Chapter

# Application Potentials of Plant Growth Promoting Rhizobacteria and Fungi as an Alternative to Conventional Weed Control Methods

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## Abstract

Weeds are the plants usually grown on unwanted places and are notorious for causing interruptions in agricultural settings. Remarkable yield losses have been reported in fields infested with weeds worldwide. So far, these weeds cause about 34% of losses to yields of major agricultural crops and pose threats to economic condition of the farmers. Conventionally, weed control was achieved by the use of chemical herbicides and traditional agronomic practices. But these methods are no more sustainable as the magnitude of threats imposed by these conventionally outdated methods such as chemical herbicides is greater than the benefits achieved and their continuous use has disturbed biodiversity and weed ecology along with herbicide resistance in some weeds. Herbicide residues are held responsible for human health hazards as well. Therefore the future of weed control is to rely on alternative approaches which may be biological agents such as bacteria and fungi. This chapter highlights the potentials of using bacterial and fungal biocontrol agents against weeds in farmer fields. Moreover, detailed review on merits and demerits of conventional weed control methods is discussed in this chapter.

**Keywords:** biological weed control, PGPR, fungi, environment, human health, economic losses

## 1. Introduction

Agriculture is an approach of deploying natural resources to grow the desired plants. Since the induction of green revolution in the 1950s, the food production has been substantially increased that helped to meet food demands for the ever-increasing world population [1]. Improved irrigation practices, tillage implements, fertilizers, and farm operations were some of the key outputs of green revolution. Nevertheless these practices have paved the way of agricultural sustainability yet there are some concerns associated with these practices as, improved irrigation have given rise to salinity of soils, intensive tillage causes deterioration of soil structure, loss of soil organic carbon and destruction of natural habitats of different flora, higher yielding crop cultivars depleted soil nutrients. With all of the outputs of green revolution, introduction of pests is also acknowledged [2]. Disturbance in agricultural production due to invasion of other living organisms for their own existence is a natural phenomenon which cannot be stopped. These living organisms that survive on others are called as pests which include insects, plant pathogens, nematodes, rodents, and weeds.

Among the agricultural crop pests, weeds are the most potent crop pests reducing crop yields by almost 34% followed by animal pests (18%) and plant pathogens (16%) worldwide [3]. Weeds are unwelcomed plants that interfere with the management of agricultural production systems, compete with the main crops for available nutrient resources and space and reduce growth, yield, and quality of agricultural produce up to a certain extent [4]. Generally, they produce a larger number of seeds, which may remain dormant in the soil seedbank for several decades, having greater plasticity and equipped with specialized seed dispersal mechanisms. Further, they exhibit the ability to invade newly disturbed areas and compete with crops for scarcely available moisture, nutrients, and light [5]. Apart from yield and production losses, they may also provide niches and harbor insects, plant pathogens, and other pests, hence increasing their incidence of attack to the main crop [6]. Weeds are the firstborn problem in agriculture since about 10,000 BC [7] representing the main hindrance in profitable agricultural production under natural resource management. The presence of weeds in natural ecosystems causes various direct and indirect losses, including interference with successful crop production, damage to biodiversity, loss of possibly fruitful land, loss of grazing areas and livestock production, obstruction of navigational and irrigation channels, and reduction of available water in water bodies. Most of the weeds belong to families Poaceae and Asteraceae. A majority of the weeds are terrestrial plants, a few are aquatic weeds and some are parasitic weeds [8]. Globally, reduction losses of wheat yield due to weed infestation are 23% [2]. The economic losses incurred due to this wheat yield reduction amount to Rs. 146 billion [9].

In the light of the abovementioned properties and harmful effects of weeds, it becomes important to control them. Appropriate weed control strategy in arable soils employs both the direct and indirect methods. Direct methods include those with the prime objective of weed control such as mechanical, manual, chemical and biological weed control and indirect being the cultural and preventive practices reducing germination, growth and vigor of weeds [10]. Many practices are available to control and manage weeds in agricultural crops. In ancient times when synthetic herbicides were not introduced, people tried polyculture, crop rotation, and other management practices that have shown sustainability with low inputs [11]. Until recently, weeds were being controlled by manual, mechanical, and chemical methods [12]. However there were drawbacks associated with each of these methods that severely limited their practical use, for example, herbicides cast detrimental effects on environment, humans, and animals [13]. They also cause contamination of water bodies and pollute natural resources like air, soil, and plants, thus destroying nontarget entities such as wildlife [14]. Also due to repeated herbicide applications, there is an increasing trend in herbicide-resistant weed species [15]. Mechanical weeding on the other side requires several repetitions and is only feasible for crops sown in rows; therefore weeds grown near to crop plants and within rows are escaped of control [10]. Similarly, hand weeding needs a huge number of labor and hence cannot be applied on a large scale [10]. Therefore, repeated manual weeding and nonavailability of labor make this method unfeasible and uneconomic [16].

Hence, the prevailing situation demands some weed control measures other than chemicals, and in this context, biological control is gaining much importance around the world. Biological control is a general term used to define the introduction of organisms mostly bacteria and fungi in order to solve one or more problems in the farmer's field [17, 18]. Biological control using bacteria (bacterial herbicides), fungi (mycoherbicides), and viruses has recently gained much attention. Different kinds of fungi showing herbicidal activity are potential candidates of *Phoma* and *Sclerotina* genera. Among the bacteria some members of *Pseudomonas* and *Xanthomonas* depict these attributes.

Broadly speaking the control of weeds using microbes in green areas is a green approach that may reduce costs, decrease dependence on synthetic chemicals, and lower the negative impressions of chemicals on the environment. Microorganisms in the form of bioherbicides can be more selective than synthetic chemicals (herbicides) and target only the desired species [19]. Bioherbicides also lessen the chances of induction of resistance in the target weed species, due to the involvement of a number of mechanisms [20]. Therefore, keeping in view the abovementioned (even more) limitations of conventionally outdated methods necessitates the adoption of newer methods based on biological agents that are environmentally safer, friendly, economic, and feasible. We tried to highlight the need for adoption of innovative methods of weed control with higher efficacy. We then focused on harmful aspects of the judicious use of herbicides that in turn causes threats to environmental quality, food security, and human health followed by future research aims for improvement.

## 2. Weed control options

About one third of the total costs in field crop production is taken away by the weed management. There exist a variety of weed control strategies that can be applied depending upon various cropping systems [21]. Traditional farming practices generally rely on the application of herbicides and manual weeding. Generally, weed control measures include physical, chemical, and biological methods.

### 2.1 Physical weed control

Physical approaches of weed control include mechanical (tillage), manual methods, crop rotations, and crop fertilization and are separately discussed with possible limitations.

An increase in the density of weed species has been observed where monocropping was adopted. However due to the diverse nature of crop rotations, the density of such weeds can be tackled for profitable crop production [22]. Using a cover crop in rotation with the main crop is an attractive solution to cope with weed infestations [23, 24]. The integration of cover crops with no-till system has shown significant reduction (78%) in weed density in the USA [25]. The weeds with similar life cycles that match with the crop pose serious threats to crop production. These cover crops when used properly in rotation with the main crop compete with weeds for available nutrients, light, space, and water sources, hence reducing their emergence and numbers [26]. However the ability of cover crops to control weeds is largely governed by the growth habit and performance of the cover crop in a desired area [27]. That makes the use of this method to be only a small scale.

Increasing the competitive ability of crops against weeds is an important aspect to avoid field losses due to weeds and has been seen as a strategy for integrated weed management systems [28]. It can be achieved through manipulating fertilizer timing, rate of fertilizer, and placement methods effectively [29]. Nitrogenous fertilizers have been known to involve in the activation of dormant weed seeds, thus directly affecting specific weed densities. The most agricultural weeds have shown growth rates equal to that of wheat in response to the added nitrogen [30]. However, it is not well known that phosphorus levels of soil affect weed growth and crop as well, but it is a fact that the crop-weed competition is considerably affected by phosphorus fertilizations, for instance, Bansal [31] reported that weed-crop (fenugreek) competition was increased with higher P levels. Similarly, Santos et al. [32] reported that lettuce (*Lactuca sativa* L.) showed a higher competitive ability than the common purslane (*Portulaca oleracea* L.) but not smooth pigweed (*Amaranthus hybridus* L.) with higher P levels than lower levels. Therefore, due to this uncertainty, this method is not widely adopted and acceptable.

Manual weed control methods involve plucking, uprooting, and hoeing with and/or without hand-driven machines [33] and are in use since ancient times. Manual weed control is one of the most efficient methods and is applicable in areas where the labor is easily available. However, immediate availability of labor before the weeds have grown in crops [10], repeated hand weedings [16] and adoptation on only small scale farming are the major limitations of this method to be adopted. Mechanical methods use tillage implements such as cultivators, weeders, and different types of harrows which are being drawn by animals (in the past) or by engines (until recently) around world [34]. Tillage practices in the field affect weed management, weed seed bank in the soil, and soil disturbance patterns. Deep cultivation can be used to burry weeds that germinate in the upper soil layers such as *Phalaris minor* in wheat. However, timely sown wheat in integration with zero tillage has shown significant results in the reduction of *Phalaris minor* infestations, obtaining higher grain yields of wheat [35, 36]. Tillage for weed control is not suitable for all crops and is only limited to crops sown on rows with suitable row-to-row spacing. Weeds that grow in close association with crop plants are not managed properly by this method, and those weeds which are grown within crop rows cause more losses than those sown in between crop rows [10, 37]. Moreover, some weeds may regenerate which are not completely uprooted, and root injury to main crop may occur [38]. However, the use of tillage implements for weed control are associated with adverse environmental impacts such as deterioration of soil structure, disturbed soil biological processes and soil erosion [39], leaching of nutrients which would otherwise be available to plants and eutrophication [40]. Therefore the efficiency of mechanical weed control measures is less than that of chemical weed control [22, 38]. Tillage practices done for weeding aggravate more soil compaction than other tillage operations due to a shorter cover of wheel tracks [38].

### 2.2 Chemical weed control

The application of synthetic chemicals for crop protection began after the second world war when most of the selective herbicides for broad-leaved weeds were commercialized in 1946 [41]. However, with the advancement in crop protection measures usually at the start of the twentieth century, copper and sulfuric acid containing herbicides were developed [42]. Herbicides are chemical compounds which kill or control weeds and are largely synthesized by crop protection industries nowadays available for almost all cultivated crops. They were rapidly adopted by farming communities as they do not require much labor and hence are not costly; no risks of soil erosion and energy efficiency are further advantages of herbicides [43]. The most widely used chemicals in wheat to control grassy and non-grassy weeds are clodinafop, tralkoxydim, Atlantis (meso-/iodosulfuron), sulfosulfuron, and pinoxaden. However, for the control of broad-leaved weeds, major chemical herbicides are carfentrazone, 2,4-D, and metsulfuron [44]. Herbicides account for 44% of all pesticides worldwide [45]. Nevertheless, chemical methods have controlled the weeds resultantly improving the yields of diverse crops from 10 to 50% [4]. However, the continuous application of such herbicides had led to intraspecific selection of weeds and caused the development of herbicide-resistant biotypes of weeds [46, 47]. Approximately, 300 herbicide-resistant weeds have been reported in 15 families of synthetic herbicides [45, 48, 49] (**Table 1**).

A major portion of applied herbicides falls on nontarget species and soil [50]. Some herbicides like triazines and sulphonylureas may persist in soil long enough to affect the growth of subsequent sensitive crops [38]. Herbicides have also caused toxicity and diseases to exposed animals [51]. Herbicides in soil however may not reduce the population of soil microflora and microfauna but may induce intraspecific and interspecific selections [38].

The magnitude of issues caused by herbicides is much bigger than the outcomes of herbicides (**Figure 1**). Therefore it is a dire need of the hour to move toward some newer methods other than chemicals that can ensure environmental safety and resource conservation and sustain crop production economically.

| Herbicide-resistant weeds | Common names | Herbicide (s) |
|---|---|---|
| *Eichhornia crassipes* | Water hyacinth | 2,4-D,Glyphosate |
| *Chenopodium album* | Common lambsquarters | Triazine |
| *Salsola kali* | Russian thistle | Sulfonylurea |
| *Senecio vulgaris* | Common groundsel | Triazine (atrazine) |
| *Sesbania exaltata* | Hemp sesbania | Glyphosate |
| *Cyperus* | Purple nutsedge | Sulfonylureas |
| *Avena fatua* | Wild oat | Glyphosate |

**Table 1.**
*Some worst weeds that evolved resistance against chemical herbicides.*

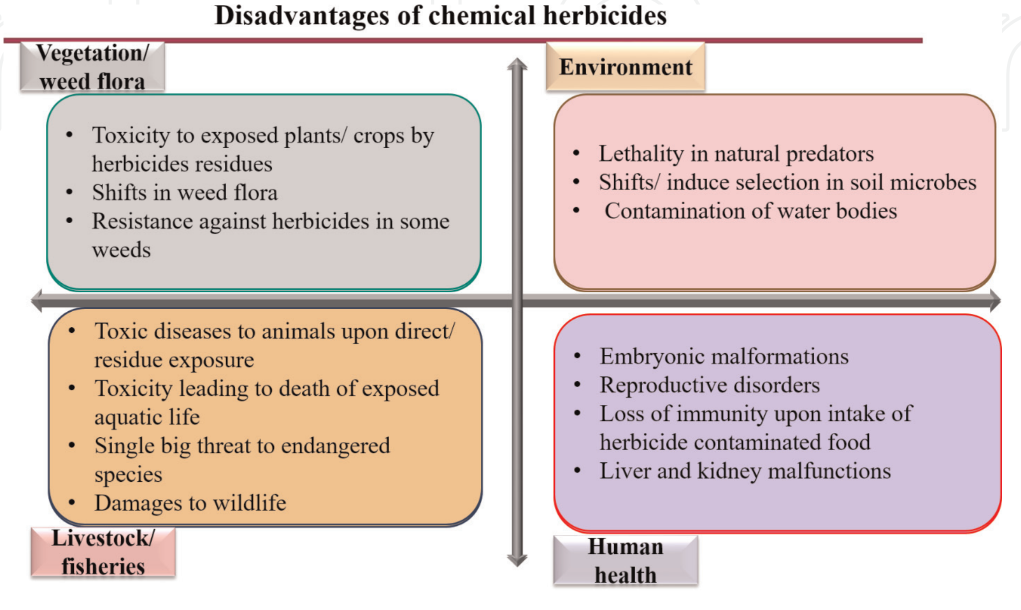


**Figure 1.**
*Disadvantages of herbicides to all life forms. Modified and redrawn from [1].*

### 2.3 Biological weed control

Biological control is the intentional use of biological agents (living organisms) to control plant pathogens or weeds in fields [52, 53]. The application of herbicides for sustaining agricultural production has created so many problems such as contamination of groundwater, destruction to the nontarget species, and induction of resistance against herbicides in a number of weed species [45], and other control methods become even more unsuitable where the land value is small and unaccessible with widespread weed infestations. This situation paved the way of researchers to move toward biological control as an alternative option in weed management. The chemical herbicides can persist in soil for longer periods of time, have limitations for crop rotation, and cause damage to the nontarget organisms [54]. Microbial herbicides on the other hand are more selective and affect only the target species [19]. The other advantage of using microbial agents is the reduced chance of induction of resistance in the target species [20].

Primarily there are two fields of application within the context of biological weed control, viz., the classical and augmentative or inundative. Classical biological control is the introduction and subsequent discharge of a natural enemy of a pest predator with the objective to reduce its virulence without becoming a pest itself [55]. This method is suitable for the control of perennial weeds that grow over a range of large areas such as in the forests, rangelands, along waterways, and roadsides and where reduction in weed competitiveness is required [56]. Several agents might be used in this strategy such as insects, fungi, mites, and different herbivores. The inundative biological control also called as bioherbicide approach is the

| Trade name | Microbe(s) involved | Target weed(s) | Representative/initial report reference |
|---|---|---|---|
| BioMal | *Colletotrichum gloeosporioides* f. sp. *nalvae* | Round-leaved mallow | [60] |
| Casst | *Alternaria cassia* | Sicklepod, coffee senna | [61] |
| Biochon | *Chondrostereum purpureum* | Woody weeds | [62] |
| Collego | *Colletotrichum gloeosporioides* f. sp. *aeschynomene* | Northern joint vetch | [61] |
| *Phoma* | *Phoma macrostoma* | Broadleaf weeds | [18, 63] |
| Devine | *Phytophthora palmivora* | Strangle vine | [64] |
| Camperico poae | *Xanthomonas campestris* pv. | Annual bluegrass | [65] |
| Hakatak | *Colletotrichum acutatum* | *Hakea sericea* | [17] |
| Myco-tech | *Chondrostereum purpureum* | Deciduous tree species | [66] |
| Smolder | *Alternaria destruens* | Dodder | [67] |
| Dr. Biosedge | *Puccinia canaliculata* | Yellow nutsedge | [68] |
| Lubao | *Colletotrichum gloeosporioides* f. sp. | Dodder | [61] |
| Woad warrior | *Puccinia thlaspeos* | Dyer's woad | [69] |
| Chontrol | *Chondrostereum purpureum* | Alders and other hard woods | [66] |
| Sarritor | *Sclerotinia minor* | Dandelion | [70] |

**Table 2.**
*Successful microbial herbicides (registered) worldwide.*

application of mass-produced fungal spores or bacterial cultures in higher concentrations with the objective to eradicate invasive weeds in a managed area [57]. The inundative biocontrol is more related to the agricultural needs and turf management because its implementation is similar to the conventional herbicides as liquid sprays and solid granules [58, 59]. A number of microbial herbicide formulations based on bacteria and fungi have been registered worldwide (**Table 2**).

## 3. PGPR and stimulation of plant growth

Rhizosphere is the region of the soil surrounded by plant roots and often extended from the surface of roots [94]. This constituency of the soil is much wealthier in bacteria than the contiguous bulk soil [95]. The plant growth-promoting rhizobacteria are the soil bacteria that reside in the rhizosphere and are involved in the stimulation of plant growth through direct and indirect methods [96]. Agricultural production currently relies on the judicious use of synthetic fertilizers [97, 98] that have shown negative environmental impacts due to overuse of these chemical fertilizers [99]. Therefore, the use of PGPR inoculants can be considered as an environmentally sound alternative approach for the sustainable management, decreasing the use of synthetic fertilizers [100–102]. Within the context of PGPR research and their modes of actions, there has been an increasing trend in literature to search for the best PGPR candidate in order to commercialize as bio-fertilizer. Plant growth-promoting rhizobacteria are equipped with a plenty of mechanisms that can result in the promotion of plant growth. For instance, Parmar and Dadarwal [103] suggested the involvement of *fluorescent pseudomonads* to promote nodulation process and increased nitrogen fixation in chickpea [104], in another study, confirmed the ability of *Azospirillum* sp. inoculation on some significant agricultural crops in terms of increased dry weights of the root and shoot. Similarly, [105], who suggested that the foliar application of rhizobacteria in apricot and mulberry causes an increase in total surface area and chlorophyll contents as compared to uninoculated control [106], documented the growth response in wheat after the inoculation with rhizobacteria and revealed that the growth and development of wheat largely depends on the nature of PGPR and environmental factors.

Spaepen et al. [107] reported that various genera of rhizobacteria use tryptophan as a precursor to produce IAA by different pathways. However, the plant pathogenic bacteria only use the indole acetamide pathway to synthesize IAA that causes tumor formation in plants. Swain et al. [108] suggested that cultures of *Bacillus subtilis* when applied on *Dioscorea rotundata* increased the root/stem ratio and number of sprouts as compared to the uninoculated control.

A recent study by Minorsky [109] reported the excellent colonization ability of a PGPR isolate *Pseudomonas fluorescens* (B16) in tomato roots. The positive effects were increased plant height, enhanced flowering, and increased fruit weight. Castro et al. [110] proposed that PGPR stimulates growth and development of crops both by direct and indirect methods. The direct methods of growth promotion may include biological nitrogen fixation, solubilization of mineral phosphorus and iron, production of phytohormones, and synthesis of enzymes and siderophores. Indirect growth promotion occurs through the production of antibiotics and fungal-degrading enzymes and competition for niche exclusion in the rhizosphere [111, 112].

As for the higher uptake of nutrients that is concerned through application of bacterial inoculants, Qin et al. [113] reported the ability of rhizobacteria to dissolve fixed phosphate is related to the rhizosphere acidification. The rhizobium inoculation in soybean plants causes increased availability of phosphorus as compared to non-inoculated plants, hence positively influencing plant growth. Ambrosini et al. [114] suggested that sunflower-associated *Burkholderia* strains were found to be solubilizing $Ca_3(PO4)_2$, hence availing phosphorus for plant use. The management of soil, plant, and environmental interactions evidenced by boosted crop yields is gaining much attention globally. Moreover, agricultural inoculants (cultures) contain plant beneficial bacteria that help plants to meet the demands for nutrients.

## 4. Bacteria in biological weed control

A number of bacterial species have been studied due to their potential against weed management (**Table 3**). Two major classes of rhizobacteria that show herbicidal activity are *Pseudomonas* and *Xanthomonas* sp. Different rhizobacterial species have been investigated as weed control agents on different crops based on their secondary metabolites [115, 116]. As stated earlier *Pseudomonas* have gained much importance as an agent in biological weed management; there are many strains of this genera, some are plant beneficial [117] and others can have inhibitory effects on plants [118] and so can be applied in biological weed control. Production of extracellular metabolites from these strains is a key mechanism in inhibition of plant growth or germination inhibition [118–120]. However several other mechanisms showing herbicidal activity of bacteria are shown in **Figure 2**.

A strain of *Pseudomonas fluorescens* (D7) isolated from wheat and downy brome rhizosphere has shown inhibitory effects on a number of grassy weeds especially downy brome by virtue of production of a phytotoxin [116, 119, 121]. Kremer et al. [122] tested the phytopathogenic ability of different *fluorescent* and non*fluorescent pseudomonads* which were isolated from the rhizosphere of seven important weeds. About 18% of the strains show phytopathogenecity. However, the ratio of isolates that inhibited seedlings was ranged between 35 and 65%. The mechanism behind is the production of antibiotics, and about 75% of the isolates were active in siderophore production.

Kennedy et al. [121] reported the differential weed inhibition ability of *Pseudomonads* for downy brome and winter wheat. When the culture filtrates were tested on agar, about 8% of the isolates reduced the root growth of downy brome but have no effects on the root growth of wheat. However, under soil application only less than 1% inhibited the growth of downy brome. In the field study, only 0.2% of the total 1000 isolates inhibited the growth of downy brome but increased the growth of winter wheat by 18–35%. Kremer [123] worked with different cover crops associated with deleterious rhizobacteria. Seed bacterization with DRB reduces growth and biomass in weeds associated with cover crops. Adam and Zdor [124] described that rhizobacteria isolated from the rhizosphere of *Abutilon theophrasti* Medik caused growth inhibition of different weeds.

Weissmann and Gerhardson [125] suggested that the application of strain (A153) on *Chenopodium album* suppressed the growth of plants for 10–14 days; however in field conditions, this effect lasts for 2 months. Similarly Weissmann et al. [126] demonstrated excellent growth inhibition ability of a strain (A153) belonging to soil bacteria *Serratia plymuthica* when sprayed on a number of broadleaved weeds. However, in field experiment this strain showed differential effects on *C. album*, *Stellaria media*, *Polygonum convolvulus*, and *Galeopsis speciosa*. Li and Kremer [127] suggested that the inoculation of *Pseudomonas fluorescens* strain (G2–11) inhibited the growth of *Ipomoea* sp. and *Convolvulus arvensis* weeds and increased the growth of wheat and soybean crops. Zermane et al. [128] in a study stated that *P. fluorescens* has the possible potential to control *Orobanche crenata* and *O. foetida* (*Broomrape*).

| Microbe(s) involved | Target weed(s) | Growth condition(s) | Mechanism(s) | Observed effects/comments | References |
|---|---|---|---|---|---|
| *Pseudomonas fluorescens* | Sour cherry | Pot | IAA production | Significant loss in root weight | [71] |
| *Streptomyces chromofuscus* cluster | Barnyard grass | Axenic | Antibiosis and $H_2S$ production | ND | [72] |
| *Streptomyces* sp. 0H-5093 | Reddish | Axenic | Antifungal activity and production of 4-chlorothreonine | Significant growth inhibition | [73] |
| *Streptomyces* sp. | Reddish | Axenic | Cellulose inhibition and phthoxazolin A production | Significant growth inhibition due to cellulose inhibition | [74] |
| *Thermoactinomyces* sp. A-6019 | *Lemna minor* | Axenic | Herbicidal activity and 5′-deoxyguanosine production | ND | [72] |
| *Streptomyces hygroscopicus* | *Barnyard grass* | Pot | Antimicrobial and herbicidal activity due to hydantocidine production | Germination inhibition, significant reduction in stem, and leaf structure of weed | [75] |
| *Fusarium* and *Rhizoctonia* sp. | Leafy spurge | Greenhouse | Exopolysaccharide and HCN production | Biocontrol activity on leafy spurge leading to significant growth suppression | [76] |
| *Flavobacterium* sp. | Sugar beet | Axenic | IAA production | Decreased root elongation and increased shoot to root ratio | [67] |
| *Enterobacter taylorae* | Bindweed | Axenic | IAA production | 90.5% reduction in root growth, phytotoxic activity | [77] |
| *Pseudomonas fluorescens* | Leafy spurge | Field | Auxin production to phytotoxic levels | Reduced cell membrane integrity, inhibited root growth | [60] |
| *Streptomyces saganonensis* | Barnyard grass, goose grass, and tufted manna grass | ND | Herbicidine (vi) | Biocontrol activity | [78] |
| *Pseudomonas syringae* strain 3366 | Corn spurry and fireweed | Pot | Phytotoxin production | Germination inhibition, reduced root, and shoot growth | [79] |
| *Pseudomonas syringae* pv. *tagetis* | Annual bluegrass | Field | ND | Greater than 70% weed control | [57] |


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| Microbe(s) involved | Target weed(s) | Growth condition(s) | Mechanism(s) | Observed effects/comments | References |
|---|---|---|---|---|---|
| *Pseudomonas syringae* pv. *phaseolicola* | Kudzu | Greenhouse | ND | ND | [80] |
| *Fusarium tricinctum* | Dodder | Field | ND | Effectively controlled dodder at preemergence and postemergence application | [70] |
| *Trichoderma virens* | Several weeds | Field | Rhizosphere competence and production of herbicidal compound viridiol | Reduced emergence and seedling growth of different weeds up to a significant extent | [81] |
| *Colletotrichum gloeosporioides* f. sp. *malvae* | Round-leaved mallow | Greenhouse | ND | Significant biomass reduction, reduced fresh and dry weight, and inhibited root growth | [82] |
| *Fusarium solani* f. sp. | Texas gourd | Field | ND | Greater than 78% mortality, reduced vigor | [83] |
| *Nectria ditissima* | Red alder | Field | Infection | ND | [84] |
| Multiple isolates were screened belonging to *Pseudomonas* spp. and *Xanthomonas* spp. | Jointed goat grass | Axenic and field | ND | Inhibition of weeds by 71% in growth chamber and by 20–74% in different field conditions | [85] |
| *Sclerotinia sclerotiorum* | Dandelion | Field | Necrosis and discoloration | 80.7% reduction in number of dandelion plants and overall weight reductions | [86] |
| *Pseudomonas putida* | Garden asparagus | Pot | Succinic acid and lactic acid production | ND | [87] |
| *Pseudomonas fluorescens* and *P. putida* | *Striga hermonthica* (Del.) Benth. | Pot | ND | Significant reduction of weeds and improved biomass of maize | [88] |
| Collection of multiple rhizobacteria | Leafy spurge | Axenic | Phytotoxin synthesis | 30% reduction in leafy spurge growth | [89] |
| *Pseudomonas syringae* st. 1 and st. 2 | *Polypogon monspeliensis*, *Convolvulus arvensis*, and *Phalaris paradoxa* | Laboratory and field | ND | Reduction in biomass up to 47.5%, 22.8%, and 51.3%. Inhibited 40%, 32.6%, and 46.4% of biomass over control in field conditions | [90] |

| Microbe(s) involved | Target weed(s) | Growth condition(s) | Mechanism(s) | Observed effects/comments | References |
|---|---|---|---|---|---|
| *Pseudomonas aeruginosa*, *Pseudomonas syringae*, and *Pseudomonas alcaligenes* | Broad-leaved dock, common lambs' quarter | Pot and field | HCN production, IAA production, antibiotic production | Grain yield losses of infested wheat were recovered up to 11.6 to 68% in pot trial, and 17.3 to 62.9% in field trial, respectively | [34] |
| *T. harzianum*,*T. pseudokoningii*,*T. reesei*, and *T. viride* | *Avena fatua* L. | Laboratory | ND | Culture filtrates of four *Trichoderma* spp. significantly reduced root, shoot growth, and biomass of *Avena fatua* | [91] |
| *Trichoderma harzianum* Rifai, *Trichoderma pseudokoningii* Rifai, *Trichoderma reesei* Simmons, and *Trichoderma viride* Pers | *Phalaris minor* L. and *Rumex dentatus* L. | Laboratory | Synthesis of butanol, n-hexane, chloroform, and ethyl acetate | Original concentration of filtrates reduced root and shoot length and biomass of *Rumex dentatus* significantly, but effect on shoot growth of *Phalaris minor* was not significant | [92] |
| *Trichoderma virens* combined with composted chicken manure and rye | Multiple broadleaf and grassweeds | Field | Viridiol (3H)-benzoxazolinone (BOA) and 2,4-dihydroxy-1,4-(2H) benzoxazine-3-one (DIBOA) production | Significant reductions in the emergence of broadleaf and grassweeds and higher reductions in weed biomass was resulted with all treatments as compared to control | [93] |

*ND = not described.*

**Table 3.**
*Features of opportunistic bacteria and fungi in weed control under varying growth conditions.*

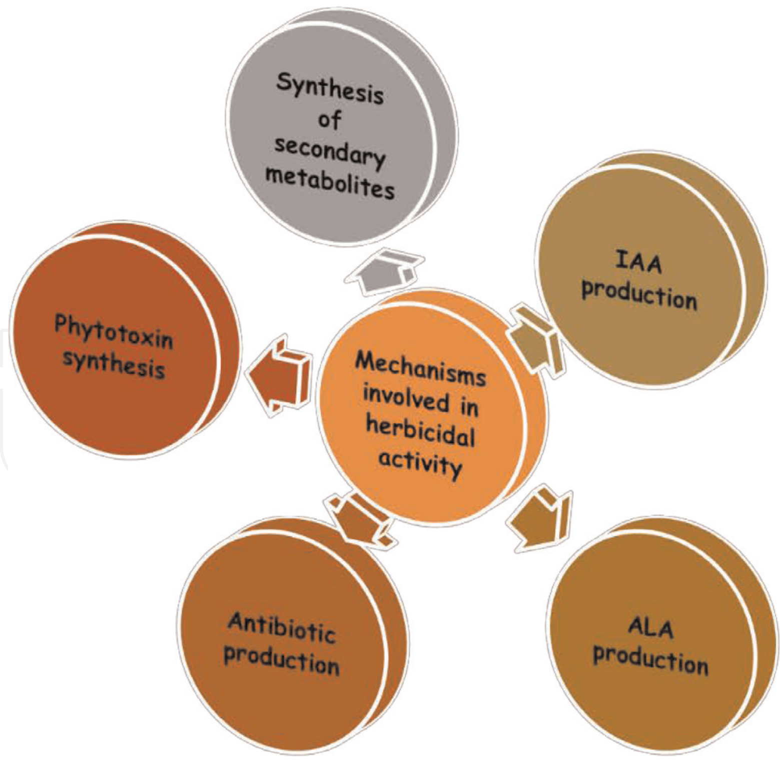


**Figure 2.**
*Possible mechanisms of plant growth-promoting rhizobacteria and fungi involved in herbicidal activity. IAA refers to indole-3 acetic acid, and ALA refers to aminolevulinic acid.*

Banowetz et al. [118] tested the germination inhibition activity in various monocot and dicot plants by the application of *P. fluorescens* (strain WH6). The germination inhibition activity was attributed due to the production of a compound called as Germination-Arrest Factor (GAF). Patil [129] screened 15 strains of deleterious rhizospheric bacteria isolated from rhizosphere of different weeds. Among these strains five isolates caused a significant reduction in root and shoot growth of weeds while showing no harmful effects on crop plants. Boyette and Hoagland [130] suggested that *X. campestris* (strain LVA-987) have shown strong growth suppressive effects against horseweed (*Conyza canadensis*). Some of the key herbicidal mechanisms shown by bacteria and fungi are shown in **Figure 2**.

## 5. Fungi (mycoherbicides) in biological weed control

A list of fungal biological weed control agents is given in **Table 3**. Within the scientific context, three genera of fungi have received worldwide attention to be used in biological weed control. In addition to the abovementioned BioMal and Collego, different other species of genus *Colletotrichum* have been researched extensively. Additionally, *C. truncatum* have been reported to control sesbania (*Sesbania exaltata*) [131] and *C. orbiculare* that has been found to control spiny cocklebur (*Xanthium spinosum*) [63, 132]. It is evident from the literature that these two *Colletotrichum* species produce indole acetic acid [133] which is a phytohormone and derivatives of which show herbicidal activity [134].

Within the genus *Phoma*, three species have a potential against weed control. *P. herbarum* is a fungus that is isolated from lesions of dandelion leaf that have shown control effects of dandelion [135]. *P. macrostoma* has also been studied for weed control due to its inhibitory effects on the dicot plants [18, 136, 137]. *P. macrostoma* strain (94-44B) has been found to control turf associated with broad-leaved weeds in Canada. Mass spectrometric analysis of *P. macrostoma* revealed the production of photobleaching of macrocidins [138] that do not have any inhibitory effects on monocot plants [18]. Despite this macrocidins an anthraquinone pigment in *P. macrostoma* has shown prominent herbicidal effects on some weeds in Central India [139]. The third species under this genus is *Phoma chenopodicola* that is studied widely for its potential against common lamb's quarter [62]. The mechanism behind its virulence against lamb's quarter is the production of diterpene and chenopodolin, a phytotoxic compound isolated from this species [62].

Two species within the genus *Sclerotinia* have been investigated for their herbicidal activity. It is evidenced by the work of Abu-Dieyeh and Watson [140] that *Sclerotinia minor* effectively controlled dandelions in turf management systems. A closely related species of this genus *S. sclerotiorum* has also shown the potential against noxious weeds [141]. Production of oxalic acid has been found by these two species that cause virulence on the host plant [142].

Apart from these three genera, there are other fungal candidates that are registered to control weeds in forest lands and ecosystem managements [143]. A worth mentioning bioherbicide is De Vine containing a fungus *Phytophthora palmivora* [144]. This formulation was registered in 1981 and again in 2006 with the EPA [144].

The mycoherbicide "EcoClear" contains *Chondrostereum purpureum*, a pathogenic fungus which should be applied after the injury to the weeds' branches to retard resprouting [145].

Soil-borne fungi also serve as an important tool in weed management. Their direct application in the soil causes decay of the seeds or emerging seedlings [146]. *Trichoderma virens* is one example that reduces weed populations in horticultural crops [81].

Khattak et al. [147] tested two fungi *Aspergillus* and *Penicillium* for their herbicidal activity against two separate weeds *Silybum marianum* L. and *Lemna minor*. Results showed excellent weed-suppressive characters in the extracts of these fungi.

## 6. Conclusion and future strategy

Biological control of weeds using bacteria and fungi should be the prime priority for mitigating the negative impressions posed by conventionally adopted weed control methods in order to ensure environmental safety and human health. These biological control agents should be adopted in areas with higher and multiple weed infestations; areas of low value land, where weeds have gotten resistance against herbicides; and areas with lack of labor and where the recommended cultural practices cannot be carried out, for example, restrictions posed by topography and narrow rowed crop cultivations. However, in special cases the combination of biological control agents with other methods could also be a promising approach as an alternative to conventional methods.

The future advancement in biological agents for weed control should be based on advancements in microbial genetics (metagenomics), microbe-plant interactions, and microbial community-level analyses. Further investigations need to be discovered in the future in order to make biological weed control more pragmatic and instrumental. In this context, additional microbe-host relationships containing a match of biological agent and its potential host at greater susceptibility of virulence should be further explored. Since the 1960s a number of formulations have been registered in the world. Formulations that can ensure greater shelf lives, efficacy, and survival of microbial agents should be investigated in the future. Investigations on microbial community structure and function can advance microbial weed control. Traditional methods of microbial community structure solely rely on phenotypic characters; molecular-level characterization should be explored in the future. In a nutshell, fatty acid profiling should be the initial step in targeted weed control. Nucleic acid tools, array pyrosequencing, metagenomics, construction of molecular probes, selection of hyper virulence, genomic studies, and host-microbe interactions should be investigated for the development of innovative weed control methods, reducing reliance on herbicide usage.

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