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# Effect of crustacean chitin on the efficacy of plant growth promoting bacteria in the control of root infecting fungi of sunflower and chickpea

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Summary

Soil amendment with crustacean chitin used alone or with *Pseudomonas aeruginosa* *Bacillus subtilis* significantly ($p<0.05$) reduced infection of *Rhizoctonia solani* *Fusarium solani* on sunflower and *R. solani* on chickpea. Crab chitin used alone or with *P. aeruginosa* or *B. subtilis* completely controlled the infection of *Macrophomina phaseolina* on chickpea. Prawn or shrimp powder used 1% w/w of soil was found phytotoxic on chickpea but not on sunflower. Maximum fresh weight of shoot was produced by *P. aeruginosa* used with shrimp powder in sunflower and with crab chitin in chickpea. *P. aeruginosa* produced greater plant height in chickpea used with shrimp chitin.

Key words: chitin, plant growth promoting bacteria, root rot

## INTRODUCTION

Chitin the most common polysaccharide when mixed with soil stimulates the micro-organisms and this chemically stable compound is mineralized in short period. Pure chitin has a narrow C:N ratio, so that the nitrogen supply exceeds the microbiological requirement (A l e x a n d e r, 1977). There are reports that soil amendement with chitin significantly control the root knot nematode (M i a n et al., 1982) and root infecting fungi (B a d e, W i c k, 1988) by changing the soil micro-organisms resulting in an increase in organisms antagonistic to the pathogens (G o d o y et al., 1983).

From the coast of Arabian sea which has an area of 885 $Km^2$, several million tons of crustaceans like shrimp, prawn and crab are caught for local consumption and export. Most of the processing byproducts of shell fishes are either not utilized or dumped as waste thus producing environmental problems (S h a h i d i, S y n o w i e c k i, N a c z k, 1992). In the recent past, fluorescent *Pseudomonas*, a plant growth promoting bacteria besides enhancing the plant growth also showed effective control of root infecting fungi of crop plants (K l o e p p e r, L e o n g, T e i n t z e, 1980; S u s l o w, S c h r o t h, 1982; E h t e s h a m u l - H a q u e, 1995). Experiments were therefore carried out to see the effect on crustacean chitin on the efficacy of plant growth promoting bacteria in the control of root infecting fungi of sunflower and chickpea.

## MATERIALS AND METHODS

Crustacean waste from shrimp, prawn and crab collected from Empress market Karachi were air dried, powdered in an electric grinder and stored in polyethylene bags until used.

For the extraction of chitin modified method of B a d e and W i c k (1988) was used where shells were demineralized in 5% HCl for 2 hrs. at room temperature and then deproteinized with 5% KOH solution for 2 hrs. at 100°C. Chitin was separated on a coarse glass sintered funnel, washed with distilled water to neutral pH and then with acetone to remove the water followed by oven drying at 105°C for 1 hr. The purified chitin was stored in polyethylene bags for further study.

Dry powders of shrimp, prawn and crab chitin were mixed in sandy loam soil, pH 8.0 0.1% w/w. Since percentage of chitin vary in shrimp (16.1%), prawn (14.5%) and crab (19.5%), the crustacean powders were mixed with soil according to percentage of chitin (0.62 g of shrimp, 0.68 g of prawn and 0.51 g of crab per 100 g soil to give 0.1% w/w. keeping the chitin content same in waste powder treatment and pure chitin treatment. The soil had a natural infestation of 3-9 sclerotia g of soil of *Macrophomina phaseoli* (Tassi) Goid as found by wet sieving and dilution technique (S h e i k h, G h a f f a r, 1975), 5-15% colonization of *Rhizoctonia solani* Kuhn on sorghum seeds used as baits (W i l h e l m, 1955) and 3200 cfu g of soil of mixed population of ium solani *F. oxysporum* Schlecht as assessed by soil dilution technique (N a s h, S n y d e r, 1962). Amended soil was transferred in 8 cm diam., plastic pots 250 g per pot. Pots were watered daily and kept at 50% W.H.C (K e e n, R a c z k o w s k i, 1921). After three weeks, aqueous suspension of *Pseudomonas aeruginosa* and *Bacillus subtilis* ($10^8$ cfu $ml^{-1}$) multiplied on Nutrient Agar were drenched in amended and nonamended pots 25 ml/pot. Five seeds of sunflower (*Helianthus annuus L.*) and chickpea (*Cicer arietinum*) were sown in each pot. Each treatment was replicated three times and the pots were randomized on a screen house bench.

Plants were uprooted after 30 days growth. After washing in tap water 5 cm long root pieces from each plant were cut, surface sterilized with 1% $Ca(Cl)_2$ for 3 minutes and transferred on to PDA plates containing penicillin (100000 units/litre) and streptomycin (0.2 gm/litre). Plates were incubated for 5 days at 28°C and incidence of root infecting fungi viz., *M. phaseolina*, *R. solani*, and *Fusarium* spp., were

recorded. Data on plant height and fresh weight of shoots were also recorded. Data were analysed and subjected to factorial ANOVA followed by Least Significant Difference (LSD) according to G o m e z and G o m e z (1984).

## RESULTS

Of the crustaceans waste used, highest chitin was recovered from crab (19.5%) followed by shrimp (16.1%) and prawn (14.5%).

Soil amendment with shrimp powder and prawn powder showed toxicity on chickpea but not on sunflower, these treatments were therefore excluded from chickpea experiments. Prawn and crab chitin significantly ($p<0.05$) reduced the infection of *M. phaseolina* on sunflower while shrimp, prawn and crab powder and shrimp chitin were found ineffective in controlling *M. phaseolina* infection, whereas chitin from crab showed complete control of *M. phaseolina* infection on chickpea. Use of *P. aeruginosa* and *B. subtilis* significantly reduced the infection of *M. phaseolina* on both sunflower and chickpea. Combined use of crustacean powder or chitin with bacteria did not produce any better result (Table 1&2).

Use of crustacean chitin or powder significantly ($p<0.05$) reduced the infection of *R. solani* on both sunflower and chickpea. Complete control of *R. solani* infection was produced where *P. aeruginosa* was used with prawn powder, shrimp or prawn chitin or where *B. subtilis* was used with shrimp or prawn powder or with shrimp or crab chitin in sunflower. In chickpea complete control of *R. solani* infection was observed where prawn chitin was used alone or where *P. aeruginosa* was used with prawn or crab chitin or *B. subtilis* was used with shrimp or crab chitin (Table 1&2).

Complete control of *F. solani* infection in sunflower was produced by shrimp chitin or *B. subtilis* used alone or where *P. aeruginosa* was used with shrimp or prawn chitin. Use of shrimp or crab powder or prawn or crab chitin also significantly reduced the infection of *F. solani* on sunflower. In chickpea significant ($p<0.05$) control of *F. solani* infection was produced by shrimp or crab chitin or where *P. aeruginosa, B. subtilis* were used alone or where *P. aeruginosa* was used with prawn or crab chitin (Table 1&2).

Maximum fresh weight of shoot in sunflower was produced in treatment where *P. aeruginosa* was used with shrimp powder followed by shrimp powder with *B. subtilis*. Whereas in chickpea greater fresh weight of shoot was produced by *P. aeruginosa* used with crab chitin followed by *P. aeruginosa* used alone. Greater plant height was produced by *P. aeruginosa* followed by crab chitin in sunflower. Use of *P. aeruginosa* with shrimp or crab chitin also produced maximum plant height in chickpea (Table 1&2).

## DISCUSSION

Chitin which is a major component of discarded shellfish is reported to have 17% in shrimp and 18.7% in crab (S h a h i d i, S y n o w i e c k i, N a c z k, 1992). In the present study use of crustacean chitin 1% or crustacean waste (having 0.1% chitin) used alone or with *P. aeruginosa* and *B. Subtilis* showed significant results in the control of root infecting fungi of sunflower and chickpea. There are reports that use of chitin alone or with other organic materials reduced the severity of fungal infection (B a d e, W i c k, 1988). Soil amendment with chitin stimulates the micro-organisms (M i t c h e l l, A l e x a n d e r, 1962) which produce toxins (M a n k a u, D a s, 1969) creating an environment adverse to plant pathogens. Decomposition of polysaccharide in soil is mediated principally through the activities of fungi and actinomycetes (D o m s c h, G a m s, A n d e r s o n, 1980; G o d o y et al., 1983; M i a n et al., 1982). R o d r i g u e z - K a b a n a, M o r g a n - J o n e s, G i n t i s (1984) reported addition of 1% or more chitin to soil showed significant control of root knot nematode. In the present study use of 0.1% chitin showed significant results in the control of soilborne root infecting fungi in sunflower and chickpea. It is interesting to note that 1% crustacean waste was found phytotoxic on chickpea. Toxicity to plants by crustacean waste is probably due to some other factors besides accumulation of nitrites and nitrate (M i a n et al., 1982; R o d r i g u e z - K a b a n a, G o d o y, M o r g o n - J o n e s, 1983) which narrows the C:N ratio of the amendments. Factors involved in phytotoxicity of crustacean waste needs further study. In the present study use of *P. aeruginosa* or *B. subtilis* with chitin in chickpea and crustacean powder showed better plant growth in sunflower and control of root infecting fungi. In soil approximately 90-99% of the chitinoclastic population are actinomycetes which digest the chitin (A l e x a n d e r, 1977). This digested chitin is then utilized by soil bacteria as nitrogen source resulting in an increase in their population in amended soil. These bacteria present in the rhizosphere inhibit root infecting fungi by producing antibiotics (A h l, V o i s a r d, D e f a g o, 1986) and enhance plant growth by producing phytohormones (B r o w n, 1972; H u s a i n, V a n c u r a, 1970). Since sufficient quantities of waste chitinous materials are available in coastal area of the world it would be suggested that use of these materials as soil amendment may provide an economical means of disposal of the waste materials with an increased supply of nitrogen to the soil and control of nematode besides control of soilborne root infecting fungi resulting in better plant growth.

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Table 1.

Effect of crustacean chitin, *Pseudomonas aeruginosa* and *Bacillus subtilis* in the control of root infecting fungi of sunflower plants.

| Treatments | *M. phaseolina* | *R. solani* | *F. solani* | Plant height (cm) | Fresh weight of shoot (gm) |
|---|---|---|---|---|---|
| | Infection % | | | | |
| Control | 91 | 83 | 75 | 24.8 | 1.6 |
| Shrimp Powder (SP) | 75 | 11 | 8 | 28.9 | 4.2 |
| Prawn Powder (PP) | 75 | 8 | 83 | 26.7 | 4.7 |
| Crab Powder (CP) | 100 | 8 | 17 | 30.9 | 4.3 |
| Shrimp chitin (SC) 0.1% w/w | 92 | 8 | 0 | 30.3 | 2.8 |
| Prawn chitin (PC) 0.1% w/w | 67 | 8 | 8 | 30.3 | 2.7 |
| Crab chitin (CC) 0.1% w/w | 50 | 8 | 17 | 31.8 | 2.7 |
| *Pseudomonas aeruginosa* | 50 | 30 | 41 | 32.4 | 1.7 |
| *Bacillus subtilis* | 11 | 61 | 0 | 30.4 | 2.0 |
| SP+ *P. aeruginosa* | 72 | 33 | 50 | 31.0 | 7.7 |
| SP+ *B.subtilis* | 53 | 0 | 55 | 31.6 | 5.4 |
| PP+ *P. aeruginosa* | 61 | 0 | 58 | 28.9 | 3.1 |
| PP+ *B. subtilis* | 88 | 0 | 66 | 27.0 | 4.5 |
| CP + *P. aeruginosa* | 75 | 17 | 41 | 26.8 | 4.7 |
| CP + *B. subtilis* | 47 | 25 | 47 | 28.2 | 3.4 |
| SC + *P. aeruginosa* | 83 | 0 | 0 | 28.0 | 2.9 |
| SC + *B. subtilis* | 92 | 0 | 8 | 29.0 | 3.4 |
| PC + *P. aeruginosa* | 83 | 0 | 0 | 30.5 | 2.3 |
| PC + *B. subtilis* | 92 | 8 | 25 | 28.4 | 3.0 |
| CC + *P. aeruginosa* | 75 | 8 | 25 | 29.2 | 2.4 |
| CC + *B. subtilis* | 58 | 0 | 33 | 28.6 | 2.5 |
| $LSD_{0.05}$ (Treatments) = 23.6 (Pathogens) = 8.9 | | | $LSD_{0.05}$ = 5.8 | | 1.9 |

Table 2.

Effect of crustacean chitin, *Pseudomonas aeruginosa* and *Bacillus subtilis* in the control of root infecting fungi of chickpea plants.

| Treatments | *M. phaseolina* | *R. solani* | *F. solani* | Plant height (cm) | Fresh weight of shoot (gm) |
|---|---|---|---|---|---|
| | Infection % | | | | |
| Control | 33 | 53 | 100 | 19.0 | 1.7 |
| Crab Powder (CP) | 30 | 33 | 100 | 18.8 | 2.3 |
| Shrimp chitin (SC) 0.1% w/w | 50 | 33 | 67 | 23.2 | 2.4 |
| Prawn chitin (PC) 0.1% w/w | 25 | 0 | 92 | 23.0 | 2.1 |
| Crab chitin (CC) 0.1% w/w | 0 | 33 | 75 | 24.1 | 2.0 |
| *Pseudomonas aeruginosa* | 0 | 55 | 58 | 24.2 | 2.9 |
| *Bacillus subtilis* | 8 | 33 | 36 | 21.9 | 1.2 |
| CP + *P. aeruginosa* | 33 | 25 | 100 | 20.1 | 2.8 |
| CP + *B. subtilis* | 11 | 47 | 80 | 20.1 | 2.2 |
| SC + *P. aeruginosa* | 67 | 28 | 92 | 27.9 | 2.6 |
| SC + *B. subtilis* | 33 | 0 | 92 | 20.5 | 2.0 |
| PC + *P. aeruginosa* | 17 | 0 | 58 | 24.5 | 2.5 |
| PC + *B. subtilis* | 28 | 11 | 92 | 23.3 | 1.7 |
| CC + *P. aeruginosa* | 0 | 0 | 72 | 26.0 | 3.1 |
| CC + *B. subtilis* | 0 | 0 | 87 | 24.3 | 2.3 |
| $LSD_{0.05}$ (Treatments) = 22.9 (Pathogens) = 10.2 | | | $LSD_{0.05}$ = 5.2 | | 0.9 |

## Wpływ chityny skorupiaków na skuteczność stymulujących wzrost bakterii w ograniczaniu infekcji korzeni słonecznika i cieciorki (*Cicer arietinum*)

Streszczenie

Potraktowanie gleby chityną otrzymaną ze skorupiaków - samą lub łącznie z *Pseudomonas aeruginosa* lub *Bacillus subtilis* – redukowało istotnie ($p<0.05$) infekcję *Rhizoctonia solani* i *Fusarium solani* na słoneczniku i *R. solani* na cieciorce. Chityna z krabów użyta osobno lub z *P. aeruginosa* lub *B. subtilis* całkowicie hamowała infekcję *Macrophomina phaseolina* na cieciorce. Proszek z ślimoraczka lub krewetki użyty w stężeniu 1% (wagowo) doglebowo był fitotoksyczny dla cieciorki, ale nie dla słonecznika. Największą świeżą masę pędów produkował słonecznik w efekcie użycia *P. aeruginosa* łącznie z proszkiem z krewetek, a cieciorka – po użyciu chityny z krabów. Najwyższe rośliny cieciorki otrzymano w wyniku użycia chityny z krewetek.