Chapter 4

Integrated biocontrol potential of Paenibacillus sp. D1 and Streptomyces sp. A6 with chemical pesticides
4.1. INTRODUCTION

Plant diseases are one of the major bottlenecks in agricultural production which result in losses up to 30% of total global food production (Strange and Scott, 2005). Plant pathogenic fungi are the most aggressive plant pathogens followed by bacteria, viruses, nematodes and protozoa. Conventionally, pesticides (insecticides, fungicides, termiticides etc.) are used to control the damage caused by pests in the crop fields. However, their excessive use which has almost tripled in last 40 years has led to several problems related to pollution and environmental degradation (Fox et al., 2007). Although, chemical pesticides provide effective protection against pests but at the same time proves lethal to several useful soil insects and beneficial microorganisms in the rhizosphere due to their non specific nature (Budi et al., 2000). Moreover, the use of chemical agents to control fungal phytopathogens is often not practical due to high costs, lack of efficacy and the emergence of resistant strains. Thus, increased societal concern towards the use of toxic agrochemicals has prompted greater interest in use of biological control that significantly reduces or eliminates the use of pesticides and is more economic, target specific and eco-friendly (Jensen and Malter 1995). However, lack of consistency is often associated with biological control systems due to environmental fluctuations and residual pesticides in fields that hinder their implementation on large scale.

In recent years, integrated pest management (IPM) has emerged as eco-friendly and more economic alternative to conventional use of chemical pesticides for control of plant diseases in agricultural fields. IPM’s main feature is minimum use of synthetic pesticides and maximum reliance on natural regulatory mechanisms to keep pest populations below the level at which they can cause economic damage (Gray et al., 2013). One of the strategies used in IPM is integrated use of biocontrol agents (BCAs) with pesticides. This approach reduces to input of pesticides in the fields and if appropriately chosen, provides better control of plant diseases than the chemical control (Kiewnick et al., 2001; Someya et al., 2007).

The first requirement for any BCA to be used in IPM is its tolerance to the pesticides with which it is to be integrated. Secondly, it is necessary to determine the type of interaction between the BCA and pesticide in control of the test pathogen. A synergistic interaction between the two control agents will provide better protection against the pathogen. *Paenibacillus* and *Streptomyces* species are widely distributed in nature and have
been reported for their inhibitory effect on bacteria and/or fungi (Kajimura and Kaneda, 1997; Seldin et al., 1999; von der Weid et al., 2003) attributed to biologically active antagonistic substances produced by these strains such as antimicrobial substances (antibiotics, bacteriocins and / or small active peptides) and cell wall degrading enzymes (p-1,3- glucanases, cellulases, chitinases and proteases) (Dunn et al., 1997; Budi et al., 2000;Crawford et al., 1993). Moreover, members both Paenibacillus and Streptomyces species can promote plant growth by atmospheric nitrogen fixation, mineral solubilization and production of siderophores or phytohormones (Glick et al., 1999; Timmusk, 2003; Chang and Yang, 2008; Dimkpa et al., 2008). Thus, strains belonging to genus Paenibacillus and Streptomyces can act as biocontrol agents with plant growth promoting ability. Moreover, their ability to form desiccation resistant spores that assist in spread and persistence during low nutrient availability and also make them easy to formulate, makes them preferred biocontrol agent (Benimeli et al., 2007)

Although, members of Paenibacillus and Streptomyces have been studied for biocontrol, studies on its pesticide tolerance are limited. Thus, the present study was focused on studying the pesticide tolerance of Paenibacillus sp. D1 and Streptomyces sp. A6 towards commonly used pesticides and to investigate its potential in IPM to control fusarium wilt of Cajanus cajan (Pigeon pea).

### 4.2. MATERIALS AND METHODS

#### 4.2.1. Organisms and culture conditions

Chitinolytic bacterium Paenibacillus sp. D1 and protease producing Streptomyces sp. A6 were used for the study. Paenibacillus sp. D1 and Streptomyces sp. A6 were cultivated in medium optimized for chitinase and protease production as described in chapters 3A and 3B, respectively. Medium composition for chitinase production by Paenibacillus sp. D1 was (g/l): Urea, 0.33; K2HPO4, 1.17; chitin, 3.75 and yeast extract, 0.65 while forprotease was (g/l): Shrimp waste, 14; FeCh, 0.035; ZnSO4, 0.065 and initial pH of 8.0. The cultures were grown at 30°C under shaking conditions (180 rpm) for 72 h. Growth was determined in terms of total cell protein (Waterborg and Matthews, 1996). The same medium was used for enzyme production and fungal biomass reduction studies.

Spores from Fusariumudum and Streptomyces sp. A6 were harvested in 0.001% aqueous
triton X-100 by cultivating the fungus in medium containing (g/1): Potato infusion, 200.0; Dextrose, 20.0; Rose Bengal, 0.0084; Agar, 15.0; pH 5.6 ± 0.2 for 7 days at 25°C and Streptomyces sp. A6 in medium containing (g/1): chitin, 5.0; yeast extract, 0.5; (NH4)2SO4, 1.0; MgSO4·7H2O, 0.3; KH2PO4, 1.36, Agar, 15.0; pH 7.2 for 7 days at 30°C (Monreal and Reese, 1969).

4.2.2. Enzyme production and assay

Cultures were centrifuged at 10,000 x g for 20 min at 4°C and the culture supernatant was subjected to ammonium sulphate precipitation (70% saturation for chitinase from *Paenibacillus* sp. D1 and 80% saturation for protease from *Streptomyces* sp. A6). The pellet was dialysed against buffer (acetate buffer pH 5.0 for chitinase and Tris·Cl buffer pH 7.2 for protease) for removing the ammonium sulphate and diluted with respective buffer to have a final activity of 100 units/ml. The enzymes were then filter sterilized and used.

4.2.3. Pesticides used in the study

List of pesticides used in the study, their active ingredients, classification based on Fungicide Resistance Action Committee (FRAC) and Insecticide Resistance Action Committee (IRAC), mode of action and recommended field concentration (RFC) according to manufacturer is given in Table 4.1.

4.2.4. Effect of pesticides on growth and enzyme production by *Paenibacillus* sp. D1 and *Streptomyces* sp. A6

Growth and enzyme production i.e. chitinase and protease, by *Paenibacillus* sp. D1 and *Streptomyces* sp. A6, respectively was investigated by growing the cultures in pesticide amended medium. The concentrations of pesticides used were RFC and 100 pg/ml of active ingredient.

4.2.5. Effect of pesticides on enzyme activities

To investigate the effect of pesticides on chitinase and protease activity from *Paenibacillus* sp. D1 and *Streptomyces* sp. A6, respectively, 100 units of enzyme preparations were incubated with different concentrations (RFC, 100 and 1000 pg/ml) of pesticides at 4°C for 5 min, followed by enzyme assay using substrate containing pesticides. Residual enzyme activity was expressed in percentage taking control without pesticide as 100%.
4.2.6. Effect of pesticides on stability of chitinase and protease

Stability of chitinase and protease at 40°C in presence of pesticides was monitored for two hours by incubating 100 units of enzymes with varying concentrations of pesticide (RFC, 100 and 1000 jig/ml). The residual enzyme activity was determined at intervals of one and two hours.

All the experiments were carried out in triplicate and the values of results were expressed as average of triplicate determinations along with the respective SD values.

4.2.7. Determination of 50% Effective Concentration (ECso) dose of Paenibacillus sp. D1 and its chitinase (C-D1); Streptomyces sp. A6 and its protease (P-A6); and test fungicides for inhibition of Fusarium spore germination and fungal biomass reduction

The dose of test fungicides (mancozeb, sulphur and carbendazim), Paenibacillus sp. DI, Streptomyces sp. A6, C-DI and P-A6 for inhibiting the fusarium spore germination by 50% was determined as described earlier by Lorito et al. (1994). 105 conidia of Fusariumudum was incubated for 96 h at 30°C with varying concentrations of test fungicides (10-80 pg/ml), Paenibacillus sp. DI (104-106 cells/ml), Streptomyces sp. A6 (104-106 spores), C-DI (100-500 units of chitinase) and P-A6 (100-500 units of protease) in a 10.0 ml system. Samples from each mixture were analyzed for spore germination. Germination of the first 100 spores observed was evaluated. ECso dose for inhibition of fungal spore germination was determined from the dosage response curve for each of the test agents.

The ECso dose of test fungicides, Paenibacillus sp. DI, Streptomyces sp. A6, C-DI and P-A6 were also determined in terms of reduction in fungal biomass. Different percentage (0.25-4%) of Paenibacillus sp. DI culture (108cells/ml), Streptomyces sp. A6 spore suspension (108 spores/ml) was incubated with 106 spores of Fusariumudum in 100 ml medium at 30°C under shaking (180 rpm) conditions. After 96 hrs fusarial biomass was obtained by filtering the media through Whatman filter paper No.1. Biomass was dried at 60°C till constant weight. EC50 dose for fungal biomass reduction was determined by regression analysis of dose response plot of percentage inoculum vs reduction in fungal biomass. Similarly, EC50 dose of C-DI, P-A6 and test fungicides were determined by supplementing different units (1000-5000 units) of filter sterilized enzymes (C-DI and P-A6) and varying concentrations.
(10-80 pg/ml) of the active ingredients of fungicides in medium.

4.2.8. Seed protection assay

Seeds of wilt susceptible variety of *Cajanus cajan* (T15-15) were surface sterilized by treatment with 0.01% HgCl and 4% NaOCl for 90 sec with intermittent washes using sterile distilled water. EC50 dose of *Paenibacillus* sp. D1 and *Streptomyces* sp. A6 for seed protection studies were determined by incubating seeds with culture/spore suspension of *Paenibacillus* sp. D1 (10^6-10^7 cells/ml) and *Streptomyces* sp. A6 (10^6-10^10 spores/ml), in presence of 1% carboxymethyl cellulose (CMC) for 4 hours while EC50 dose of test fungicides were determined by treating the seeds with different concentrations of test fungicides (mancozeb and carbendazim) (20-100 pg/ml). After treatments the seeds were air dried under sterile conditions and placed on water agar plate (5 seeds per plate) containing 10^5 spores/ml of *Fusarium udum*. Plates were incubated in dark for 96 h and observed for infection. Experiment was conducted in six replicates per treatment.

4.2.9. Field trials

The field trial was conducted (2007-2013) in a rain irrigated and well maintained *Fusarium* wilt sick plot at pulse research centre model farm, Vadodara, India, during the monsoon seasons. The details of field trials were same as described in section 2.2.14.

4.2.10. Combined application of biological antifungal agents (*Paenibacillus* sp. D1, *Streptomyces* sp. A6, C-D1 and P-A6) with chemical fungicides for control of *Fusarium* growth and infection in *Cajanus cajan*

The percentage reduction in fungal spore germination, fusarial biomass and wilt incidence during seed protection studies and field trials, in presence of combined EC50 dose of biological control agents (*Paenibacillus* sp. D1, *Streptomyces* sp. A6, C-D1 and P-A6) and test fungicides were analyzed for synergism Limpel’s formula (1) (Richer, 1987).
where, $E_e$ is the expected effect from additive responses of two inhibitory agents; $X$ and $Y$ represent the percentage inhibition caused by the inhibitory agents when used alone. The synergy factor (SF) is calculated by Abott’s formula (2) (Abbott, 1925).

$$SF = \frac{Observed\ Inhibition}{Expected\ Inhibition}$$ (2)

where, SF $>1$ for Synergistic reaction; SF $<1$ for antagonistic reaction; SF $=1$ for additive reaction.

4.2.11. Statistical analysis

The data was subjected to analysis of variance (ANOVA) followed by Fisher’s LSD test ($p < 0.05$) using SigmaStat version 3.5 (Systat Software Inc. Germany).

4.3. RESULTS

4.3.1. Effect of pesticides on growth and enzyme production by *Paenibacillus* sp. D1 and *Streptomyces* sp. A6

4.3.1.1 Effect of Fungicides

Both *Paenibacillus* sp. D1 and *Streptomyces* sp. A6 were found to be tolerant to most of the fungicides tested. However, slight variation in growth, chitinase and protease production was observed with different classes of fungicides. Supplementation of medium with fungicides belonging to M, MBC and phosphonates group had no inhibitory effect on growth of both the cultures and their chitinase and protease production. Instead, sulfur was found to stimulate the growth and chitinase production in *Paenibacillus* sp. D1 while sulfur, mancozeb, carbendazim, fosteylaluminium, and triadimefon, increased the growth and protease production in *Streptomyces* sp. A6 (Table 4.2).

Among all the DMI group of fungicides tested, tebuconazole, difenoconazole and triadimefon had little inhibitory effect on growth and chitinase production by *Paenibaciilus* sp. D1 at their respective recommended field application concentrations with maximum
inhibition of 24% by triadimefon. Increasing the concentrations of these fungicides further increased the inhibitory effect. However, Streptomyces sp. A6 exhibited strong tolerance towards all the DMI fungicides (Table 4.2).

4.3.1.2. Effect of Insecticides and termiticides

No significant inhibition (>10%) in growth and major mycolytic enzyme production by Paenibacillus sp. D1 and Streptomyces sp. A6 was observed on supplementation of medium with organophosphate and carbamate group of insecticides. Acephate increased growth of Paenibacillus sp. D1 by 23% with no effect on chitinase production while, growth and protease production by Streptomyces sp. A6 was enhanced in presence of both acephate and methyl parathion (Table 4.2). At higher concentrations (100 pg/ml), except for acephate, all other organophosphate insecticides significantly inhibited both growth and chitinase production in Paenibacillus sp. D1 while, no such decrease was observed in Streptomyces sp. A6. Instead, increase in growth by Streptomyces sp. A6 was observed in presence of acephate, methyl parathion and dichlovas.

Growth of both Paenibacillus sp. D1 and Streptomyces sp. A6 was drastically inhibited in presence of cypermethrin, a pyrethroids insecticide. Endosulfan inhibited growth of both the cultures only at higher concentrations in concentration dependent manner.
4.3.2. Effect of pesticides on activity and stability of chitinase from *Paenibacillus* sp. D1 and protease from *Streptomyces* sp. A6

No inhibitory effect on activity and stability of chitinase and protease was observed with test fungicides at RFC and higher concentrations (1000 pg/ml) except for propiconazole which inhibited chitinase and protease activity by almost 40 and 30% respectively, at 100 fg/ml concentration. Stability of chitinase was reduced at higher concentrations of propiconazole and hexaconazole. However, no decrease in stability of protease was observed in presence of these fungicides.

Both the enzymes were highly active and stable in presence of most of the insecticides and termiticide tested at RFC, except for insecticides eypermethrin which completely inhibited the chitinase activity at RFC while protease activity was decreased at higher concentrations (Table 3.4a and 4b).
Tables 4.6a and 4.6b depict the observed and expected values for fungal biomass reduction caused by combined ECso dose of enzymes (C-D1 or P-A6) and test fungicides. The observed inhibition for all the combinations was found to be greater than or equal to the expected inhibition indicating synergism between CF and test fungicides. Culture filtrates from both the cultures exhibited highest synergism with carbendazim for fungal spore germination inhibition as well as biomass reduction.

Seed protection assays and field trials revealed strong synergistic interaction between bacterial cultures (.Paenibacillus sp. D1 and Streptomyces sp. A6) and fungicides (carbendazim and mancozeb) used in seed dressing, for controlling fusarium infection and wilt incidence in Cajamtscajan (Table 4.7a and 4.7b).
4.3.3. Determination of 50% Effective Concentration (ECso) dose of test fungicides, *Paenibacillus* sp. D1 and its chitinase (C-D1); *Streptomyces* sp. A6 and its protease (P-A6) for inhibition of Fusarium spore germination and fungal biomass reduction

EC50 dose of test fungicides, mancozeb, carbendazim, fosteyi aluminum and sulphur, were 27.77, 6.78, 47.15 and 41.93 pg/ml for inhibition of fungal spore germination and 29.25, 18.34, 50.49 and 45.48 pg/ml for fungal biomass reduction, respectively. EC50 dose of test fungicides, used for seed dressing, mancozeb and carbendazim were determined as 40 and 30 pg/ml, respectively.

The EC50 dose of *Paenibacillus* sp. D1 and C-D1 was found to be 3.18 x 10^6 cells/ml and 25.03 units/ml for fungal spore germination inhibition and 2.36 x 10^6 cells/ml and 31.4 units/ml for fungal biomass reduction, respectively. The cell density of 105 cells/seed served as EC50 dose of *Paenibacillus* sp. D1 for inhibition of fungal infection of *Cajanuscajan* seeds.

The ECso dose of *Streptomyces* sp. A6 and P-A6 was found to be 1.14 x 10^6 spores/ml and 37.7 units/ml for fungal spore germination inhibition and 1.44 x 10^6 spores/ml and 40.94 units/ml for fungal biomass reduction, respectively. The spore count of 106 spores/ml served as EC50 dose of *Streptomyces* sp. A6 for prevention of fusarium infection of *Cajanuscajan* seeds.

4.3.4. Interaction between the bacterial cultures, their major mycolytic enzymes and test fungicides

The interaction between *Paenibacillus* sp. D1 or *Streptomyces* sp. A6 and fungicides was found to be highly synergistic (SF>1) as their combined ECso doses reduced the fungal spore germination and fungal biomass better than expected. *Paenibacillus* sp. D1 exhibited maximum synergism with carbanedazim for both inhibition of fungal spore germination and fungal biomass reduction while *Streptomycyes* sp. A6 exhibited maximum synergism with carbanedazim for inhibition of fungal spore germination and with mancozeb for reduction of fungal biomass (Table 4.5a and 4.5b).

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4.4. DISCUSSION

The isolates *Paenibacillus* sp. D1 and *Streptomyces* sp. A6 had strong antifungal activity against a wide spectrum of fungal plant pathogens mainly attributed to production of mycolytic enzymes and an unknown antifungal metabolite which makes it a potential biocontrol agent. Chitinase and protease were the major mycolytic enzymes produced by *Paenibacillus* sp. D1 and *Streptomyces* sp. A6, respectively. The present investigation was carried to explore the IPM potential of these biocontrol agents with commonly used pesticides. The first and most important for IPM is the compatibility of integrating components.

Enhanced growth and mycolytic enzyme production by *Paenibacillus* sp. D1 and *Streptomyces* sp. A6 in presence of commonly used pesticides suggested their compatibility with these chemicals for possible application in integrated pest management for control of phytopathogens. High tolerance towards mancozeb and carbendazim was advantageous as these fungicides are used during seed dressing mainly for pigeon pea (*Cajanus cajan*) seeds. Fungicides act by number of different mechanisms inhibiting various metabolic functions specific to eukaryotic cells as depicted in Table 4.1. Such targets are absent in prokaryotes which explains the tolerance of *Paenibacillus* sp. D1 and *Streptomyces* sp. A6 towards fungicides. Mancozeb, captan and sulphur are protectant fungicides and exert their effect by acting on number of different metabolic sites within the fungus especially by inhibiting fungal energy production. All of these fungicides are contact fungicides and work only if applied prior to infection (www.ipm.iastate.edu/ipm/icm/2006/6-12/fimgicides.html). There are several reports on interaction of rhizobia with protectant fungicides. However, no such reports are available for *Paenibacillus* and *Streptomyces* species. Olebiowska et al. (1967) reported that *Rhizobium* strains had different sensitivities to the fungicide thiram. Inhibitory effects of mancozeb on the growth of *Bradyrhizobium japonicum* and *Rhizobium* sp. were suggested by Mallik and Tesfai (1983) and Castro et al. (1997). Heinonem-Tanskiet al. (1982) reported toxic effect of many fungicides (captan, carboxin, folpet, etc.) on rhizobia. A recent report has stated reduction in efficiency of nitrogen fixation by rhizobia and the host plant even after single application of pesticide (Fox et al., 2007). Effect of these fungicides has also been studied in other microorganisms. Dillwith and Lewis (1982) suggested inhibition of growth of *E. coli* by captan in concentration dependent manner due to alterations in RNA metabolism. Ordentlich et al. (1990) observed that integrated use of *Trichoderma harzianum*
with captan was more effective in controlling *Verticillium* wilt in potato compared to use of either of them alone.

Sulphur has been shown to be very important in agriculture. It plays important role in adjusting the soil pH, thus, affecting the availability of most of the macronutrients. Furthermore, sulphur itself is a macronutrient for the crops. Enhanced growth of *Paenibacillus* sp. D1 and *Streptomyces* sp. A6 in sulfur supplemented medium may be due to its requirement for amino acid and protein synthesis thus, serving as nutrient for bacterial growth.

Tolerance of both the cultures towards fosteylaluminium and carbendazim was expected as the modes of action of these fungicides do not interfere with the bacterial growth. Fosteylaluminium acts by multisite action and carbendazim inhibits mitosis in fungal cells. Moreover, fosteylaluminium can serve as a phosphorous source which increases the growth of organism. Enhanced growth of *Trichoderma harzianum* and *Tolypothrix scytonemoides* in presence of low concentrations of carbendazim fungicide has been reported by El-Katatny et al. (2004) and Rajendran et al. (2007) however, no reports are available on growth enhancing effect of fosteylaluminium.

DMI fungicides act by inhibiting demethylase enzyme involved in sterol biosynthesis. Sterols are essential component of fungal cell membranes and inhibition of their biosynthesis inhibits fungal growth. Since sterols are not synthesized in bacterial cells no inhibition of *Paenibacillus* sp. D1 and *Streptomyces* sp. A6 was expected.

On many occasions insecticides are used to control insect pests in fields and many of these have tendency to accumulate in the soil. For a biocontrol agent to be effective infields, it is necessary that it must be tolerant to accumulated insecticides therein. Hence, tolerance of both the cultures towards number of commonly used insecticides was checked. Both the cultures exhibited considerable tolerance towards organophosphate and carbamate group of insecticides at concentrations recommended for field application. Organophosphate, carbamate and pyrethroid compounds constitute the largest class of pesticides currently used in industrialized and developing countries. Organophosphate and carbamate group of insecticides display neurotoxicity in mammals and insects by inhibiting acetylcholine esterase (Sogorb and Vilanova, 2002). There are few reports on tolerance of bacteria towards
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organophosphate and carbamate insecticides. Digrak and Kazanici (2001) have reported enhanced total viable count of soil bacteria on application of organophosphorous insecticides isofenphos, fonofos and phorate.

Effect of pesticides on activity and stability of major mycolytic enzymes i.e. chitinase and protease from Paenibacillus sp. D1 and Streptomyces sp. A6 was also investigated. Enzymes were more tolerant than the cultures themselves. Moreover high stability of enzymes in presence of pesticides at average temperatures (40°C) in fields during summer in Uttar Pradesh, India, suggested their applicability for agricultural purposes. El-Katatny et al. (2004) reported that carbendazim fungicide benomyl had no significant inhibition on chitinase activity of Trichoderma harzianum in range of 0-1500 pg/ml. Bhushan and Hoondal (1999) have reported compatibility of chitinase from Bacillus sp. BG-11 in most of the commonly used fungicides and insecticides. However, there are no reports on stability of chitinase in presence of pesticides at temperatures prevailing in fields.

The effect of cultures, their culture filtrates and fungicides on fungal spore germination, fungal biomass reduction and Cajanus cajan seed protection against Fusarium udum was investigated. High EC50 dose of Paenibacillus sp. D1 required for inhibiting fungal spore germination compared to that required for fungal biomass reduction may be due to faster growth and higher chitinase production by the bacterium under shaking conditions (for biomass reduction studies). On the contrary, low EC50 dose of Streptomyces sp. A6 was required for fungal spore germination inhibition compared to that required for fungal biomass reduction. This can be attributed to higher production and stability of an antifungal compound by the bacterium under static conditions (for spore germination inhibition studies). In case of enzymes (chitinase and protease) and test fungicides, low EC50 doses was required for inhibiting fungal spore germination compared to EC50 doses required for fungal biomass reduction suggesting better interaction of mycolytic enzymes/fungicides with fungus under static incubation than under shaking conditions. The interaction of cultures and their major mycolytic enzymes with test fungicides was found to be highly synergistic with respect to inhibition of fungal spore germination, fungal biomass reduction, fusarial infection of seeds and wilt incidence of Cajanus cajan. Paenibacillus and Streptomyces species are known to degrade components of fungal cellwall by producing mycolytic enzymes such as chitinases, proteases and glucanases. Digestion of fungal cell wall by such enzymes may enhance the uptake of chemical fungicides and serve as the basis of synergism. Paenibacillus sp. D1 was found to
be high chitinase producer. *Streptomyces* species are also known to produce several other antimicrobials. *Streptomyces* sp. A6 produced a strong antifungal metabolite, inhibiting a range of fungal pathogens. Culture supernatant containing antifungal principles of *Streptomyces* sp. A6 exhibited higher synergism with fungicides under static conditions. The level of synergism may also be affected by the mode of action of fungicides. Mancozeb has broad spectrum activity and acts by contact while, sulphur once taken up by the fungus, disrupts the transfer of electrons reducing sulphur to hydrogen sulfide (H2S), which is toxic to most cellular proteins (McCallen, 1949). Carbendazim acts by interfering with tubulin function, which is crucial for fungal growth (McMahan et al., 2001).

*The control of fusarium* infection by combined EC50 dose of cultures with fungicides, observed during laboratory studies and field trials, was much higher than both these antifungal agents used alone. These results suggested that the dosage of fungicides can be reduced by more than 50% without compromising the efficiency of disease control. Several other reports have also indicated the synergistic phenomenon involved in control of pathogens using the integrated application of fungicides and biocontrol agents to be more efficient and long lasting than that achieved through biocontrol agents or fungicides alone. Lorito et al. (1994) had reported enhanced inhibition of spore germination in *Botrytiscinerea*, due to synergism between fungal cell wall degrading enzymes and fungicides. Kiewnick et al. (2001) have reported better control of rhizoctonia crown and root rot of sugar beet with integrated use of fungicides and antagonistic bacteria. Efficient control of cabbage yellow caused by *Fusariumoxysporum* has been achieved by combined application of *Pseudomonas fluorescens* and low dosage of benomyl (Someya et al., 2007). Chien-Jui and Chen (2008) reported synergistic interaction between chitinase ChiCW and fungicides against plant fungal pathogens.

Thus, the present investigation revealed that *Paenibacillus* sp. D1 and *Streptomyces* sp. A6 were tolerant to most of the commonly and frequently used pesticides, especially those belonging M and DMI group of fungicides and organophosphate group of insecticides, at concentrations much higher than recommended for field application. Moreover, chitinase from the *Paenibacillus* sp. D1 and protease from *Streptomyces* sp. A6 was even more tolerant than the organisms themselves against most of the pesticides at temperature prevailing under field conditions. This suggested the potential of both the cultures for use in integrated pest management. Furthermore, synergistic interaction of cultures and their antifungal principles with test fungicides would be advantageous for developing new fungicide formulations and application strategies that can reduce the dosage of toxic agrichemical in agriculture.