like soil pH, texture, and organic carbon [27], [12], soil water [28], and temperature [31]. The distribution of bacteria on root systems also may vary with temporal and spatial variations in root exudates quality or quantity [15] and microfloral competition. The frequency of presence of DRB in the soil is also influenced by tillage. In conventional or reduced tillage, more DRB inhibitory effects towards downy brome (*Bromus tectorum*) and jointed goatgrass *(Aegilops cylindrica*) were noticed compared to no-tillage [23]. Continuous monoculture flourishes certain kinds of rhizospheric microorganisms [51]. The higher proportion of DRB was associated with some agronomic practices that result in relatively higher organic matter through perennial forage and pasture systems, organic farming, and integrated cropping systems. [21] experimented with different cover crops associated with deleterious rhizobacteria and reported that some DRB reduced the growth and biomasses of weeds associated with cover crops. Reference [53] reported that weed suppression by deleterious bacteria was affected differently by formulations and soil properties. Corn gluten meal and semolina flour formulated with selected DRB was reported to be good formulation source for weed suppression. For the successful biocontrol with DRB, it is important to match the most virulent stage of biocontrol agent with the susceptible stage of weeds and an active growth period of the host [46].

IV. Criteria of a successful bio-agent

An effective biological control agent should possess certain characteristics. Most importantly, biological control agents must first have fairly narrow host specificity or selectively suppress a target organism without adversely affecting non-target species. The host range has to be broad enough to include similar weed species. Suppression of the pest does not necessarily need to be 100 percent effective. Secondly, the agent must survive and function in an environment where it is introduced. It is very easy to find antagonistic relationships in the laboratory, but often these relationships do not persist in field situations under varying environmental conditions. The microorganism must survive and proliferate on the leaf or in the soil and tolerate fluctuations in moisture and temperature. Formulation and application technology can be developed to enhance the survival and bioactivity of biocontrol microorganisms. Thirdly, the biocontrol agent must suppress the target organism at a critical point in its growth. Weed characteristics are critical for biocontrol success. These include factors that influence seed, root, or leaf colonization; infection by the biocontrol organism, and the competition of the weed with the crop. Information on economic suppression and the biological interaction with herbicides and other stresses is also needed. Finally, the biological control procedure must be practical, economical, and compatible with other methods of weed control [17].

V. Symptoms of infected weeds by DRB

DRB can inhibit root and shoot growth and yet may not produce any obvious symptoms. Foliar symptoms, when present, resemble nutrient deficiencies. DRB may also cause browning and discoloration of roots, necrotic reactions, distortion of leaves and roots, as well as inhibition of root hair development [41]. Root browning does not necessarily indicate necrosis, although necrosis may occur with virulent isolates of DRB. Generally, DRB does not cause seedling death, although seedling mortality has been recorded in some laboratory and pot experiments [9], [2]. Chlorosis, necrosis, distortions of leaves and roots are the distinct symptoms that might be due to the high DRB inoculum numbers [41].

VI. Constraints of using bioherbicides:

The success of using bioherbicides has been limited by several factors [44], viz., narrow spectrum of activity, poor survival or activity of the introduced DRB due to various environmental impact, the lower shelf life of DRB, difficulty in large-scale production, storage and commercialization. Moreover, the introduced DRB may have an antagonistic effect by the chemical herbicides, and it is also difficult to avoid injury to non-target organisms.

VII. Conclusion

The plant rhizosphere consists of both plant growth-promoting rhizobacteria (PGPR) and deleterious rhizobacteria (DRB). Proper management is thus necessary to enhance the population of DRB to suppress weed growth, which indirectly promotes crop due to the competitive advantage gained by the crop. In organic agriculture, DRB has the potential to be used as a bioherbicide replacing chemical herbicides. Their application in the field in correlation with crop plants requires vigorous work and investigation to avoid the adverse effect of DRB on crops under different conditions. Extensive investigations are needed to gain an understanding of the mechanisms involved in weed suppression by DRB and inoculum response in diverse cropping systems and edaphic factors. Moreover, appropriate inoculum technology has to be developed to accomplish targeted weed control through the introduction of DRB as biocontrol agents.

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