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Allelopathic growth stimulation of plants and microorganisms

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CONTENTS

ABSTRACT

Growth promotion of plants by other plants and microorganisms, as well as that of microorganisms by plants and other microorganisms, is discussed. *Agrostemma githago* in mixed culture with wheat enhances growth and yield of wheat. Allantoin, a purine derivative and the principal component of agrostemin released from *A. githago*, is the growth factor. Soil amended with shoots of *Solanum nigrum*, enhances the

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soybean growth and nodulation. Growth and yield of several legumes are enhanced by mixed culture with *Heliotropium peruvianum*.

Triacontanol isolated from alfalfa, and brassinolide from rape and alder pollen, stimulates the growth and yield of several crops. Chromosaponin I, isolated from etiolated pea seedlings, stimulates the growth of lettuce by 190 %. Petunioside M stimulates the growth of cucumber and suppresses the cucumber mosaic virus. Strigolactones, isolated from host or non-host plants, promote seed germination of angiospermous parasites. Unidentified allelochemicals from *Chenopodium album* and *Setaria viridis* enhances the growth of *Bradyrhizobium japonicum* in broth culture.

Seed inoculation with selected strains of *Pseudomonas capacia* and *P. putida* enhances the growth and yield of wheat under field conditions. Inoculation of soybean seeds with a strain of *Bacillus cereus* enhances the growth and nodulation of soybean by indigenous nodulating bacterium. Several soil microbes, with appropriate precursors, produce plant growth regulators that enhance the plant growth. Tomatoes grown in soil amended with L-ethionine at the appropriate concentration produce more and larger fruits than the untreated soil. Pearl millet inoculated with *Azospirillum brasilense* and grown in solution culture amended with tryptophan produces more lateral roots with greater root hair density than the control. Microbial metabolites enhance the growth of several *Rhizobium* species and promote reproduction in certain fungi. These examples indicate that the allelochemicals from plants and microorganisms have potential to enhance the yield of agricultural products, while promoting sustainable agriculture.

Key Words: Allantoin, auxins, cytokinins, ethylene, gibberellins, nitrogen fixation, petunioside, plant-microbe interaction, rhizosphere microorganisms, saponin, strygolactones.

1. INTRODUCTION

Although Molisch (35) defined allelopathy to include both beneficial and harmful effects of one plant or microorganism on another, the majority of studies on allelopathy are concerned with inhibitory effects. Scientists involved in allelopathy research, have generally ignored the stimulatory effects, possibly because stimulatory effects are often not as spectacular as inhibitory effects. However, there are reports of stimulation of plants by other plants, of plants by microorganisms and vice-versa and of microorganisms by other microorganisms. An extensive review of allelopathic literature covering these stimulatory effects is not intended. References cited here are exemplary to draw the attention of researchers to allelopathic stimulation, with a view to exploit the phenomenon where feasible in agriculture and in biological research.

2. PLANTS STIMULATORY TO OTHER PLANTS

2.1. Non-parasitic Plants

Inhibitory allelopathic effects have been the principal focus of allelopathy research due to its potential in weed control. The beneficial effects have received little attention. However, several reports of allelopathic growth promotion of one plant by another indicate that this phenomenon has potential in agriculture. Nicollier *et al.* (44) bioassayed water and methanol extracts of 90 weed and crop species at 3, 30 and 300 ppm (wt./vol.) to determine their possible allelopathic effects on turnip (*Brassica rapa*) root growth. Of these, six species (*Boltonia diffusa*, *Conyza canadensis*, *Erigeron*

philadelphicus, *E. strigosas*, *Geranium carolianum* and *Helianthus hirsutus*) were reported to be stimulatory. Neill and Rice (43) reported that rhizosphere soil from western ragweed (*Ambrosia psilostachya*) stimulated the growth of several plant species that occurred in the same field (Table 1). This indicated that the rhizosphere soil from western ragweed

Table 1. Stimulation of plant growth by rhizosphere soil from *Ambrosia psilostachya* collected in the field during July[a]

Test Species	Control[b]	Test
Amaranthus retroflexus	42 ± 8.0	95 ± 10.0[c]
Andropogon ternaries	25 ± 1.4	33 ± 2.1[c]
Bromus japonicus	22 ± 1.1	46 ± 3.3[c]
Digitaria sanguinalis	56 ± 6.2	117 ± 7.7[c]
Leptroloma cognatum	20 ± 1.9	36 ± 1.8[c]
Rudbeckia hirta	16 ± 0.8	25 ± 1.6[c]
Tridens flavus	27 ± 1.6	43 ± 3.2[c]

[a]Modified from Neill and Rice (43). [b]Control soil was collected in the same field at least 1m away from the *A. psilostachya* plants. [c]Difference from control significant at the 0.05 probability level or greater.

contained a stimulatory factor that was absent in the soil, not near the plant roots. Russian knapweed (*Centaurea repens*), a perennial herb, is a common weed in the U.S. and Canada. Stevens and Merrill (60) reported that several sesquiterpene lactones (acroptilin, repin, solstitiolide and centaurepensin) from this weed were stimulatory to lettuce root elongation at 10-ppm, but were inhibitory at higher concentrations. Sugha (62) found that soaking pea seeds in aqueous extracts of superb lily (*Gloriosa superba*) for 4 h stimulated growth of pea plants, produced more pods per plant at 57 days and increased the number of seeds per pod and seed weight. However, soaking the pea seeds for 8 h had an inhibitory effect. Gajic (18) reported stimulating effects of corn cockle (*Agrostemma githago*) on biomass production and yield of wheat. Stimulation was more pronounced in the growth phase than in the tillering phase (Figure 1). The growth factor in corn cockle is agrostemin, which consists of allantoin, the principal component, several amino acids and purines. The Yugoslav government patented agrostemin as a commercial product. The effect of allantoin on wheat growth and seed yield was analogous with corn cockle grown in mixed cultivation with wheat. This might indicate that the stimulating factor in agrostemin is allantoin, a purine derivative (Figure 2). When both wheat and corn cockle seeds were planted in close proximity on agar plates, wheat coleoptile growth was enhanced. This indicated that a diffusible, stimulatory factor from corn cockle seedlings was involved in stimulation of wheat seedlings (19). Rice (55) tested root exudates of ground ivy (*Glechoma hederaceae*) for its allelopathic effect against downy brome (*Bromus tectorum)* and radish (*Raphanus sativus*) as assay plants. The experiment was conducted in U-shaped tubes containing Hoagland's solution (26). The test plants, selected for uniformity, were inserted in one arm, one seedling per arm (seedlings were held with a cotton plug) and a ground-ivy plant in the other arm. In the control tubes, both arms received test plants. Root exudates of ground ivy stimulated the radish shoot and root growth by 50%, but also inhibited the downy brome growth (Table 2).

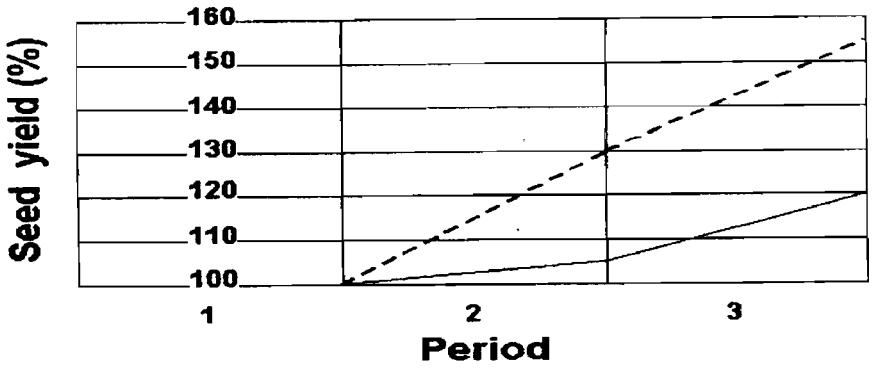

Figure 1. Yield of wheat seed grown in association with and without corn cockle on brown soil (solid line) and smonitsa (dashed line) at different wheat growth periods (wheat as a single crop, 1; tillering, 2; and ripening, 3). Modified from Gajic (18).

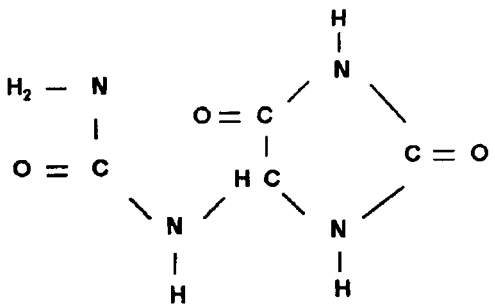

Figure 2. Chemical structure of allantoin.

Table 2. Effects of ground-ivy root exudates on downy brome and radish seedling shoot and root dry weight.[a]

Plant spp.	Control (mg)[b]	Test (mg)[c]	Stimulation/ Inhibition (%)
	Shoot		
Downy brome	71.4 ± 16.8	53.6 ±12.4[d]	25
Radish	239.8 ± 100.0	352.5 ± 118.6[d]	47
	Root		
Downy brome	22.8 ± 9.4	15.1 ± 5.8[d]	34
Radish	206.4 ± 141.2	325.0 ±147.4[d]	58

[a]Modified from Rice (55), [b]Mean of 20 replicates. [c]Mean of 10 replicates, [d]Significantly different from the control at the 0.05 probability lever or greater.

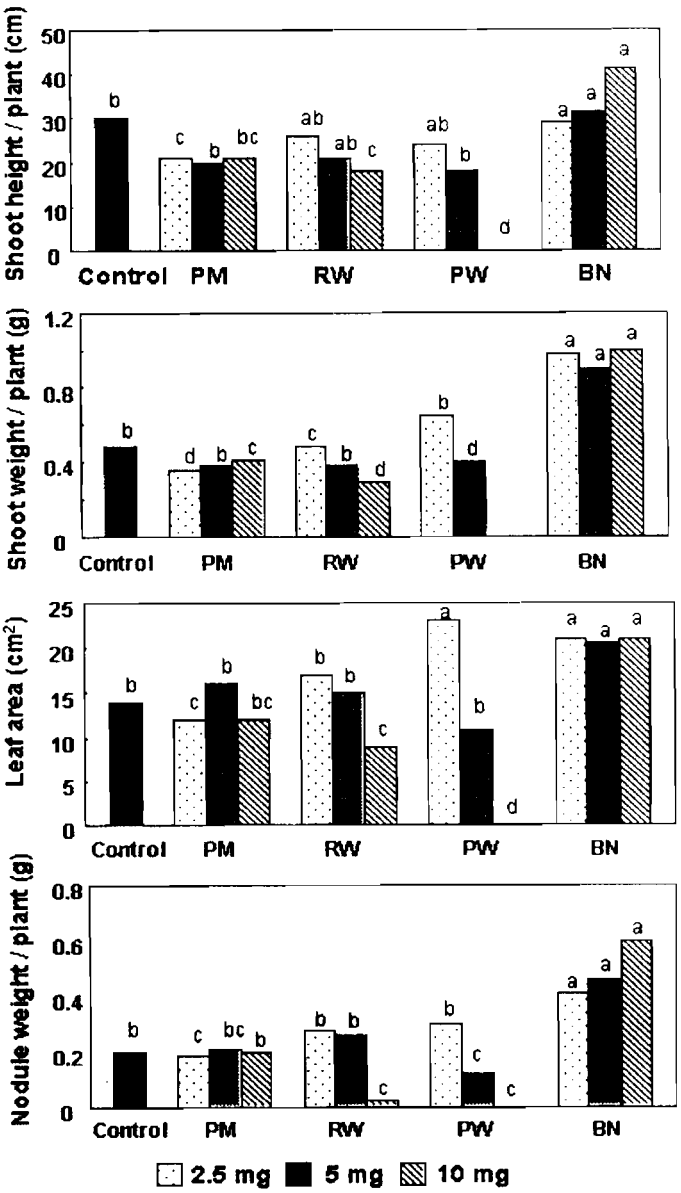

Figure 3. Soybean growth in sand supplemented with peat moss (PM) and residues of ragweed (RW), pigweed (PW) and black nightshade (BN) at 2.5, 5.0 and 10.0 mg/g of sand. Means followed by the same letter are not significantly different at the 0.01 probability level as determined by the Duncan's multiple range tests. Modified from Mallik and Watson (39).

Mallik and Watson (39) investigated the allelopathic potential of black nightshade (*Solanum nigrum*), redroot pigweed (*Amaranthus retroflexus*) and ragweed (*Ambrosia artemisifolia*) using soybean as the test plant (Figure 3). These weeds are common in soybean fields of Oklahoma, U.S. Soybean seedlings were grown in pots containing pulverized shoot residues of the three weeds incorporated at 2.5, 5.0 and 10.0 mg/g of silica sand. Equal amounts of peat moss were for comparison. The pots were held in a growth chamber (14-h day length, photosynthetic photon flux density of 490 μ mol.m^{-2} s^{-1} at top of plants, day/night temperature 34/28 °C). Seedlings were inoculated 3 ml/pot with broth culture of *Bradyrhizobium japonicum* held at mid-exponential phase that supplied at least 10^8 cells/plant. Each pot held one soybean plant. Black nightshade residue at all three levels significantly enhanced the growth and nodulation of soybean. At 10 mg/g, the soybean plants were the tallest among all the treatments and flowered at 28 days after planting; buds (leaf and flower) were observed at every node indicating healthy growth. Also, the average leaf area of soybean plants was significantly larger at all three levels of black nightshade than those in peat moss at similar concentrations. Nodulation at all three levels was enhanced; at 10 mg/g, nodule weight increased almost three times compared with peat moss. Ragweed residue at 10 mg/g reduced growth and nodulation; pigweed residue at the same level was lethal to soybean plants, although at 2.5 mg/g nodulation was not reduced compared to peat moss treatment. In addition, Mallik and Watson (39) tested the aqueous extracts of these three weeds. Soybean seedlings of uniform size were grown in growth pouches held in a growth chamber with a N-free solution amended with aqueous extracts of the three weeds at 10 and 20 mg/ml concentrations. The seedlings were inoculated with broth culture of *B. japonicum*. Black nightshade extract at both concentrations significantly enhanced soybean growth and nodulation (Figure 4); at 10 mg/ml, the nodule weight increased by almost one and a half times the control. Ragweed was highly inhibitory to growth and nodulation. Stunting of growth was noticeable 2 weeks after amendment of nutrient solution and abnormal swelling of roots was observed at harvest, which might have suppressed the nodulation. Pigweed at the 20 mg/ml severely inhibited the growth and completely suppressed nodulation. At the lower concentration, pigweed marginally affected growth, but completely suppressed the nodulation. These results indicated water soluble allelopathic factors in these weed residues. It is noteworthy that fresh black nightshade residue incorporated in sand, was very stimulatory to soybean growth and nodulation. In an agro-ecosystem, mulching by black nightshade residue might be beneficial to soybean. We have not found another reference of stimulation of soybean growth and nodulation by weed residues in the literature.

2.1.1. Crop mixtures: Modern agriculture has abandoned multiple cropping practices in favour of monoculture, principally because of mechanization. The following examples of beneficial effects of mixed cropping are found in Putnam and Duke (52), who indicate that this Russian research is available through English summaries. Several legumes, particularly late maturing varieties, grown together in mixed culture with corn (*Zea mays*) have been shown to enhance corn yield. Corn productivity increased in biculture with *Vicia faba*; the increased yield was correlated with greater microbial activity. This was attributed to increase in exchange of root exudates monitored by P^{32}. Higher yields of several crops were reported from mixed culturing with (addition of 1-2 kg/ha seeds) white

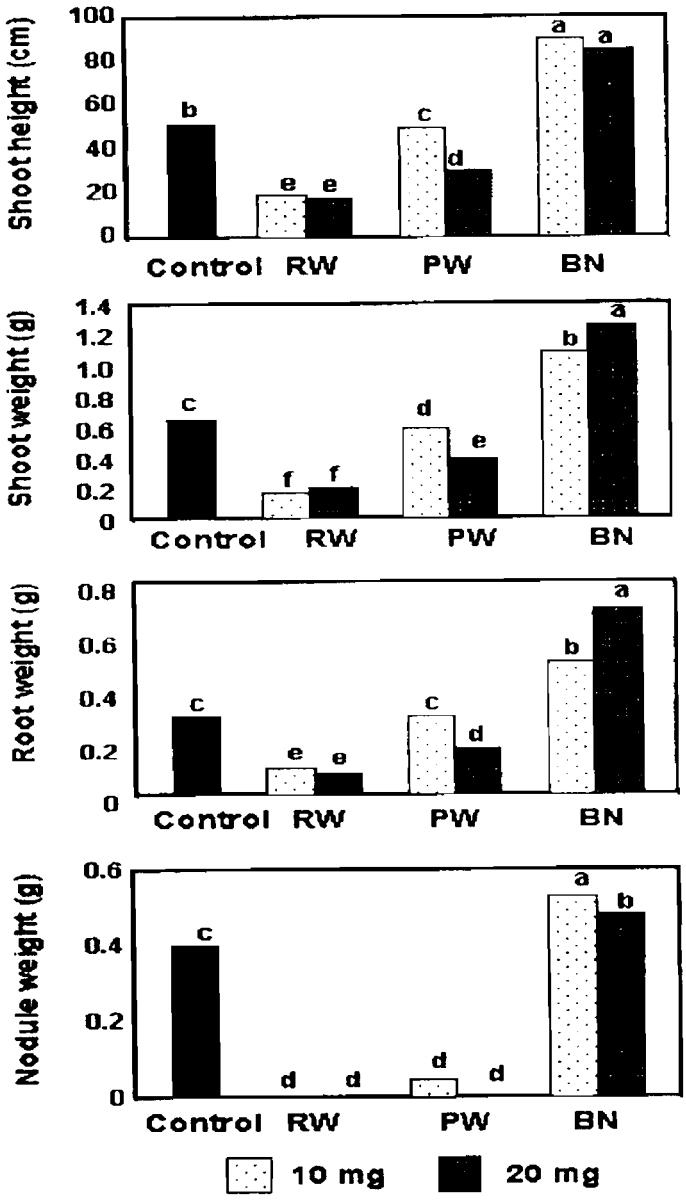

Figure 4. Soybean growth in seedling pouches containing nutrient solution supplemented with an aqueous extract of plant residues at 10 and 20 mg/ml concentrations. (RW, ragweed; PW, pigweed; BN, black nightshade) Means followed by the same letter within a parameter are significantly different at the 0.01 probability level as determined by the Duncan's multiple range tests. Modified from Mallik and Watson (39).

mustard. Likewise, the addition of 1 to 2 kg/ha wild heliotrope (*Heliotropium europasum*) seeds increased the yield of several legumes. Heliotrope reduced the weed population and insect pests. The beneficial effect of mixed cropping is associated with an allelopathic mechanism. Improved uptake of phosphorus and potassium by cereal crops resulted from mixed cropping with legumes. Root exudates from one plant can influence ion absorption and accumulation by another plant in a mixed cropping system. This was demonstrated by using ^{14}C labeled plants that exchange of root secretions among species was 1.5 to 7 times greater in mixed culture than in monoculture. These examples of the benefit of mixed cropping may draw attention of agricultural scientists and stimulate further investigation in this area.

2.1.2. Growth regulators: Ries *et al.* (56) found that chopped alfalfa (*Medicago sativa*) added to soil stimulated the growth of tomato, cucumber, lettuce and several other crops. They isolated the growth-promoting factor from a chloroform extract of alfalfa meal and identified it as triacontanol, a primary alcohol containing a straight chain of 28 carbons. The allelochemical was found to stimulate the growth of rice, corn and barley by foliar spray, as well as applied in nutrient solution and soil (Table 3).

Table 3. Response of rice, grown in nutrient solution, and barley and corn, grown in soil, to application of crystals isolated from alfalfa[a]

Alfalfa crystals (mg/liter)	Rice nutrient culture		Soil with foliar application	
	Dry weight (mg/plant)		Shoot dry weight (mg/plant)	
	Filter paper	Foliar Spray	Barley	Corn
0.00	109	110	58	355
0.01	132	118	88	466
0.10	135	123	65	405
1.00	139	132	71	429
$LSD_{0.05}$	18	15	17	66

[a]Modified from Ries *et. al.* (56).

The synthetic triacontanol produced comparable results (in rice and tomato) with those of the natural allelochemical isolated from alfalfa. The increased growth of several plants by foliar spray and root application led the authors to suggest that the allelochemical may be involved in growth process, possibly affecting membrane permeability. Unfortunately, the growth stimulation in subsequent experiments was not consistent. Later, it was found that addition of calcium or lanthanum salts to triacontanol solution partially corrected the inconsistency. A preparation of the allelochemical dissolved in acetone and water and applied at 10 mg/acre produced more consistent stimulation (40). Another growth promoting allelochemical, brassinolide, originally discovered at USDA's Beltsville laboratories, was isolated from rape (*Brassica napus*) and alder tree (*Alnus sp.*) pollen. It is a steroid with a lactone ring. Synthetic brassinolide (synthesized in Palo Alto and in Tokyo), as well as its analogues (brassinosteroids), are commercially available. The allelochemical applied at 1 ng/plant was reported to enhance growth and yield of radish by 15%, lettuce by 30%, potato by 25%, and bean and pepper by 6 to 7% (40).

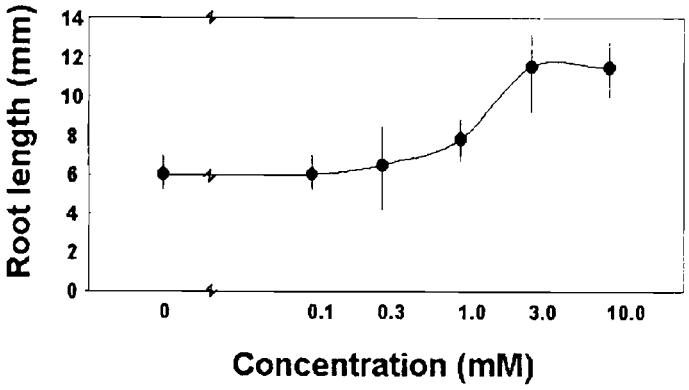

Figure 5. Promotive effects of chromosaponin I on the growth of lettuce roots in 50 mM MOPS buffer (pH 6.7). Root length was measured 42 h after treatment. Values are the mean (n = 20 to 24 measurements) and the standard error. Modified from Tsurumi and Tsujino (64).

Saponins are a class of natural products composed of aglycones and sugars. They are widely distributed in plants and are thought to have some regulatory functions. Chromosaponin I, a conjugate of soysaponin I and γ-pyrone, was isolated from 7-day-old etiolated pea seedlings (*Pisum sativum*) and purified by HPLC (64). The allelochemical was found to stimulate lettuce seedling (*Lactuca sativa*) growth (Figure 5). Another experiment (65) reported that chromosaponin enhanced the growth of chrysanthemum, leaf mustard, timothy, Italian rye grass, white clover, salt green, alfalfa, milk vetch and Japanese hornwort. It is effective on most plants at 3 mM concentration and the authors suggested that the growth stimulation resulted from cortical cell elongation. Continuous cropping of mung bean (*Vigna radiata*), an allelopathic plant, causes growth reduction of the plants by 10 to 25%. However, Waller *et al.* (66) reported that crude saponin of mung bean (principal component, soysaponin I) at 1, 10 and 100 ppm concentration stimulated lettuce and mung bean seed germination on filter paper. Partially purified mung bean saponin applied to soil at 10, 150 and 450 ppm stimulated mung bean germination and enhanced plant growth, but did not increase the yield. Waller *et al.* (67) isolated soysaponin I from roots, leaves and stem of mung bean plants of different ages. The partially purified saponin was bioassayed using mungbean and lettuce seedlings. The mung bean bioassay results were mixed; allelochemicals from leaves and roots were stimulatory, but not from stem, indicating the importance of plant part extracted. Regardless of plant age, only the leaf saponin stimulated lettuce seedlings. Steroidal glycosides are known to possess wide range of biological activity including plant growth promotion. Shvets *et al.* (58) isolated 10 petuniosides (steroidal glycosides) from *Petunia hybrida* seeds. Potential of petunioside M for growth promotion and resistance to green eye-spot mosaic virus of cucumber was tested. The cucumber seeds were soaked with petunioside M at concentrations ranging from 0.1 to 0.001%. The authors reported that the allelochemical at 0.01% concentration stimulated seed germination and root length by 37

and 60%, respectively (Table 4). The seeds soaked in the 0.005% concentration reduced the virus infection by 61.2% (Table 5).

The beneficial effect of triacontanol, brassinolide, chromosaponin I, soysaponin I and petunioside M cited here indicates that these allelochemicals have the potential of enhancing crop yield and being useful in experimental botany and agriculture. Further exploitation of these allelochemicals for enhancing plant growth and yield is highly desirable.

Table 4. Phytostimulation of petunioside M on cucumber seed emergence and rootlet length[a]

Treatment	Seed		Rootlet	
	Emergence (%)	Per cent of Control	Length (cm)	Percent of Control
Petunioside (%)				
0.1	90	123	5.4	104
0.08	86	118	6.8	130
0.01	100	137	8.3	160
0.005	83	114	5.2	100
0.0001	86	118	5.7	110
Control	73	100	5.2	100

[a]Modified from Shvets *et. al.* (58).

Table 5. Effect of presowing petunioside M treatment of cucumber on the rate of cucumber infection with VGESMC[a]

Treatment	Number of infected plants	Average degree of infection (Score)	Decrease in the average degree of infection	
			Score	%
Water	100	1.47	--	--
$KMnO_4$ 1.0 %	100	1.16	0.31	21.1
Petunioside (%)				
0.08	97	1.00	0.47	32.0
0.01	100	0.91	0.56	38.1
0.005	78	0.57	0.90	61.2
0.001	95	0.93	0.54	36.7

[a]Modified from Shvets et al. (58).

2.2. Parasitic Plants

The parasitic angiosperms are distributed in eight families. Agriculturally important parasites mostly belong to *Striga*, *Orobanche* and *Alectra*. They affect members of Graminae, principally corn, wheat, rice, sugarcane, sorghum and warm season grasses, legumes and vegetables (42). These root parasites are distributed mainly in Mediterranean countries, west Asia, east and south Africa, and cause great losses to agriculture. The feature common to these parasitic plants is the development of haustorium that establishes morphological and physiological connection between the roots of the host and the parasite. Haustorial induction is initiated by the root exudates of the host plants.

2.2.1. Crop germination stimulants: The parasitic flowering plants are photosynthetic and are capable of producing seeds without hosts, but rarely do so under field conditions. The application of herbicides (2,4-D and others) to control these parasites is ineffective because their seed remain viable and dormant in soil for several years. An attractive control strategy is application of chemical stimulants to the field prior to sowing the crop to induce the parasitic seed to germinate, "suicidal germination," in absence of the host. At least three seed germination stimulants, strigolactones, are known. Strigol was first isolated from cotton root exudates, a non-host, and from sorghum, a host. Its chemical structure was determined by Cook *et al.* (13). It is a stimulant to *Striga asiatica* (witch weed) and *S. harmonthica*. Sorgolactone, structurally related to strigol, was isolated from *Sorghum bicolor*, a natural host of several parasitic flowering plants. The stimulant triggers seed germination of several species of *Striga*, *Orobanche aegyptiaca* and *Alectra vogeli* (25). Alectrol was isolated from *Vigna unguiculata*, a host of *A. vogelli* and *S. gesneriodes*. Yokota *et al.* (68) isolated from *Trifolium pratense*, a host of *Orobanche* spp., alectrol and a strigol isomer, tentatively named orobanchol. Both are germination stimulants of *Orobanche* spp. Minor stimulants isolated from sorghum includes sorgolene and sorghumol. In addition to the natural stimulants listed above, ethylene is a stimulant to seed germination of *Striga* spp. Berner *et al.* (7) found that *Pseudomonas syringae* strain glycinea, synthesizes large amounts of ethylene and that this and other ethylene-producing bacteria are highly effective in promoting *Striga* spp. seed germination.

3. PLANTS STIMULATORY TO MICROORGANISMS

3.1. Root Exudates

Enhanced microbial growth and population density in the rhizosphere zone called "rhizosphere effect" results from the release of a variety of organic compounds and mucilage by plant roots. The organic compounds include amino acids and low molecular weight carbohydrates through root exudation. Plant mucilage consists of Golgi bodies, root cap cells and microbial degradation products of dead epidermal cells released through secretion and lysate from lyses of epidermal cells.

The relative abundance of various taxonomic and nutritional groups of microorganisms in the rhizosphere zone differs considerably from non-rhizosphere soil. Denitrifiers and nitrifiers in particular were reported to be more abundant in wheat rhizosphere than in non-rhizosphere soil (8). Thus, there is a direct influence of the organic compounds released in the rhizosphere on microbial growth and activities. A 55-fold increase in the microbial number was found in the root zone of *Eucalyptus calophylla* than in soil obtained outside the root zone of the plant (9).

Several investigators have documented the rapid multiplication of root nodulating bacteria in the rhizosphere zone of their specific legume hosts (51, 57). The explanation of this phenomenon is that root exudates of legume hosts contain a stimulatory factor for their homologous root nodulating bacteria. Geitte *et al.* (20) demonstrated this by fractionating root exudates of *Cicer arietinum* into cationic, anionic and neutral fractions. The chemotactic response of *Rhizobium* sp. isolated from *C. arietinum* was greatest with the cationic fraction, least with the anionic fraction and intermediate with the neutral fraction. The cationic fraction, which was the bulk of the exudates, contained all the amino acids.

Nutman (49) reported that decapitation of inoculated *Trifolium fragiferum* 2 days after germination, almost completely suppressed the root hair infection by *Rhizobium trifolii*. Whereas, decapitation 3 days after germination had no adverse effect indicating that the factor necessary for root hair infection and nodulation process comes from the plumule of the seedling.

3.2. Plant Extracts

Extracts of selected plants were found to contain factors stimulatory to microorganisms. Singh and Rai (59) reported that aqueous extracts of mustard and barley leaves stimulated the several saprophytic and plant pathogenic fungi.

The filter sterilized aqueous extracts of shoots of lambsquarters (*Chenopodium album*) and green foxtail (*Setaria viridis*) enhanced the growth of soybean root nodule bacteria (*Bradyrhizobium japonicum*, 3I1b110) in yeast extract-mannitol broth culture (37). Growth of the bacterium was almost linear up to 6 mg/ml concentration. Growth stimulation reached its maximum of 4.1 times the control at the 12 mg/ml concentration after 72 h incubation. Generation time (mean doubling) at that concentration was 6.5 h, approximately 50% of the control (Figure 6). Significant growth enhancement was noted for *Aspergillus niger* and *Saccharomyces cerevisiae* in Czapek's broth and *Streptomyces griseus* in glucose peptone broth, each supplemented with 10 mg/ml of aqueous extracts of lambsquarters shoot or root (unpublished).

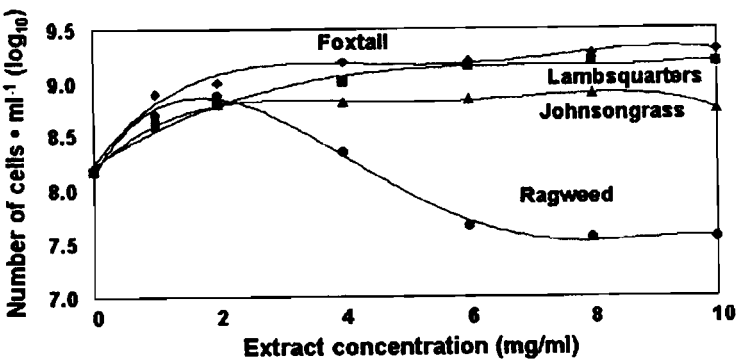

Figure 6. Growth stimulation of *Bradyrhizobium japonicum* in yeast extract-mannitol broth supplemented with filter-sterilized water extracts of plant residues at different concentrations. Modified from Mallik and Tesfai (37).

Subsequently, Mallik and Tesfai (38) found that aqueous extracts of lambsquarters shoot were more stimulatory than methanol extracts; neither ether nor butanol extracts were stimulatory. Comparable growth stimulation was also noted in four other strains of *B. japonicum*. To purify the growth factor, aqueous extracts of lambsquarters shoot were sequentially extracted and fractionated with organic solvents (hexane, ether, ethyl acetate and butanol) and the residual aqueous fraction still retained the stimulatory factor (Figure 7). Paper chromatographic separation of the dialysate (MWCO<1000) from the residual

aqueous extract yielded two bands (UV short-wave). The eluate from the band with Rf 0.89-0.91 showed the greatest stimulation of the bacterium. We surmise that the factor has the potential of being useful in the commercial production of legume inoculants and in the fermentation industry.

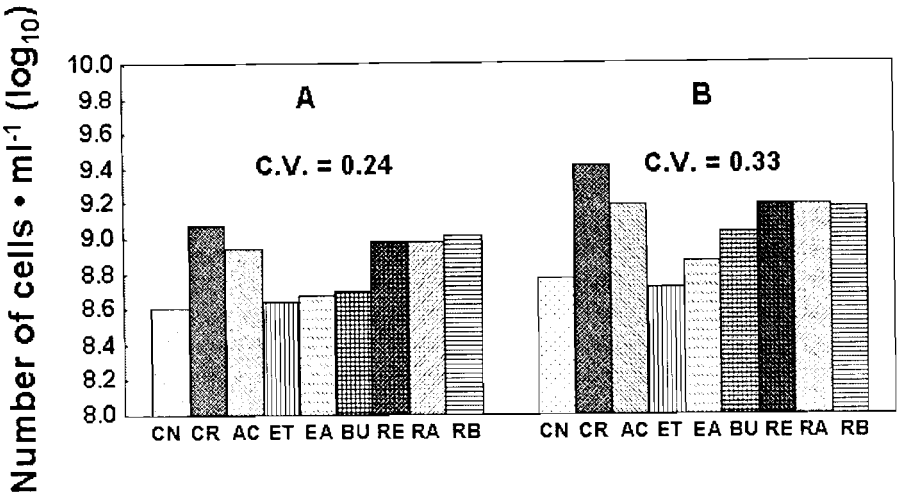

Figure 7. Growth response of *Bradyrhizobium japonicum* in yeast extract-mannitol broth supplemented with different fractions from sequential partitioning of aqueous extracts of lambsquarters shoots (A) and root (B). Incubation period 72 h. (CN, control; CR, crude aqueous extract before acetone extraction; AC, extract after acetone extraction; ET, ether extract; EA, ethyl acetate extract; BU, butanol extract; RE, aqueous extract after ether extraction; RA, aqueous extract after ethyl acetate extraction; RB, aqueous extract after butanol extract.) Modified from Mallik and Tesfai (38).

4. MICROORGANISMS STIMULATORY TO PLANTS

4.1. Bacteria

4.1.1. *Pseudomonas* spp.: There is ample evidence of growth stimulation of plants by allelopathic beneficial bacteria introduced as seed inoculant or otherwise (excluding N_2-fixing bacteria). De Freitas and Germida (14) reported significant increase of growth and grain yield of wheat under field conditions in north Canada by seed inoculation with selected strains of *Pseudomonas cepacia* and *P. putida*. The authors reported that the introduced bacteria colonized the wheat rhizosphere and survived through winter. The mode of action of the bacteria was not suggested. Hussain and Vacura (29) found IAA, gibberellin-like substances and few B-vitamins in the culture fluids of several rhizosphere and rhizoplane bacteria of corn. The largest amounts of these growth regulators were found in the cultures of *Pseudomonas fluorescens* and *Bacillus brevis*. Several bacterial cultures or their supernatants significantly enhanced seed germination and plant growth.

Significant corn yield and dry matter increases were obtained from seed inoculation with *P. fluorescens* and *Chromobacterium violeceum.*

4.1.2. Azotobacter: Seed inoculation with bacteria was first introduced with *Azotobacter* as it was thought to add nitrogen to the plants. Later, it was found that growth promotion of plants by the inoculant was due to production of plant growth regulators in addition to fixed nitrogen (61). Gonzales-Lopez *et al.* (21) reported that culture supernatants of *Azotobacter vinelandii* ATCC 12837, especially in dialyzed soil medium, contained auxins as well as three gibberellin-like and three cytokinin-like substances. This might suggest that selected strains of *Azotobacter* have the potential of stimulating growth by supplying plant growth regulators.

Azotobacter paspali, a N_2 fixing bacterium, improves the growth of *Paspalum notatum* in pastures due to addition of nitrogen to the grass by *A. paspali.* Brown (12) showed that the bacterium promoted growth of the grass primarily by producing growth regulators: IAA and gibberellin-like and cytokinin-like substances. Living and cell-free culture broth of *A. paspali* was equally effective in promoting growth of the grass. Hussain *et al.* (27) reported an increase in yield of corn inoculated with different strains of *Azotobacter* in fertilized and unfertilized fields. The authors concluded that the increase was due to the production of growth regulators by the bacterium rather than to nitrogen fixation.

4.1.3. Other bacteria: Tien *et al.* (63) identified indole acetic acid, indole lactic acid, a small but biologically significant amount of gibberellins and three cytokinin-like substances in broth cultures (with added tryptophan) of *Azospirillum brasilense*, a N_2-fixing bacterium associated with various grass species. The production of these growth regulators by the bacterium was verified by bioassay with pearl millet (*Pennisetum americanum*). Plant growth in solution cultures inoculated with the bacterium had more lateral roots, which were densely covered with root hairs, than the control plants. When the three growth regulators were added as treatment, the pearl millet root morphology was similar to that produced by the bacterium.

The rhizosphere harbours appreciable numbers of microorganisms. Several bacteria, which were abundant on roots of older plants (*Achromobacter*, *Alcaligenes*, *Arthobacter*, *Brevibacterium* and *Nocardia*), isolated from rhizosphere of wheat and root free soil produced plant growth regulators *in vitro* resembling IAA and gibberellins (11). The growth regulators were identified by paper chromatography. Bioassay (elongation of dwarf pea seedlings for gibberellins and oat coleoptile for IAA) of plants inoculated with soil suspension containing growth promoting bacteria produced comparable responses with those seedlings treated with GA_3 and IAA and differed from control without the additives.

4.2. Exogenous growth regulators

Plants can indigenously synthesize growth regulators and are also capable of absorbing introduced growth regulators in the system. Four growth regulators (auxins, gibberellins, cytokinin and ethylene) will be discussed.

4.2.1. Auxins: Many microorganisms are capable of producing more than one type of auxin. A variety of bacteria, actinomycetes and fungi can synthesize auxin. Arshad and Frankenberger, Jr. (4) provide a list of auxin-producing microorganisms. More microorganisms found in the rhizosphere than those found in root-free soil are auxin producers and many of these produce auxin only in the presence of tryptophan. Indole acetic acid as a secondary metabolite was isolated and identified from the ectomycorrhizal fungus *Pisolithus tinctorius* (17). In addition they (17) reported significant growth stimulation of *Pseudotsuga menziesii* (Douglas fir) inoculated at seedling stage with the fungus and supplied with tryptophan at low concentration (0.34 -34 µg/kg soil).

Dubeikovsky *et al.* (15) inoculated soft wood cuttings of black currant (*Ribes nigrum*) and sour cherry (*Prunus cerasus*) by dipping the cuttings in the cultures of wild and IAA- producing recombinant strains of *P. fluorescens*. They found significantly greater root growth in cuttings inoculated with recombinant strain compared with the wild strain in black currant. However, this response was not observed in sour cherry. It was concluded that IAA produced by the bacterium stimulated the root growth.

4.2.2. Gibberellins: Gibberellins, plant growth-promoting regulators originally isolated from a culture filtrate of *Gibberella fujikuroi* (the imperfect stage of *Fusarium moniliforme*), are a classic example of fungal stimulation of plant growth. Later, gibberellins were identified in several bacteria, fungi, and tissues of green plants. Several gibberellins have been isolated; of these, gibberellic acid or GA_3 is the most common. Application of synthetic gibberellins in agriculture is well known. For more information on agricultural application and chemical composition readers are referred to Rappaport (54) and Railton (53). Indigenous supply of gibberellins to plants by seed inoculation with selected gibberellin-producing microbes along with substrate manipulation should receive attention in allelopathy research.

Several bacteria (principally the genera *Arthrobacter, Azospirillum, Azotobacter, Bacillus* and *Pseudomonas*) and fungal species (principally the genus *Fusarium*) produce gibberellin-like substances. Greater quantities of gibberellin-like substances were found in root nodules of *Lupinus luteus* than in the roots (16). They demonstrated that bioproduction of IAA from tryptophan in the nodules is promoted by GA_3.

In a growth pouch study, Kucey (31) found responses of two cultivars of wheat plants inoculated with *Azospirillum brasiliense* and an isolate of *Bacillus* (C-11-25) were similar to those caused by the addition of gibberellic acid. Dead cells or cell-free culture filtrates of the bacteria elicited similar responses as the live cultures of the bacteria indicating that production of growth hormone was a normal activity of the bacteria. It was concluded that both species of bacteria were capable of producing gibberellic acid in the root region.

4.2.3. Ethylene: Of the four plant growth regulators (auxins, gibberellins, cytokinins and ethylene) more studies have been made on ethylene than on any other microbial growth promoter. Ethylene-producing microbes are prevalent in nature. High soil organic matter content is conducive to ethylene production. Lynch and Harper (34) suggested that fungi, including yeast, are the primary producers of ethylene in soil; aerobic bacteria play a lesser role. Examples of some prolific producers of ethylene in culture include: Bacteria (*Enterobacter, Erwinia, Escherichia, Klebsiella, Pseudomonas*) and fungi (*Aspergillus,*

Candida vartiovaaria, Curvularia, Fusarium, Mucor hiemalis, Penicillium, Rhizopogon and *Trichosporon cutaneum*) [6,33,50]. Soil microorganisms can produce ethylene from many substrates, e.g. methionine and its analogues, other amino acids, organic acids, carbohydrates and protein. Methionine is the preferred ethylene precursor. The combination of glucose and methionine strongly influences the ethylene production in soil (31,33).

Of the five fungal isolates from the rhizosphere of corn, *Acremonium falciforme* was the best ethylene producer in presence of methionine as precursor in broth culture (2). In an earlier experiment, etiolated pea seedlings were exposed to ethylene produced by the fungus in presence of L-methionine. Ethylene induced reduction in stem elongation, swelling of the hypocotyl and horizontal curvature of the stem (Table 6).

Table 6. Influence of L-methionine derived C_2H_4 produced by *Acremonium falciforme* on etiolated pea seedlings grown in autoclaved soil[a]

Treatment	Seedling	
	Length[c] (cm)	Diameter[c] (mm)
Control	6.23 b	1.97 ab
$AgNO_3$ (240 mg/L)[b]	7.57 b	4.87 a
Inoculated	6.67 b	2.07 b
L-Methionine		
10 mM	7.22 b	2.10 b
10 mM + inoculation	2.58 a	2.44 c
10 mM + $AgNO_3$ (240 mg/L)	6.11 b	1.93 ab
10 mM + inoculation + $AgNO_3$ (240 mg/L)	5.77 b	2.07 b

[a]Modified from Arshad and Frankenberger (1), [b]Treatments not subject to $AgNO_3$ received $NaNO_3$ (240 mg/l), [c]Means followed by the same letter are not significantly different at the 0.05 probability level as determined by the Duncan's multiple range test.

Exposure to pure ethylene produced similar effects as those shown in Table 6 (1). These results demonstrated that microbially produced ethylene could influence plant growth and development. Arshad and Frankenberger, Jr. (3) demonstrated that the application of L-methionine and L-ethionine to soil enhanced the ethylene production by 299- and 313-fold, respectively, compared with the unamended soil. A treatment of L-methionine at 1.85 mg/kg influenced corn growth in several parameters, e.g. shoot and root fresh and dry weights, enhanced internodal distance and stem diameter (Table 7). A treatment of L-ethionine at 0.002 to 2 mg/kg enhanced growth and reproduction of tomato plants, e.g. maximum fresh fruit weight at 0.2 mg, most fruits at 0.02 mg and greater average weight of fresh fruit at 2 mg (Table 8). It was concluded that the responses of both corn and tomato were probably due to substrate-supplied ethylene released by the indigenous microflora or uptake of the precursors by the plants and their metabolisms within the plant tissues.

4.2.4. Cytokinins: More than 40 cytokinins and their metabolites have been identified. Nieto and Frankenberger, Jr. (45) have reviewed microbial production of cytokinins. Cytokinins affect plant growth and development by enhancing root and root hair growth

Table 7. Effect of L-methionine applied to the soil on the growth of kandy korn[a]

L-Methionine ($mg\ kg^{-1}$)	Shoot		Root dry weight (g)	Internodal Distance (cm)	Stem Diameter (mm)	Leaf Width (cm)	Resistance to stem breaking (relative units)
	Height (cm)	Dry weight (g)					
Control	134a[b]	26.1a	4.1a	9.1a	15.4a	6.2a	3.41a
185.0	143ab	26.7a	4.1a	11.6b	16.8ab	7.0bc	3.66ab
18.5	155bc	29.9ab	4.6ab	13.1bc	16.9ab	6.7abc	3.77ab
1.85	173d	34.5	5.4c	14.6c	17.4b	7.4c	4.35 b
1.85×10^{-1}	160cd	31.8ab	4.9bc	12.6b	17.0ab	7.1bc	3.66ab
1.85×10^{-3}	160cd	30.8ab	4.4ab	11.6b	16.9ab	6.8abc	3.67ab

[a]Modified from Arshad and Frankenberger (2), [b]Mean of eight replicates. Means followed by the same letter are not significantly different at the 0.05 probability level as determined by the Duncan's multiple range test.

Table 8. Effect of L-ethionine applied to the soil on the plant dry weight and fruit yield of 'Bonny Best' tomato[a]

L-Ethionine ($mg\ kg^{-1}$)	Plant Dry Wight		Fresh fruit yield			
	Shoot (g)	Root (g)	Total Yield (g)	Total number	Mean fruit weight (g)	Number of ripe fruit[b]
Control	106.7ab[c]	4.7a	261ab	7.0ab	37.3ab	1.8ab
60.0	104.4ab	4.0a	229ab	4.8a	47.5abc	0.3a
20.0	93.7a	4.6a	351abc	6.85ab	50.1abc	3.2c
2.0	107.9ab	4.5a	445bc	7.2ab	62.1c	2.7bc
2.0×10^{-1}	93.8a	4.7a	477c	8.7ab	55.0bc	2.5bc
2.02×10^{-2}	95.0a	4.8a	358abc	9.3b	38.4ab	2.3bc
2.0×10^{-3}	104.9av	4.4a	296abc	8.2ab	36.2ab	2.2abc

[a]Modified from Arshad and Frankenberger (2), [b]Includes breaker, pink, light orange, orange, red-orange and red tomatoes, [c]Means followed by the same letter are not significantly different at the 0.05 probability level as determined by the Duncan's multiple range test.

and development, initiating shoot development, delaying leaf senescence, increasing flower formation and increasing transpiration and translocation of assimilates. Rapid absorption of natural cytokinins sprayed on plants often results in larger fruits and greater yields. The high cost of natural cytokinins precludes their use in agriculture as a standard practice.

In culture, a variety of bacteria (*Azotobacter, Azospirillum, Bacillus, Escherichia, Pseudomonas, Rhizobium* and several others) and fungi (*Dictyostillum, Exobasidium, Glomus, Monilia, Plasmodiophora, Rhizopogon* and *Taphrina*) produce cytokinins when a suitable precursor is supplied. Kampert and Strzelczyk (30) detected cytokinin-like compounds in the culture fluid of *Arthrobacter* isolated from the rhizosphere and mycrorhizosphere of pine (*Pinus sylvestris*) and *Bacillus* sp. from adjacent soil. The majority of the microorganisms that produce cytokinins, especially diazotrophs (N_2-fixer), also produce auxins and gibberellins, or both. The use of these bacteria as inoculants is likely to benefit plants by supplying fixed N_2 and phytohormones.

Azotobacter chroococcum, among five bacteria tested, was found to be a prolific producer of cytokinins in culture media; and adenine together with isopentyl alcohol was

the best precursors for this biosynthesis (47). The cytokinins (zeatins and isopentyl adenine and its ribosides) were identified using HPLC and UV spectrometry. Verification of cytokinins production in the broth was demonstrated with a radish seedlings bioassay compared to a known concentration of t-zeatin. Nieto and Frankenberger, Jr. (48) found that an application of adeninine (135 μg/kg soil), isopentyl alcohol (88.1 mg/kg soil) and inoculum *A. chroococcum* enhanced the production of zeatin riboside and t-zeatin. In further greenhouse and field experiments, radish growth (dry weights of shoot and root, leaf area and chlorophyll content) was increased by the application of 0.2 mg/kg adenine and 13 mg/kg isopentyl alcohol with the inoculum, as compared to treatments with inoculum or precursors only. It was concluded that applying suitable precursors to the soil could enhance microbial biosynthesis of cytokinins resulting in enhanced plant growth (46).

It appears that the application of suitable precursors with selected soil microbes can improve plant growth and development. Application of synthetic plant growth regulators in agriculture is not feasible due to the high expenses of application. Application of carefully selected precursors to stimulate microbial synthesis of growth regulators in the rhizosphere zone may be studied to achieve improved plant growth and development. Selection of suitable and inexpensive precursors for stimulation of microbially produced plant growth regulators in a given soil is highly desirable, before testing the practical application in agriculture.

4.3. Actinomycetes

Mishra *et al.* (41) screened metabolites from 796 actinomycetes for plant growth promoting properties using an alga *Chamydomonas reinhardtii* as the test organism. Metabolites of 30 isolates (or 7.8% approx.) stimulated algal growth by more than 50%. The most frequent promoters were isolates of *Nocardia, Promicromonospora, Thermononaspora* and *Rhodococcus.* Solvent extracted broth from six isolates was sprayed on corn, cucumber, soybean, tomato and sorghum. The dry weight of plants was increased by 11 to 20% (Table 9).

Table 9. Stimulation of plant growth by six actinomycete isolates expressed as percent of control.[a]

Isolate number	Actinomycete	Sensitive species	Dry weight (Percent of control)[b]
516	*Micromonospora sp*	Corn	13
		Soybean	14
		Cucumber	17
533	*Nocardia sp.*	Tomato	11
538	*Thermononospora sp.*	Soybean	18
		Cucumber	12
576	*S. hygroscopius*	Soybean	15
580	*Micromonospora sp.*	Cucumber	20
		Sorghum	12
4002	*Actinomadura sp.*	Sorghum	12
		Cucumber	(23 decrease)

[a]Modified from Mishra et. al. (41), [b]All observations were significantly different from the control at the 0.01 probability level.

5. MICROORGANISMS STIMULATORY TO OTHER MICROORGANISMS

Examples of inhibitory effects of microorganisms on other microorganisms (e.g. antibiosis) abound in the literature, but examples of stimulatory effects are rare. Hattingh and Louw (24) isolated 246 bacteria and actinomycetes and 107 fungi from the rhizosphere of two inoculated (nitrogen fixing rhizobia) and uninoculated clovers and adjacent root-free soil. They tested these isolates for stimulatory activities against five strains of *Rhizobium trifolii*. Only three of the five strains were stimulated. Of the 246 isolates, 16% were stimulatory to one or more of the strains. The majority of the stimulating microbes were gram negative, short rod and non-sporing bacteria mostly from the rhizosphere. Stimulating actinomycetes were obtained mainly from the adjacent soil. Only one fungus was stimulatory to one of the strains.

Leuck and Rice (32) tested 28 isolates of bacteria from *Aristida oligantha* rhizosphere, against three strains of *Rhizobium* sp. and three of *Azotobacter* sp. Several of the isolates were inhibitory, but only two stimulated at least one strain of *Rhizobium* sp. *Bacillus cereus* stimulated growth of *Rhizobium* sp. ATCC 10703; *B. megaterium* stimulated growth of the same strain and of *R. japonicum* ATCC 10324.

Thirty-nine fungal species and 18 bacterial isolates from *Trifolium alexandrinum* rhizosphere were tested against *Rhizobium trifolii* (28). Six fungal species stimulated growth of *R. trifolii*, but none of the bacteria were stimulatory (Table 10).

Table 10. Growth stimulation of *Rhizobium trifolii* by factors in a culture medium of fungi isolated from the rhizosphere of *Trifolium alexandrinum* L[a]

Fungi	Rhixobal cell number (cell/ml x 10^6)
Control (no metabolites)	77
Rhizopus sp.	133
Fusarium sp.	125
Aspergillus candidus	99
A. ustus	98
A. nidulans	87
Penicillium funiculosum	83

[a]Modified from Hussain and Mallik (28).

Mallik and Hussain (36) tested 41 fungal, 24 actinomycetal and 66 bacterial isolates from the rhizosphere of *Melilotus alba* against two strains of *Rhizobium meliloti*. Three fungal species stimulated the growth of both strains of *R. meliloti* and several of these were inhibitory (Table 11). It was observed that addition of small dose of cell free culture broth of the stimulatory fungi substantially reduced the generation time of *R. trifolii*. It may be worthwhile to investigate the possibility of using the cell free metabolites from the stimulatory microorganisms as an additive to reduce the generation time in the production of rhizobial inoculant.

Table 11. Growth stimulation of two isolates of *Rhizobium meliloti* by untreated, autoclaved or charcoal filtered factors in a culture medium of three fungi isolated from the rhizosphere of *Melitousta alba*[a]

Fungi	Optical density					
	Untreated		Autoclaved		Charcoal filtered	
	Isolate I	Isolate II	Isolate I	Isolate II	Isolate I	Isolate II
Control (no metabolites)	0.270	0.270	0.270	0.270	0.270	0.270
Aspergillus terreus	0.470	0.500	0.460	0.450	0.430	0.425
Mucor sp.	0.380	0.375	0.350	0.325	0.285	0.295
Pencillium sp.	0.345	0.350	0.332	0.300	0.325	0.310

[a]Modified from Mallik and Hussain (36).

Phytophthora cinnamomi, a plant pathogen with a wide host range and two related species do not produce sporangia and oospores unless an unknown stimulant is supplied to the growth medium. Zentmyer (69) reported that aqueous soil extracts contained the stimulant. Later, it was found that the stimulant was of microbial origin and that metabolites of *Chromobacterium violaceum* contained the stimulant. Acetone extracts of soil, mixed population of soil microbes, and a soil pseudomonad at aqueous dilution of 10^{-9} induced *P. cinnamomi* to form sporangia (5). The unidentified stimulant is non-volatile, polar, thermostable, as well as soluble in water and in several organic solvents. The factor is eliminated by bacteriological filtration of the soil extract and aerated steam treatment (50° C for 10 min.) of the soil.

A morphogenetic factor is involved in microsclerotia formation in fungi. Brandt and Reese (10) found that an isolate of *Verticilliium albo-atrum* produced a diffusible morphogenetic allelochemical that stimulated microsclerotia and melanin production and inhibited the hyphal elongation. The allelochemical is water soluble and insoluble in ether, ethanol and methylene chloride.

Handelsman *et al* (23) isolated a wild-type *Bacillus cereus* UW85 from alfalfa roots that can control the alfalfa damping-off disease caused by *Phytopthora megasperma*. It was found that seed treatment with *B. cereus* UW85 significantly enhanced soybean nodulation by indigenous *Bradyrhizobium japonicum* in three field experiments (22). Soybean seeds, co-inoculated with the strain UW85 and *B. japonicum*, were planted in sterilized soil in a phytotron. Nodulation and acetylene reduction were significantly increased in treated plants as compared to the control plants (without UW85). The authors suggest that *B. cereus* UW85 enhanced nodulation most likely by "stimulating bradyrhizobial infection or by suppressing abortion of infection process."

The stimulation of microorganisms by metabolites of other microbes may be exploited to enhance nodulation and symbiotic N_2-fixation, to reduce the growth-period of the inoculum in commercial production of legume inocluant and to induce reproduction of plant pathogenic fungi, thereby facilitating control measures.

6. CONCLUSIONS

Growth inhibition is the primary focus of allelopathy research, while growth stimulation is generally ignored or incidentally mentioned. Overall, allelopathic stimulation is grossly under explored. Here we provide several examples of allelopathic stimulation, but more are available in the literature, and many more await discovery. Multiple cropping, with careful attention to the selected plant components, could provide higher yields and reduce weed and pests. Seed germination stimulants from host and non-host plants and microbially produced ethylene are highly efficacious and environmentally safe tools to control angiospermous plant parasites. Microbial plant growth regulators are cost effective and are more environmentally friendly compared to synthetic compounds. Application of selected microbial inoculum, along with the appropriate precursors and substrates, could supply phytohormones in the root zone to enhance crop yield. Seed inoculated with selected bacteria besides diazotroph has been reported to enhance crop yield. Further exploration in this area is desirable. Microbial metabolites stimulatory to other microorganisms have been found useful in developing strategies for the control of plant pathogens. The usefulness of these metabolites in the production of legume inoculant and in the fermentation industry, as well as the identification of these metabolites, needs further investigation. If carefully exploited, allelopathic stimulation could be used to enhance crop yield, reduce reliance on synthetic pesticides and promote sustainable agriculture.

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