Management of major sucking pests in cowpea *Vigna unguiculata* (L.) Walp. with entomopathogens and plant defense inducing rhizobacteria

KAVITHA, S. J.

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DEPARTMENT OF AGRICULURAL ENTOMOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM- 695 522

DECLARATION

I hereby declare that this thesis entitled **"Management of major sucking pests in cowpea *Vigna unguiculata* (L.) Walp. with entomopathogens and plant defense inducing rhizobacteria"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani,
28/01/2010 .

KAVITHA, S. J.
(2007-11-114)

CERTIFICATE

Certified that this thesis entitled **"Management of *Aphis craccivora* Koch and *Riptortus pedestris* Fab. infesting cowpea *Vigna unguiculata* (L.) Walp. with entomopathogens and rhizobacteria** is a record of research work done independently by Ms. **KAVITHA, S. J.** (2007-11-114) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Vellayani,
28/01/2010.

Dr. M. H. Faizal
(Chairman, Advisory Committee)
Associate Professor,
Department of Agricultural Entomology,
College of Agriculture, Vellayani,
Thiruvananthapuram - 695522.

APPROVED BY

Chairman:

Dr. M. H. FAIZAL
Associate Professor,
Department of Agricultural Entomology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

Members:

Dr. M. S. SHEELA
Professor and Head,
Department of Agricultural Entomology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

Dr. K. SUDHARMA
Professor
Department of Agricultural Entomology,

College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

Dr. K. N. ANITH
Associate Professor
Department of Agricultural Microbiology
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

Dedicated to

My Parents…

Mr. D.H.Srirama

and

Mrs. Jaya Srirama

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Introduction

1. INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp, is an ancient Neolithic African crop grown throughout the tropics and subtropics as vegetable, pulse, fodder and cover crop. In India cowpea is mainly cultivated in the states of Karnataka, Tamil Nadu, Andra Pradesh and Kerala. It is a nutritionally rich and highly priced vegetable and pulse in the domestic markets of Kerala.

The crop is damaged intensively by a large number of insect pests at various stages of its growth. Though the crop harbours an array of pests, sucking pests, predominantly aphids and pod bugs often inflict severe damage to the economically viable parts. Cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae), one of the most common aphid species in the tropics, is a cosmopolitan, polyphagous pest with marked preference for leguminouse plants and is a serious pest of cowpea. The nymphs and adults of this pest suck sap from the under surface of tender leaves, growing tips, flower stalks and pods causing distortion of attacked portion and reduction in plant growth. It is the major pest of cowpea in Asia resulting in 20 to 40 per cent yield loss (Singh and Allen, 1980). In addition, it also acts as a vector of many plant viruses such as rosette, mottles, stunt and stripe (Porter et al., 1984). *Riptortus pedestris* (Fabricius) (Heteroptera: Coreidae), the most destructive pod bug of leguminous crops, desap tender shoots and pods of cowpea. The attacked seeds shrink and shrivel up within the pods and become discoloured. Damage to pods and seeds by this pest ranges from 60 to 70 per cent (Krishna et al., 2005).

Management of *A. craccivora* and *R. pedestris*, the two most destructive sucking pests that severely curtail yield is of paramount importance for successful production of cowpea. Farmers often resort to application of chemical pesticides

as a single track measure to contain them. This strategy, though provides initial relief, is not only counter productive on a long run but also leaves toxic residue in the produce posing health hazards to consumers, warranting development of viable, sustainable and environmentally benign alternatives.

Microbial control employing application of entomopathogens particularly fungi have been attempted and found successful against several sucking pests (Rabindra and Ramanujam, 2009). Broad spectrum fungal pathogens *viz., Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorok were reported to be effective against a number of sucking pests (Garcia et al., 1990) including *A. craccivora* (Ekesi et al., 2000; Nirmala et al., 2007) and *R. linearis* (Hu et al., 1996).

Plant growth promoting rhizobacteria (PGPR), a subset of root colonizing microflora have been recognized to bring about beneficial effect on plant development by exerting physiological and biochemical changes (Kloepper et al., 1980) and are utilized in crop production for better yield. PGPR, in addition to growth promotion, fortify the mechanical and physical strength of cell wall as well as change the physiological and biochemical reaction of host plant leading to the synthesis of defence chemicals against challengers inducing resistance against pathogens and insect pests (Ramamoorthy et al., 2001). Many PGPR strains belonging to *Bacillus*, *Pseudomonas* and *Serratia* effectively colonize roots of various crop plants and offer protection from a variety of crop pests (Zehnder et al., 1997).

The present study is an attempt to biologically manage major sucking pests in cowpea by utilizing potential entomopathogens producing epizootics and PGPR capable of triggering plant mediated defence responses against them with the following objectives.

1. To identify the potential PGPR in cowpea and to evaluate their efficacy in enhancing plant resistance against the major sucking pests.

2. To test bioefficacy of entomopathogens against sucking pests.
3. To study the interaction and compatibility of PGPR and entomopathogens.
4. To develop and evaluate dual application of selected PGPR and entomopathogens to contain the sucking pests.

Review of Literature

2. REVIEW OF LITERATURE

Cowpea, *Vigna unguiculata* (L.) Walp. is cultivated as grain, vegetable and fodder crop in the semi-arid tropics covering Asia, Africa, Southern Europe, Southern United States and Central and South America. It is a major cheap source of protein in human diet with the grains containing about 23 to 25 per cent protein (Bressani, 1985). The whole seeds have been reported to contain phenolic acids, such as p-hydroxybenzoic acid, protocatechuic acid, 2,4-dimethoxybenzoic acid and cinnamic acid derivatives, such as p-coumaric acid, caffeic acid, cinnamic acid and ferulic acid which serve as antioxidants (Cai et al., 2003).

The estimated world wide area under cowpea is over 14 million ha, with over 4.5 million tonne annual production. India is the largest cowpea producer in Asia (FAO, 1999). In spite of its importance in food and farming, it is rarely grown as an entire crop in the country. The production potential of cowpea is limited by numerous factors. Large numbers of insect pests, covering the main phytophagous taxa between them attack all parts of the plant at all stages, from seedling to harvest and beyond. In this sucking pests play great role in reducing yield (Singh and Vanemden, 1979).

2.1 SUCKING PESTS IN COWPEA

Sucking pests *viz*., the black cowpea aphid, *Aphis craccivora* Koch; green leafhopper, *Empoasca kraemeri* Ross and Moore; coreids, *Clavigralla* spp. and *Riptortus* spp.; Pentatomids, *Nezara viridula* (L.) and Mirids, *Lygus hesperus* Knight, are recorded in cowpea (Jackai and Daoust, 1986). Gurjar et al. (2007a) studied population dynamics of pests of cowpea at weekly intervals and recorded the incidence of sucking pests, *A. craccivora*, *Empoasca kerri* Pruthi and *Bemisia*

tabaci Gennnadius, the populations of which peaked three, eight and seven weeks after sowing respectively.

2.1.1 Cowpea Aphid*, Aphis craccivora*

The Cowpea aphid, *A. craccivora* (Homoptera: Aphididae) is a sporadic pest, serious throughout the crop season. The colonies and scattered aphids feed on leaves, flower buds, pods and branches of cowpea (Srivastava and Singh, 1976). Serious damage occurs at high populations. Infestation greatly reduces pod formation and the entire plant may even be destroyed (Kabir, 1978).

Direct damage by *A. craccivora* to host plant is due to depletion of assimilates by the removal of sap coupled with increased respiration rate. Large numbers can cause damage resulting in distorted leaves and stunted plants with small, poorly nodulated root system. An indirect damage is the transmission and spread of viruses which severely reduces the yield (Singh and Vanemden, 1979). Aphid is reported as major pest in Asia causing an estimated loss of 20 to 40 per cent in yield and as a minor pest in Africa causing a loss up to 35 per cent (Singh and Allen, 1980). Gurjar et al. (2007b) reported *A. craccivora* as a major pest attacking cowpea in Gujarat with a total life period varying from 14 to 24 days.

2.1.2 Pod Bugs

Pod sucking bugs are the most serious pests during the post flowering phase of cowpea causing considerable economic loss by affecting both quantity and quality of the produce.

Lefroy (1909) reported *Clavigralla gibbosa* Spinola and *Clavigralla horrens* Dohrn as pests occurring in the reproductive phase of cowpea. Pigeon pea, Lab lab, Cowpea and Cluster bean were reported as the most preferred host plants of *C. gibbosa* (Singh et al., 1988). This pest has been recorded from Delhi,

Karnataka, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu and Uttar Pradesh damaging cowpea pods (Srivastava, 1996). *Clavigralla tomentosicollis* Stal. was recorded as a severe pest of cowpea in tropical Africa causing premature drying and shriveling of developing pods and production of half filled pods resulting in yield loss exceeding 80 per cent, if left uncontrolled (Ekesi, 1999).

Faleiro et al. (1986) observed regular incidence of the coried bug *Cletus* sp. in cowpea. Population build up of *N. viridula* was reported in cowpea field of Uttar Pradesh exhibiting positive correlation with maximum, minimum temperatures and relative humidity (Singh et al., 2002).

2.1.2.1 Riptortus spp.

Several species in the genus *Riptortus viz*., *Riptortus clavatus* Thnb (Sawada, 1988) *Riptortus pedestris* (Fab.) (Chand, 1995) *Riptortus dentipes* (Fab.) (Koona et al., 2001) and *Riptortus linearis* (Fab.) (Krishna et al., 2005) were reported as serious pests of cowpea in Asia and Africa.

Visalakshi et al. (1976) observed severe incidence of *R. pedestris* in cowpea fields of Kerala. Tender pods failed to develop fully and older pods were rendered unfit for consumption due to presence of feeding punctures. Prayogo and Suharsona (2005) reported *R. linearis* as the most destructive pod sucking bugs causing yield loss up to 79 per cent. Studies were conducted during kharif 2003 and 2004 to monitor the incidence of the pod sucking bug *R. pedestris* on pulse crops, particularly cowpea and field bean, in the southern zone of Andhra Pradesh and it was found that damage to pods and seeds ranged from 60 to 70 per cent (Krishna et al., 2005). Bharathimeena et al. (2008) studied the seasonal occurrence of different species of pod bugs and their natural enemies in vegetable cowpea in Kerala and noticed high populations of *N. viridula, R. pedestris* and *R. linearis* positively correlated with minimum temperature.

2.2 ENTOMOPATHOGENIC FUNGI AS BIOCONTROL AGENT

Naturally occurring entomopathogens are important regulatory factors in insect populations and many species are employed as biocontrol agents of insect pests primarily from the perspective of safety to non target organisms. Under natural conditions, fungi are frequent natural mortality factor in insect populations. Unlike other potential biocontrol agents, fungi do not have to be ingested to infect their host but invade directly through the cuticle and hence can be used for the control of all insects including the sucking pests (Ignoffo, 1978). Biological control with entomopathogenic fungi offers a sound management strategy for reducing yield losses caused by insect pests on cowpea (Ekesi et al., 2002).

Approximately 700 species of fungi in 90 genera are known to be entomopathogenic (Charnley, 1989). Entomopathogenic fungi are reported from most of the insect taxa like Lepidoptera, Isoptera, Coleoptera, Hemiptera, Diptera and Orthoptera. These fungi have a wide host range including many important pests of cowpea. Widely studied entomopathogenic fungi belong to genera *Beauveria, Metarhizium, Verticillium, Hirsutella, Erymia (Zoopthora), Nomuraea, Aspergillus, Aschersonia, Paecilomyces, Tolypocladium, Leptolegnia, Culicinomyces, Coelomomyces,* and *Lagenidium* (Moore and Prior, 1993).

2.2.1 Fungal Pathogens of Sucking Pests

Fungal diseases are regular feature among natural populations of sucking pests. Epizootics are noticed at times, though usually low incidences prevail. The possibility of controlling sucking pests by microorganisms is probably restricted to fungi since they are less amenable to control by others such viruses and bacteria.

Species of hyphomycetes demonstrate activity against a broad range of insect pests and are the main contenders for use against homopterous pest insects.

Several species *viz*., *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff.) Sorokin, *Verticilium lecanii* (Zimmermann) Viegas, *Paecilomyces fumosoroseus* (Wize) Brown and Smith, *Metarhizium flvoviridae* Gams and Rozsypal, *Nomuraea rileyi* (Farlow) Samson and *Aschersonia aleyrodis* Webber are currently in use or development (Lacey et al., 2001).

2.2.1.1 Aphids

Control of aphids heavily relies on chemical insecticides though the use of alternatives is emphasized in IPM. Entomopathogenic fungi offer environmentally benign alternative to chemical insecticides and are considered as best candidate for biological control of aphids (Latje and Papierok, 1988). Entomopathogenic fungi like *Pandora neoaphidis* (Remaudiere and Heenebert), *Zoophthora radicans* (Brefeld) Batko, *Z. occidentalis* Batko, *B. bassiana,* and *V. lecanii* were found to be infective to seven species of cereal aphids (Feng et al., 1990). In a survey on entomopathogenic fungi of aphids in South Africa, Hatting et al. (1999), recorded eight species of fungi including *B. bassiana* which infected and killed the aphids on cereals. Feng and Johnson (1990) demonstrated the pathogenicity of various isolates of *B. bassiana* against the Russian aphid, *Diuraphis noxia* (Mordvilko) and obtained 95 per cent mortality with the most pathogenic isolates at eight days post inoculation.

Kish et al. (1994) noticed infection of *V. lecanii* in *M. persicae* and *B. bassiana* in aphids on potato. Application of *B. bassiana* twice at 10^8 spores per ml resulted in 72 to 86 per cent mortality of *M. persicae* on canola plant at six days after the treatment (Miranpuri and Khachatourians., 1998).

Mathew et al. (1998) exposed cardamom aphid *Pentalonia nigronervosa* f. *caladii* to *B.bassiana*, *B. brongniartii, V. chlamydosporium* and *M. anisopliae* and observed mortality of both apterous adults and nymphs ranging from 37.0 to 96.6 and 32.8 to 75.4 per cent, respectively. *B. bassiana* produced the highest mortality of both adults and nymphs. During regular surveys carried out at Karnataka,

several dead cardamom aphids *P. nigronervosa* colonies were noticed, which showed infection by pathogenic fungi, *Penicillium fellutanum, Paecilomyces pilacinus, Fusarium oxysporum, Aspergillus parasiticus* and *V. lecanii*. Pathogenicity tests revealed that, all the five fungi induced heavy mortality to both adults and nymphs of cardamom aphid (Mathew et al., 1999).

Laboratory bioassays demonstrated the virulence of several isolates of *B. bassiana, P. fumosoroseus* and *M. anisopliae* against the brown citrus aphid, *Toxoptera citricidus* (Kirkaldy) and *B. bassiana* applied as the mycoinsecticide, Mycotrol® successfully controlled the aphid populations under a humid Florida conditions (Poprawski et al., 1999a). Poprawski et al. (1999b) tested *B. bassiana* (strain GHA) based mycoinsecticide Mycontrol ES against brown citrus aphid *T. citricidus* and obtained 79.8 to 94.4 per cent morality at five days after spraying. The proportion of mycosis was 0.67 and 0.8 at 2.5×10^{13} and 5×10^{13} conidia per ml respectively. Zhang et al. (2001) found that *F. lateritium* at 7.1×10^{7} spores/ml gave 91.80 per cent mortality of citrus aphid *T. citricidus*.

Pandey and Kanaujia (2003) observed highest and lowest mortality of aphid *Lipaphis erysimi* (Kalt.) up to 98.33 to 76.66 per cent respectively when treated with *B. bassiana* under laboratory condition.

The field evaluation made by Nagarathna (2004) using *Fusarium* sp. on sugarcane wooly aphid, *Ceratovacuna lanigera* (Zehntner) proved to be highly effective causing 90 per cent mortality within 12 days after spray. Mikunthan (2004) reported pathogenicity of *F. semitectum* on nymphs and adults of sugarcane wooly aphids. Aswini (2006) assayed *F. semitectum* on *C. lanigera* under laboratory conditions. LC_{50} for adult sugarcane wooly aphid was 1.50×10^{8} spores per ml and highest mortality (59.32 per cent) was at 2.7×10^{9} spores per ml. Efficacy of fungal pathogen, *F. semitectum* against *C. lanigera* under laboratory and greenhouse conditions was evaluated by Aswini et al. (2007). The results showed that earlier instars of woolly aphid were more susceptible to the fungus and recorded mortality of 35.57 per cent. Four isolates in each of *B. bassiana,*

M. anisopliae and *V. lecanii* were tested for their pathogenicity and mycosis was observed with six isolates *viz*., *B. bassiana*, Bb4 (10 per cent), Bb5a (19.8 per cent), Bb6 (8.3 per cent) and *M. anisopliae*, Ma2 (4.7 per cent), Ma3 (16.2 per cent) and Ma4 (42.3 per cent) (Nirmala et al., 2007).

2.2.1.1.1 Cowpea aphid, A. craccivora

Hareendranath et al. (1987) noticed natural infection of *A. craccivora* by the fungus *Fusarium pallidoroseum* (Cooke) Sacc. He observed cent per cent mortality of nymphs and adults when sprayed with a spore suspension prepared from pure culture of the fungus. Mass production of *F. pallidoroseum* was attempted and found successful in several media like rice bran + tapioca bits (Mathai et al., 1988), broken maize grains (Hareendranath, 1989) wheat bran and rice bran (Faizal and Mathai, 1996).

Faizal et al. (1996) evaluated dust and wettable powder formulations of *F. pallidoroseum* employing talc and diatomaceous earth as inert material and found that to be as effective as insecticide quinalphos 0.05 per cent in controlling *A. craccivora*.

Several fungicides inhibited growth and sporulation of *F. pallidoroseum in vitro*, where as insecticides monocrotophos and mercaptothion allowed fairly good sporulation and growth (Faizal and Mathai, 1997).

Sunitha et al. (1999) evaluated the efficacy of different formulations and concentrations of *F. pallidoroseum* against cowpea aphid and identified spore suspension and wettable powder formulation at 7×10^6 conidia / ml to be superior.

Ekesi et al. (2000) reported that an isolate of *B. bassiana* CPD 11 and two isolates of *M. anisopliae* CPD 4 and 5 caused high mortality of *A. craccivora* ranging between 58 to 91 per cent, 64 to 93 per cent and 66 to 100 per cent, respectively at 7 days post treatment, the LC_{50} of which were 6.8×10^5, 3.1×10^5 and 2.7×10^5 conidia /ml respectively.

Suresh (2005) reported the bio efficacy of entomopathogens, *B. bassiana, F. solani, V. lecanii and P. fumosoreous* against *A. craccivora* nymphs under laboratory conditions. *V. lecanii* @ 1×10^8 conidia/ml gave the highest mortality of 57.73 per cent, followed by *F. solani* with 54.72 per cent.

Nirmala et al. (2006) in Bangalore studied the pathogenicity of twelve fungal isolates belonging to *B. bassiana, M. anisopliae* and *V. lecanii* against *A. craccivora, Aphis gossypii* (Glov.) and *R. maidis* using the detached leaf bioassay technique. All the twelve isolates of the fungi were found to be pathogenic to *A. craccivora* and *A. gossypii* at a concentration of 1×10^7 spores per ml. All isolates except Bb3 and Bb4 of *B. bassiana* were pathogenic to *R. maidis*. The mortality ranged from 2 to 74 per cent in *A. craccivora*, 14 to 80.8 per cent in *A. gossypii* and 6 to 50 in *R. maidis*. Bb5a isolate of *B. bassiana* caused highest per cent mortality in *A. gossypii* (80.8 per cent) and *R. maidis* (50 per cent) indicating its broad spectrum action.

Tamo et al. (2002) while discussing the role of biological contol in IPM programme for cowpea in Africa emphasized the importance of entomogenous fungi in management of *A. craccivora* and *C. tomentosicollis* the two predominant sucking pests infesting the crop.

2.2.1.2 Pod sucking bugs

Pod sucking bugs being seed feeders are major pests affecting yield and quality of seeds of leguminous crops. Currently they are almost exclusively managed by application of broad spectrum insecticides. However, alternative strategies including the development of fungi as microbial insecticides are gaining importance.

Leite et al. (1987) studied the pathogenicity of fungi *B. bassiana* and *Paecilomyces* sp. to fifth instar nymph of the pentatomid pest *N. viridula*. When sprayed with a suspension containing 10^7 conidia/ml, *B. bassiana* showed greater

pathogenicity, giving 66.7 per cent mortality after 14 days as compared with 22.5 per cent for *Paecilomyces* sp.

Moscardi and Correa-Ferreia (1988) observed that population of stink bugs are naturally infected by enomopathogenic fungi, especially *B. bassiana* and *M. anisopliae*, but usually at very low incidences.

Hu et al. (1996) investigated the pathogenicity of *B. bassiana* to *R. linearis* and found it pathogenic to third, fourth and fifth instar nymphs and adults though the adult and third instar nymphs were less susceptible. They observed highly significant linear relationship between the dose of *B. bassiana* and the mortality of *R. linearis* within the range of 4.5×10^3 to 4.5×10^5 conidia/ml.

The ability of *M. anisopliae* and *B. bassiana* isolates to infect *N. viridula, Piezodorus guildinii* and *Euschistus heros* was investigated in Brazil. In laboratory bioassays, the mean time to mortality by *M. anisopliae* applied at 10 per cent w/w was 4.3, 4.6 and 7.4 days for *P. guildinii*, *N. viridula* and *E. heros*, respectively with per cent mortality of 41, 48 and 33, respectively, where as in the field, time to mortality increased to 23.8, 17.8 and 25.6 days, respectively (Sosagomez and Moscardi, 1998).

The virulence of eight isolates of entomopathogenic hyphomycetes against adult and fifth instar nymph of cowpea pod bug *Clavigralla tomentosicollis* was evaluated in the laboratory at four different concentrations of inoculum. At all concentrations, *B. bassiana* CPD 9 and *M. anisopliae* CPD 5 caused the highest mortality in adult bug ranging from 58 to 97 per cent and 53 to 100 per cent, respectively at seven days post inoculation. A significant reduction in feeding in both developmental stages treated with fungi was observed two days after treatment with the greatest reduction occurring in insects treated with *B. bassiana* CPD 9 and *M. anisoplia* CPD 5. Percentage pod and seed damage were significantly lower in fungal treated plants than in the control and grain yield was significantly higher in fungal treated ones than in the control (Ekesi, 1999).

Mathai (1999) reported that *Rhizopus oryzae* at 5×10^6 spores/ml was pathogenic to *Coptosoma cribraria*, *N. viridula*, *R. pedestris* and *A. craccivora*.

The ovicidal activity of eight isolates of entomopathogenic hyphomycetes was evaluated in the laboratory against *Maruca vitrata* and *C. tomentosicollis.* At a concentration of 1×10^8 conidia ml^{-1}, three isolates *B. bassiana* CPD 9 and *M. anisopliae* CPD 5 and 12 were highly pathogenic to eggs of *C. tomentosicollis*, resulting in 91 to 94 per cent mortality. These isolates also caused high larval and nymphal mortality ranging from 91 to 100 per cent. Grain yield per plant was significantly higher in fungal treated plants, which exhibited reduced pod and seed damage (Ekesi et al., 2002). Prayogo and Suharsono (2005) observed that control of pod sucking bug *R. linearis* using entomopathogenic fungus, *V. lecanii*, as the most promising biocontrol tactic due to environmental safety.

2.3 PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

Of late, the rhizosphere occupying microflora particularly rhizobacteria are recognized to induce resistance against herbivores in addition to their long identified role of plant growth promotion and thus emerging as an important component of bio-intensive management of crop pests, along with entomopathogens.

The rhizosphere of plants is a zone of intense microbial activity and some bacteria from this zone, termed rhizobacteria, exhibit active root colonization in the presence of the existing native microflora. Rhizobacteria that exert beneficial effects on plant development are referred to as plant growth promoting rhizobacteria (PGPR) (Kloepper et al., 1980), because their application is often associated with increased rates of plant growth. They promote plant growth and yield either directly or indirectly (Kloepper et al., 1989; Glick, 1995). The direct mechanisms of plant growth promotion may involve the synthesis of substances by the bacterium or facilitation of the uptake of nutrients from the environment (Glick et al., 1999). The indirect promotion of plant growth occurs when PGPR

lessen or prevent the deleterious effects of plant pathogens or insect pests on plants by production of inhibitory substances or by increasing the natural resistance of the host (Cartieaux et al., 2003).

The direct growth promoting mechanisms are nitrogen fixation, solubilization of phosphorus, sequestering of iron by production of siderophores, production of phytohormones such as auxins, cytokinins, gibberellins and lowering of ethylene concentration (Kloepper et al., 1989). The indirect mechanisms of plant growth promotion by PGPR include antibiotic production, depletion of iron from the rhizosphere, synthesis of antifungal metabolites, production of fungal cell wall lysing enzymes, competition for sites on roots and induced systemic resistance (Dunne et al., 1993; Liu et al., 1995; Glick et al., 1999).

Most strains of the PGPR like *Serratia*, *Bacillus* and *Pseudomonas* can effectively colonize plant roots and protect plants from variety of crop pests (Tomczyk, 2006; Hanafi et al., 2007; Siddiqui et al., 2007).

2.3.1 Root Colonization

The term “root colonization” denotes an active process whereby bacteria survive inoculation into seeds or soil, multiply in the spermosphere in response to seed exudates rich in carbohydrates and amino acids (Kloepper et al., 1980). Electron microscopic studies as well as the use of marked strains revealed a non-uniform distribution of bacteria on the root. While some areas, such as the root tip, are almost free from bacteria, other areas may be heavily colonized. Areas intensely colonized by bacteria are usually junctions between epidermal root cells or sites of side roots (Chin-A-Woeng et al., 1998). Root colonization, which is a complex process, is under the influence of various parameters such as bacterial traits, root exudates, biotic and abiotic factors (Benizri et al., 2001).

Seed coating, dipping and scanning electron microscopy (SEM) were employed to study bacterial (*Bacillus* spp.) colonization of the seeds and

rhizoplane of maize during the early stages of growth. The bacterial colonization of the spermosphere was 90 per cent. When the coated seeds were fully germinated, bacteria moved to the emerging radicle. Virtually no bacteria occurred on the root tip both for the treated and untreated. However, colonization was 20 per cent in the basal portion of the roots close to the seed-root junction. SEM observations showed that the bacterial cells were arranged linearly and laterally on the growing root axis. The results indicate that attachment to the seed coat and the rhizoplane by the PGPR is an important factor in the successful colonization of the rhizoplane (Ugoji et al., 2005).

2.3.2 Plant Growth Promotion

Over the last 25 years, there has been an increasing number of reports on promotion of plant growth following treatment of seeds, roots, cutting or soil with rhizobacteria (Whipps,1997), particularly species of *Pseudomonas* and *Bacillus*. Growth promotion has been expressed in various ways, but most commonly as an increase in germination, emergence, shoot length, fresh or dry mass of roots or shoots, root length, flowering and yield.

Plant growth promoting rhizobacteria (*Pseudomonas putida, P. putida biovar B, P. fluorescens*, *Arthrobacter citreus*, and *Serratia liquefaciens*) treatment in canola maximize the yield up to 57 per cent more than the controls and also increased seedling emergence and vigor under field conditions (Kloepper et al., 1988). Some *Serratia* strains such as *S. proteamaculans* 1-102 and *S.liquefaciens* 2-68 had beneficial effects on growth of legume crops (Chanway et al., 1989; Zhang et al., 1996). Co-inoculation of some *Pseudomonas* and *Bacillus* strains along with effective *Rhizobium* spp., stimulates chickpea growth, nodulation and nitrogen fixation (Parmar and Dadarwal, 1999). *Pseudomonas* inoculants significantly increased root dry weight in spring wheat (Walley and Germida, 1997), and yield in sugar beet (Çakmakçi et al., 2001).

Enhancement in the productivity of *Bacillus* treated Geranium by 88 per cent over untreated control was reported by Abdul et al. (2003). *Bacillus* isolates were found to promote growth in chickpea, brinjal, okra and chilli among which the isolate SE34 enhanced maximum germination and seedling vigor (Amruthesh et al., 2003). Dey et al. (2004) found that application of nine isolates of *Pseudomonas* spp. resulted in significantly enhanced pod yield (18–28 per cent) of peanut (*Arachis hypogaea* L.). Other attributes like root length, pod number, 100-kernel mass, shelling out-turn and nodule number were also enhanced.

The PGPR strains Sp7 (*Azospirillum brasilense*) and UPMB10 (*Bacillus sphaericus*) significantly increased the bunch yield and fruit physical attributes, i.e. finger weight, length and diameter, and pulp/peel ratio, besides inducing early flowering by three weeks in Banana (Mia et al., 2005). In the greenhouse, inoculations with PGPR increased sugar beet root weight by 2.8-46.7 per cent. Leaf, root and sugar yield were increased by the bacterial inoculation by 15.5-20.8, 12.3-16.1, and 9.8-14.7 per cent, respectively (Çakmakçi et al., 2006).

In betelvine (*Piper betel* L.) under greenhouse conditions PGPR (*S. marcescens* NBRI1213) resulted in significant growth increase in shoot length, shoot dry weight, root length, and root dry weight, averaging 81 per cent, 68 per cent, 152 per cent, and 290 per cent, respectively, greater than untreated controls (Chauhan et al., 2006).

Root inoculation with *Bacillus* M3 and *Bacillus* OSU-142 strains significantly increased cumulative yield (26.0-88.0 per cent), fruit weight (13.9-25.5 per cent), shoot length (16.4-29.6 per cent) and shoot diameter (15.9-18.4 per cent) of apple (*Malus domestica* L.) compared with the control. In addition, all nutrient element contents (N, P, K, Mg, Ca, Fe, Mn and Zn except Mg) was significantly affected by bacterial applications compared with the control (Karlidag et al., 2007).

In Sugarcane, plant growth-promoting bacteria belonging to *Pseudomonas* significantly increased the number of tillers by 31 per cent and the fresh shoot weight by 26 per cent above the uninoculated control. Inoculated plants were significantly taller and accumulated significant amounts of biomass. Shoot dry weight increased up to 61 per cent and root dry weight up to 67 per cent (Villegas and Paterno, 2008).

2.3.3 Induced Resistance in Plants

According to Agrios (1988) resistance is the ability of an organism to exclude or overcome, completely or in some degree, the effect of a pathogen or insect pest or other damaging factors. Induced resistance is a physiological state of enhanced defensive capacity elicited by specific environmental stimuli, where by the plants innate defenses are potentiated against subsequent biotic challenges. Two types of induced resistance namely, systemic acquired resistance and induced systemic resistance are recognized in plants (van Loon, 1997).

2.3.3.1 Systemic acquired resistance

"Systemic acquired resistance" (SAR) is a term first introduced by Ross (1961) to describe induction of resistance in tobacco by prior inoculation with tobacco mosaic virus. Since then, the term SAR has been commonly used in cases where induced resistance results from prior inoculation with necrotizing pathogens or application of chemical agents. Induction of SAR is characterized by an accumulation of salicylic acid (SA) and pathogenesis-related (PR) proteins (van Loon et al., 1998).

2.3.3.2 Induced systemic resistance by PGPR

The term Induced systemic resistance is used to denote induced resistance by non pathogenic root colonizing rhizobacteria. So this ISR is also called as Rhizobacteria-mediated induced systemic resistance (RMISR). RMISR does not involve the accumulation of pathogenesis related proteins and salicylic acid but

instead depend on pathways regulated by jasmonic acid and ethylene. ISR has been demonstrated in many plant species like bean, carnation, cucumber, radish, tobacco, canola, tomato, rice, and the model plant *Arabidopsis thaliana* (van Loon et al., 1998). The ability to develop ISR in response to selected strains of rhizosphere bacteria has been documented for many different plant species and appears to depend on the host-rhizobium combination. Specific recognition between the plant and the ISR inducing rhizobacterium is required for the induction of ISR (Pieterse et al., 2001).

2.3.3.2.1 Against pests

PGPR induces resistance in plants against insects (Zehnder et al., 1997), spider mites (Tomczyk, 2006) and nematodes (Siddiqui et al., 2007).

Pseudomonas maltophila affects the growth of larval stage of *Helicoverpa zea*, the corn earworm, leading to more than 60 per cent reduction in adult emergence while pupae and adults that emerged from bacteria-infected larvae were smaller (Bong and Sikorowski, 1991). Induction of systemic resistance by PGPR strains, viz., *P. putida* strain 89B-27, *S. marcescens* strain 90-166, *Flavomonas oryzihabitans* strain INR-5 and *Bacillus pumilus* strain INR-7 have significantly reduced populations of the striped cucumber beetle, *Acalyma vittatum* and the spotted cucumber beetle, *Diabrotica undecimpunctata* Howardi on cucumber. Among these strains, *S. marcescens* strain 90-166 was more effective in reducing the population of both the beetles and its efficacy was better than application of the insecticide fenvalerate (Zehnder et al., 1997). Similarly, the relative growth rate, consumption rate and digestibility of feed by *Helicoverpa armigera* have been affected when larvae fed on cotton plants treated with *P. gladioli* due to an increase in their polyphenol and terpenoid content (Qingwen et al., 1998).

Bergen (2006) reported that two PGPR strains, *P.chlororaphis* (PA23) and *Bacillus amyloliquifaciens* (BS6) induces resistance against flea beetle and diamond back moth in Canola.

Melvin and Muthukumaran (2008) observed under the pot culture condition that tomato leaves treated with combined foliar application of JA and *P. aeruginosa* caused maximum *S. litura* larval mortality followed by JA and SA as compared to the untreated check. Pupation rate was reduced to the minimum in case of leaves treated with JA and *P. aeruginosa.* Adult emergence and adult longevity was also reduced on the same treatment. JA and *P. aeruginosa* has a negative impact on growth and development of *S. litura.* The JA treatment strongly affected the activity of proteinase inhibitors, moderately the PPO activity and to a lesser amount the lipoxygenase activity but the peroxidase activity was even less as compared to control on the other hand SA treatment could induce the peroxidase activity to a higher extent.

Hanafi et al. (2007) found out that *B. tabaci* proliferate less on tomato plants that have been inoculated with *B. subtilis* (BS) than in the control. This indicates that the inoculation of plants with BS confers some type of resistance or avoidance behavior which results in less proliferation of *B. tabaci* on the BS inoculated plants. It is probable that BS has some impact on plant nutrient absorption by the roots.

Plant growth promoting fluorescent pseudomonas strains Pf1, TDK 1 and PY 15 were evaluated for their efficacy against leaf folder, *Cnaphalocrocis medinalis* in rice plants under field conditions individually and in combinations. Application of mixture of *P. fluorescens* strains significantly reduced the leaf folder damage in rice plants compared with untreated control. Natural enemy population in plots treated with *P. fluorescens* was greater than the chemical and untreated controls. This was because of higher activity of polyphenol oxidase and lipoxygenase in plants treated with *P. fluorescens* mixture. Further, fluorescent

pseudomonad mixture increased the rice yield compared with individual strain and non- bacterized treatment (Saravanakumar et al., 2008).

2.3.3.2.1.1 Sucking pests

Several *Bacillus* PGPR species applied to tomato as seed treatments were found to reduce whitefly nymph densities by 40–43 per cent (Murphy et al., 2000).

White clover and Medicago plants grown in the presence of a *Pseudomonas*-like PGPR were better able to resist effects of blue-green aphids, *Acyrthosiphon kondoi* Shinji (Kempster et al., 2002).

Stout et al. (2002) speculated that the delay in population growth and population size of cotton aphids, *Aphis gossypii* Glover, on cucumbers was due to a *Bacillus* containing PGPR treatment.

Four *Pseudomonadas* isolates were tested to study their biocontrol ability against aphid (*Aphis gossypii* Glover) and leaf hopper (*Amrasca biguttula biguttula* Ishida) pests of okra. All the four PGPR isolates reduced the incidence of pests remarkably, out of which *Pseudomonas* B 25 was found to be the most efficient biocontrol agent against both pests. The populations of aphids and leaf hoppers were reduced by about 79 and 81 per cent, respectively, when sprayed with B 25 isolate. The okra yield was improved by 53 per cent over uninoculated control (Jagadeesh et al., 2007).

Yao (2007) reported that *B. subtilis* and its metabolites induced resistance against broad bean aphid, *A. fabae* and wheat aphid, *Rhopalosiphum padi*. *Bacillus subtilis* and *Bacillus amyloliquefaciens* were evaluated for impact on germination and initial growth of bell pepper plants and for efficacy against the green peach aphid, *M. persicae*. Plants grown in the presence of *Bacillus* spp. exhibited substantial tolerance to aphids in addition to greater yield than the control treatment (Herman et al., 2008).

Swarnali and Senapati (2008) reported that among the eleven *B. subtilis* strains evaluated, nine showed the ability to promote the growth of mung bean. Two strains, including one local isolate, showed promising results by rendering resistance against *A. craccivora*, inducing biochemical changes in mung bean by enhancing the phenol and peroxidase concentrations.

2.3.3.2.1.2 Nematodes

B. subtilis has induced protection against *Meloidogyne incognita* and *M. arenaria* in cotton (Sikora, 1988). Similarly, *P. fluorescens* has induced systemic resistance and inhibited early root penetration of *Heterodera schachtii*, the cyst nematode in sugar beet (Oostendorp and Sikora, 1989, 1990). Application of the bacterium, *P. chitinolytica* reduced the root-knot nematode infection in tomato crop (Spiegel et al., 1991). The level of infestation of root-knot nematode *M. incognita* on tomato was reduced with fewer galls and egg masses in the soil following root dipping with *P. fluorescens* strain Pf1 (Santhi and Sivakumar, 1995).

Treatment of rice seed with PGPR alone or in combination with chitin and neem cake has reduced the root and soil population of the rice root nematode, *Hirschmanniella oryzae* (Swarnakumari and Lakshmanan, 1999; Swarnakumari et al., 1999). Anitha and Rajendran (2005) reported soil application of *P. fluorescens* at the time of sowing resulted in significant reduction (53.03 per cent) in *M. graminicola* Golden and Birchfield infecting rice.

Siddiqui et al. (2007) reported that *Pseudomonas putida* caused greater inhibitory effect on the hatching and penetration and higher reduction in galling and multiplication of *M. javanica* followed by *P. alcaligenes, P. polymyxa and B. pumilus*. Kavitha et al. (2007) reported *P. fluorescens and B. subtilis* significantly decreased *M. incognita* population on sugar beet and was as effective as carbofuran.

2.3.3.2.1.3 Mites

Tomczyk (2006) conducted glass house experiment to evaluate resistance inducer *Psedomonas fluorescens* against two spotted spider mite *Tetranychus urticae* Koch. He observed lower preference of spider mites for the plants treated with *P. fluorescens*. Six weeks after infestation of plants with spider mites, the density of the mite population was two fold lower on plants treated with bacteria. Fecundity of mite females also decreased on leaves of the plants treated with bacteria.

Materials and Methods

3. MATERIALS AND METHODS

The experiment on the "Management of major sucking pests in cowpea *Vigna unguiculata* (L.) Walp. with entomopathogens and plant defense inducing rhizobacteria" was carried out at the Department of Entomology, College of Agriculture, Vellayani during 2007-2009.

The details of the materials used and methods followed during the course of investigation are mentioned below.

3.1 MAINTENANCE OF CULTURES OF ENTOMOPATHOGENS AND RHIZOBACTERIA

The initial culture of the entomopathogens were obtained from Department of Entomology, College of Agriculture, Vellayani.

Table 1. List of Entomopathogens, Rhizobactria and media used for maintenance

No.	Entomopathogen	Isolated from	Media
1	*Fusarium pallidoroseum*	*Aphis craccivora*	Potato Dextrose Agar (PDA)
2	*Beauveria bassiana*	*Odoiporus longicollis* Oliver	PDA
3	*Metarhizium anisopliae*	*Odoiporus longicollis*	PDA
4	*Serratia marcescens*	*Paradasynus rotratus* Dist.	Nutrient agar (NA)
	PGPR	**Procured from**	**Media**
1	*Pseudomonas putida* strain 89B61	Auburn University, Alabama, USA	King's B (KB)
2	*Pseudomonas* sp. strain PN026R	Department of Microbiology, College of Agriculture, Vellayani	KB
3	*Bacillus subtilis* strain GB03	Auburn University, Alabama, USA	NA
4	*Bacillus pumilus* strain SE34	Auburn University, Alabama, USA	NA
5	*Serratia marcescens*	Department of Entomology, College of Agriculture, Isolated from *Paradasynus rotratus*	NA

200 ml of each culture medium was taken in separate 500 ml conical flasks, autoclaved at 121°C (15 lbs) for 20 minutes. The plates were prepared by pouring 20 ml of media per plate and each isolate was inoculated in separate plates and were incubated at 25°C.

The virulence of the entomopathogens were maintained by passing them periodically through *A. craccivora* and *R. pedestris* and reisolating them in fresh cultures. For this purpose spore suspension of the entomopathogens were prepared aseptically by pouring 10 ml of sterile distilled water into heavily sporulated one-week old culture plates. After shaking the plates the resulting spore suspension was sprayed on host insects. The mortality of host insects was noticed after two to four days. Later the dead insects showing fungal growth were collected, surface sterilized with 0.1 per cent mercuric chloride, washed in sterile water three times and placed at the center of petri dishes containing medium and incubated at room temperature. When growth was visible, it was subcultured and maintained in plates and slants for further studies.

3.2. SCREENING OF PGPR FOR GROWTH PROMOTION AND PEST TOLERENCE AT SEEDLING STAGE

3.2.1 Preparation for Planting

Sterilized sand was used as substrate to grow cowpea seedlings (Variety-Kanakamony). Sand was washed thoroughly till water flows colorless from it, dried to attain desirable moisture, packed in polypropylene bags and autoclaved for two consecutive days at 121ºC (15 lbs) for 2 hours. Sterilized sand was filled in plastic cups of diameter 6 cm and kept in glass house after sowing. Experimental plants are shown in Plate 1.

Five PGPR strains *viz., P. putida* (B_1), *Pseudomonas* sp. (B_2), *B. subtilis* (B_3), *B. pumilus* (B_4) and *S. marcescens* (B_5) were applied following seed treatment (M_1) and soil drenching (M_2). Foliar application (M_3) was tried after establishment of aphid population to asses direct effect of PGPR on the pest. The

Plate 1. Experimental plants

experiment was conducted as 6 × 3 factorial CRD replicated thrice. PGPR used in this experiment are showed in Plate 2.

3.2.2 PGPR treatment

Bacterial cell suspension was prepared by pouring 10 ml of sterilized water into two days old culture plates and scrapping the culture by using a glass spreader. Optical density of bacterial suspension was measured in spectrophotometer at 650 nm and was adjusted to 1.0 to have approximately 10^9 cells/ml, by diluting with sterile water.

3.2.2.1 Seed Treatment

Seeds were soaked in respective bacterial suspensions for 30 minutes and sown. Seeds sown after soaking in sterile water for 30 minutes, served as control.

3.2.2.2 Soil Drenching

At 12 days after sowing, plants in cups kept for soil drenching were drenched with 0.5 ml of respective rhizobacteria by using a micro pipette. Plants drenched with sterile water served as control.

3.2.2.3 Foliar Application

Foliar application of bacterial suspension was carried by using an atomizer after establishment of *A. craccivora* at 25 days after sowing. Plants sprayed with sterile water served as control.

3.2.3 Biometric observations

Number of days for germination was recorded. Number of fully opened leaves were recorded at 15 days after sowing. Plant height was recorded at 10, 15 and 25 days after sowing.

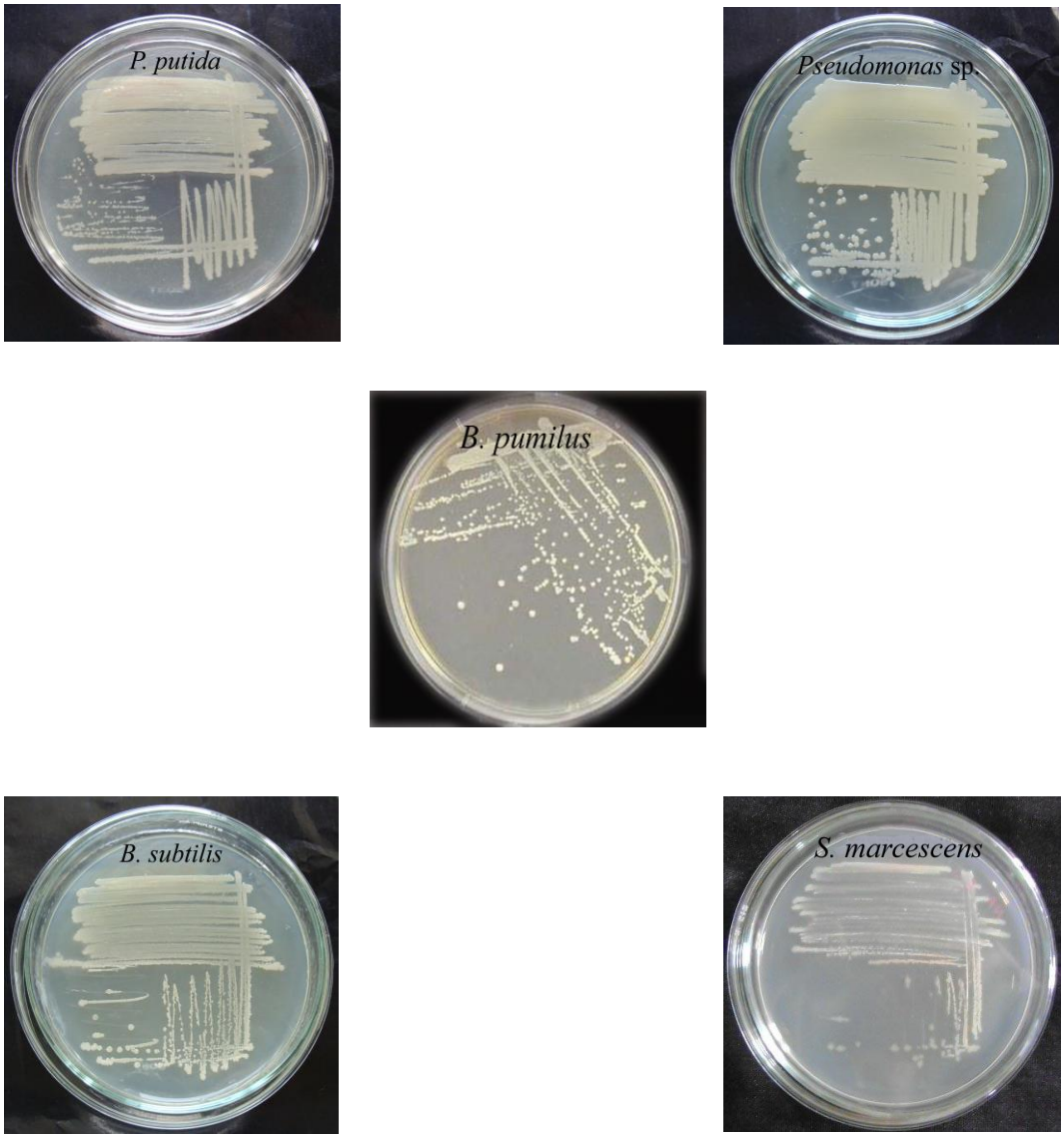


Plate 2. PGPR used in screening experiments

3.2.4 Monitoring for *A. craccivora* resistance

Since natural occurrence of aphid population was very low in experimental plants, aphids were collected from field and were released at the rate of 5 aphids per plant by using camel hair brush on 20th day after sowing. Number of aphids per plant were recorded at five, seven and ten days after release.

3.2.5 Biochemical observations

Leaf sample was taken from 30 days old plant to record content of chlorophyll and epicuticular wax.

3.2.5.1 Chlorophyll Content

500 mg of leaf sample was ground with 10 ml of 80 per cent acetone using a pestle and mortar. The homogenate was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and made upto 25 ml with 80 per cent acetone. The optical density value of the extract was measured at 663 nm and 645 nm with 80 per cent acetone as blank in a spectrophotometer. The amount of the pigment was calculated using the following formulae and expressed as milligram of pigment per gram of fresh leaf as described by Sadasivam and Manickam (1996).

Total chlorophyll content: 20.2 (OD at 645) + 8.01 (OD at 663) × V/1000×W mg/g.

Chlorophyll a: 12.7 (OD at 663) – 2.69 (OD at 645) ×V/1000×W mg/g.
Chlorophyll b: 22.9 (OD at 645) – 4.689 (OD at 663) ×V/1000×W mg/g.

where,

OD - Optical density at specific wavelength.
V - Final volume of chlorophyll extract in 80 per cent acetone.
W - Fresh weight of tissue extracted.

3.2.5.2 Epicuticular Wax

For epicuticular wax estimation 5 cm^2 leaf samples were carefully dipped into clean 50 ml pre weighed beakers containing 20 ml of chloroform and stirred so that the wax from the leaves are extracted in chloroform. This was done for 40 sec. Then the beakers were heated at 50ºC to evaporate the chloroform completely. Weights of the beakers were noted until a stable weight was obtained. Wax content was calculated by subtracting the pre weight of the beaker from the weight of the beaker with wax and expressed in mg/cm^2 leaves.

3.3 BIOEFFICACY OF ENTOMOPATHOGENS ON SUCKING PESTS

Three entomopathogenic fungi and one entomopathogenic bacteria (Plate 3) were tested for their pathogenicity to *A. craccivora* and *R. pedestris*. LC_{50} value was fixed with the help of literature available.

1. *Beauveria bassiana* - 6.8×10^5spores/ml (Ekesi et al., 2000)
2. *Metarhizium anisopliae* - $3.2x10^6$ spores/ml (Mohan, 2001)
3. *Fusarium pallidoroseum* - 7×10^6 spores/ml (Sunitha et al., 1999)
4. *Serratia marcescens* - 2.9×10^9 cells/ml.

3.3.1 Preparation of Spore Suspension of Entomopathogens

The fungi *viz*., *B. bassiana, M. anisopliae* and *F. pallidoroseum* were grown on PDA. From seven days old cultures, stock suspension of spores were prepared. The spores of fungi were harvested by flooding the plate with 10 ml sterile distilled water containing a little soap powder and scraping the surface with sterile spatula. The required spore concentration was adjusted with the help of haemocytometer.

The bacterium, *S. marcescens*, was grown on NA. Two day old culture was used. For preparing the spray suspension, 10 ml of sterile distilled water was

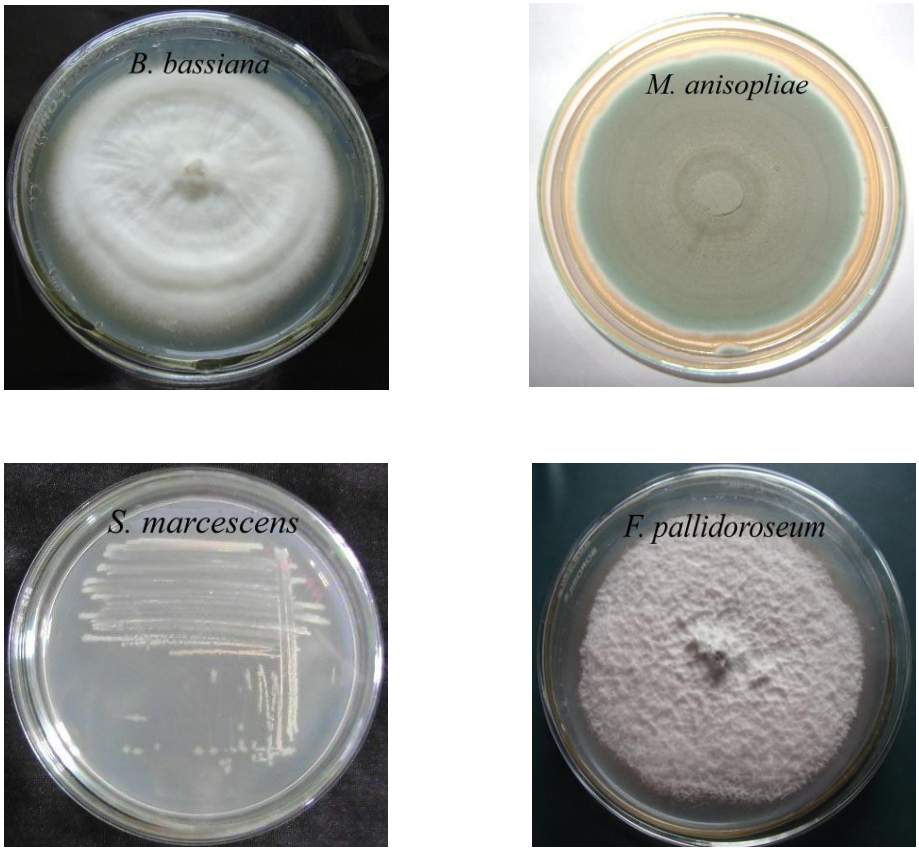


Plate 3. Entomopathogens used in bioefficacy experiments

poured into culture plate and scraped using spatula. The required cell concentration was adjusted with the help of haemocytometer.

3.3.2 Application of Spore Suspension on *A. craccivora*

Cowpea plants were raised in plastic cups of diameter 6 cm filled with soil in the glass house. Twelve replications were maintained for each treatment. Field collected *A. craccivora* was maintained on cowpea plants. Ten aphids were released to each plant, allowed for multiply for seven days. Pre-count of aphid was recorded. The spore suspension was sprayed uniformly on aphids using atomizer. Control was maintained by spraying aphids with sterile water.

3.3.3 Application of Spore Suspension on *R. pedestris*

Uniform staged bugs collected from field were used for the experiment which was conducted with five replications each with five insects. Ten ml of spore suspension was sprayed using atomizer. After 20 minutes, treated insects were transferred into fresh cowpea pods placed in plastic jars secured with muslin cloth at the top. An untreated control was maintained by spraying the bugs with sterile water.

3.3.4 Observations

The treated insects were examined daily for their mortality. Observation on mortality of aphids were recorded at two, four and seven days after spray. Observation on mortality of bugs were recorded at five, seven, nine, eleven and thirteen days after spray. Dead insects were transferred to petri plates containing moist tissue paper and observed for mycelial growth on the cadavers or symptoms of bacterial infection. Pathogenecity was further confirmed by Koch's postulates.

The per cent mortality was worked out using the following formulae.

$$\text{Per cent mortality} = \frac{\text{Initial population - Final population}}{\text{Initial population}} \times 100$$

3.4 ASSESSMENT OF COMPATIBILITY OF PGPR AND ENTOMOPATHOGENS

The compatibility of different PGPR and entomopathogens between themselves and between each other was studied by observing the *in vitro* interactions.

3.4.1 Between PGPR Strains

In vitro interaction between five rhizobacterial strains were tested by dual culture plate assay on mixed medium of NA and KB. *Pseudomonas* sp. and *P. putida* were grown on KB and *B. pumilus*, *B. subtilis* and *S. marcescens* on NA medium for obtaining single colonies. Sterile petri plates were poured with molten medium and allowed to solidify. A heavy inoculum of the individual rhizobacterial strain was applied as a band at the center of petri plate. Then all other rhizobacterial strains were streaked perpendicular to first one. This is repeated in all combinations. Five replications were maintained. The inoculated plates were incubated at 28°C. After 48 hours the zone of inhibition if any was recorded.

3.4.2 Between Entomopathogens

In vitro interaction between entomopathogens were tested by dual culture plate assay on PDA medium. Sterile petri plates were poured with molten PDA medium and allowed to solidify. Two fungi were tested at a time in single plate. Mycelial discs of five mm diameter from seven-days old culture of entomopathogens grown on PDA were cut out with a cork borer and placed on two opposite edges of the PDA medium. Plate containing single fungi served as control. Five replications were maintained. The inoculated plates were incubated at 28°C and observation on the mycelial growth of entomopathogens were taken after a period of seven days.

Scoring of zone of inhibition	Description
+++	Zone of inhibition >5 mm
++	Zone of inhibition <5 mm
+	Entomopathogen touches another entomopathogen

3.4.3 Between PGPR and Entomopathogens

In vitro antagonism of the five rhizobacterial strains against *B. bassiana, M. anisopliae* and *F. pallidoroseum* were tested by dual culture plate assay on two mixed culture media. *Pseudomonas* sp. and *P. putida* were grown on King's medium B and *B. pumilus, B. subtilis* and *S. marcescens* on nutrient agar medium for obtaining single colonies. Sterile petri plates were poured with molten mixed medium of either King's medium B + PDA or nutrient agar + PDA and allowed to solidify. A heavy inoculum of the individual rhizobacterial strain was applied as a band of 1.5 cm length equidistantly on two opposite edges of the agar medium in the petri plate using an inoculation loop. Mycelial discs of five mm diameter from seven-day old culture of entomopathogens grown on PDA were cut out with a cork borer and placed at the center of the petri plate. Eight replications were maintained. Plates containing the entomopathogen alone served as control. The inoculated plates were incubated at 28°C and observations on the mycelial growth of entomopathogens were taken after a period of five days.

Scoring of zone of inhibition	Description
+++	Zone of inhibition >5 mm
++	Zone of inhibition <5 mm
+	Entomopathogen touches PGPR
-	Entomopathogen grows over PGPR

3.5 POT CULTURE EVALUATION OF EFFECT OF SELECTED PGPR AND ENTOMOPATHOGENS

A pot culture experiment was conducted to evaluate the efficacy of combined use of selected plant growth promoting rhizobacteria and entomopathogens against major sucking pests of cowpea (*A. craccivora* and *R. pedestris*). Three PGPR strains namely *B. subtilis*, *S. marcescens* and *B. pumilus* and three entomopathogens viz, *F. pallidoroseum*, *B. bassiana* and *S. marcescens* found promising in the laboratory experiments were evaluated in the pot culture experiment. The experiment was conducted in 4×5 factorial CRD with PGPR as first factor and entomopathogen as second.

96 pots were filled with sand : soil : cow dung in the ratio 1 : 2 : 1. Each pot was sown with four cowpea seeds (variety: Kanakamony) and maintained following KAU package of practice recommendations (2007).

3.5.1 PGPR treatment

Cell suspensions (10^9cells/ml) of selected PGPR *viz*., *B. subtilis* (T_1), *S. marcescens* (T_2) and *B. pumilus* (T_3) were prepared as described in 3.2.2 and applied as seed treatment by keeping the seeds immersed in the suspension for 30 minutes prior to sowing. Three weeks after sowing, the base of each plant was drenched with 0.5 ml of respective rhizobacteria to fortify the initial seed treatment. Seeds and plants treated with sterile water served as control (T_4).

3.5.2 Application of entomopathogens

Spore suspensions of selected entomopathogens *viz*., *F. pallidoroseum* (E_1), *B. bassiana* (E_2) and *S. marcescens* (E_3) at respective field dose were prepared as described in 3.3.1 and sprayed on to the plants after establishment of *A. craccivora* (P_1) and *R. pedestris* (P_2) at 45 days after sowing and 60 days after sowing respectively. Application of quinalphos

0.03% served as chemical check and application of sterile water serve as absolute control.

3.5.3 Biometric observations

Biometric observations like, number of days for germination, plant height, number of leaves and root characters were recorded as mentioned below and was subjected to one way ANOVA with four treatments *viz*., *B. subtilis* (T_1), *S. marcescens* (T_2), *B. pumilus* (T_3) and control (T_4) with 24 replications. Pots kept for observation on efficacy of entomopathogens were considered as replications.

3.5.3.1 Days for germination

Number of days taken for emergence of seedlings was recorded.

3.5.3.2 Plant height (cm)

The length of the plant from ground level to the growing tip was measured at 1, 2, 4, 6 and 8 weeks after sowing.

3.5.3.3 Number of leaves

Number of fully opened leaves in each plant were counted at 1, 2, 3 and 4 weeks after sowing.

3.5.3.4 Root length (cm)

Two plants from each pot were uprooted at 20 days after sowing and root length was measured. After final harvest (80 days after sowing) the remaining plants were also uprooted and root length was recorded.

3.5.3.5 Number of root branches

As mentioned in 3.5.3.4 both at 20 and 80 days after sowing number of root branches were counted.

3.5.3.6 Number of root nodules

As mentioned in 3.5.3.4 both at 20 and 80 days after sowing number of root nodules were counted.

3.5.4 Population assessment of sucking pests and their natural enemies

3.5.4.1 A. craccivora

The presence or absence of aphids in PGPR treated plants was individually recorded at 15 and 20 DAS and percentage of plants in each treatment showing presence of aphids worked out.

The number of aphids per plant were counted and recorded at 25, 30, 35, 40 and 45 DAS. Population of aphids were also recorded at 2, 4, 7 and 10 days after treatment with entomopathogens. The data was subjected to square root transformation and factorial analysis was performed on post treatment population of aphids.

3.5.4.2 R. pedestris

Percentage of plants in each treatment showing presence of pod bug was worked out as in 3.5.4.1.

Since infestation was very low, bugs were collected from field and released onto experimental plants. The number of adults and nymphs of *R. pedestris* per plant was recorded two week after release (55 DAS) before application of entomopathogens.

Number of *R. pedestris* nymphs and adults were also estimated at three and seven days after treatment with entomopathogen and statistical analysis was done as in 3.5.4.1.

3.5.5 Observations on yield parameters

Yield parameters like days to first flowering, pod length, number of pods per plant, number of seeds per pod, number of seeds per plant, 100 seed

weight and dry weight of pods per plant were recorded. Data were subjected to factorials analysis.

3.5.5.1 Days to first flowering

Days taken for first flowering was recorded in each plant.

3.5.5.2 Pod length (cm)

The length of the pods was measured in each plant and their average value was recorded.

3.5.5.3 Number of pods per plant

The number of pods per plant was counted and the mean value was recorded.

3.5.5.4 Number of seeds per pod

The number of seeds in each pod was counted and their mean value was recorded.

3.5.5.5 Number of seeds per plant

The number of seeds per plant was counted and recorded.

3.5.5.6 Hundred seeds weight

Two lots of seeds, each of hundred numbers were counted and mean weight of the lot was recorded and expressed in gram.

3.5.5.7 Dry weight of pods per plant

Dry weight was taken after drying the samples to a constant weight in a drying oven at 60°C.

Results

4. RESULTS

4.1 SCREENING OF PGPR FOR GROWTH PROMOTION AND PEST RESISTANCE AT SEEDLING STAGE

Five PGPR strains *viz*., *Pseudomonas putida*, *Pseudomonas* sp., *Bacillus subtilis, Bacillus pumilus* and *Serratia marcescens,* were evaluated to know their ability to enhance plant growth and suppress sucking pests.

4.1.1 Effect of PGPR on plant growth

All the PGPR treatments significantly enhanced plant growth compared to control (Table 1 and Table 2) (Plate 3).

4.1.1.1 Number of days taken for germination

Least number of days to germination (3.03) was observed in *B. subtilis* (B_3) seed treatment, which was on par with *S. marcescens* (B_5) (3.17), *B. pumilus* (B_4) (3.23), *Pseudomonas* sp. (B_2) (3.47) and *P. putida* (B_1) (3.55).

4.1.1.2 Number of opened leaves at 15 days after sowing

Plants treated with *B. subtilis* (B_3) recorded maximum numbers of opened leaves (3.79) which was on par with *S. marcescens* (B_5) (3.78), *P. putida* (B_1) (3.52), *Pseudomonas* sp. (B_2) (3.47) and *B. pumilus* (B_4) (3.44).

Seed treatment (M_1) (3.78) was significantly superior to soil drenching (M_2) (3.19).

B. subtilis seed treated (B_3M_1) plants beard maximum number of opened leaves (4.52) which was on par with B_5M_1 (4.25) and B_4M_1 (3.98).

Table 1. Effect of PGPR seed treatments on germination and plant height of cowpea seedlings

Treatments		Days for germination	Plant height at 10 DAS (cm)
P. putida (B_1)		3.55	4.12
Pseudomonas sp. (B_2)		3.47	5.09
B. subtilis (B_3)		3.03	5.59
B. pumilus (B_4)		3.23	5.51
S. marcescens (B_5)		3.17	5.68
Control (Water) (B_6)		3.89	4.09
CD values	Treatments	0.523	0.841
	Treatment Vs Control	0.383	0.617

DAS: Days after sowing

Table 2. Growth promotion in cowpea seedlings by different PGPR treatments

PGPR	Mean number of opened leaves at15 DAS			Plant height (cm)					
				15 DAS			25 DAS		
	Seed treatment	Soil drenching	Mean	Seed treatment	Soil drenching	Mean	Seed treatment	Soil drenching	Mean
P. putida (B_1)	3.60	3.43	3.52	6.38	6.28	6.33	11.65	11.68	11.67
Pseudomonas sp. (B_2)	3.38	3.55	3.47	7.02	6.32	6.67	12.83	12.00	12.41
B. subtilis (B_3)	4.52	3.07	3.79	7.89	6.05	6.97	16.47	12.13	14.30
B. pumilus (B_4)	3.98	2.90	3.44	7.38	6.44	6.91	14.20	12.04	13.12
S. marcescens (B_5)	4.25	3.30	3.78	7.53	6.22	6.88	14.69	12.31	13.50
Control (Water)	2.93	2.90	2.92	5.46	5.38	5.42	9.73	8.52	9.13
Mean	3.78	3.19		6.94	6.12		13.26	11.45	
CD values (0.05)	PGPR : 0.496			PGPR : 0.396			PGPR : 1.217		
	Method : 0.286			Method : 0.229			Method : 0.703		
	Interaction : 0.701			Interaction : 0.560			Interaction : 1.721		

4.1.1.3 Plant height

Ten days after sowing

At ten days after sowing, *S. marcescens* (B_5) treated plants recorded maximum plant height (5.68 cm), which was on par with *B. subtilis* (B_3) (5.59 cm), *B. pumilus* (B_4) (5.51 cm) and *Pseudomonas* sp. (B_2) (5.09 cm). *P. putida* (B_1) treated plants recorded least plant height of 4.12 cm (Table 2).

15 days after sowing

The maximum mean plant height of 6.97 cm was observed in plants treated with *B. subtilis* (B_3), which was on par with *B. pumilus* (B_4) (6.91 cm), *S. marcescens* (B_5) (6.88 cm) and *Psedomonas* sp. (B2) (6.67 cm).

Seed treatment (M_1) improved plant height (6.94 cm) more than soil drenching (M_2) (6.12).

B. subtilis seed treatment (B_3M_1) recorded maximum plant height (7.89 cm) which was on par with B_5M_1 (7.53 cm), B_4M_1 (7.38 cm) and B_2M_1 (7.02 cm).

25 days after sowing

Maximum plant height was observed in plants treated with *B. subtilis* (B_3) (14.30 cm) which was on par with *S. marcescens* (B_5) (13.50 cm) and *B. pumilus* (B_4) (13.12 cm).

Seed treatment (M_1) (13.26 cm) was significantly superior to soil drenching (M_2) (11.45 cm) irrespective of bacterial treatments.

B. subtilis seed treatment (B_3M_1) showed maximum plant height (16.47 cm) which was superior over rest of the treatments.

4.1.2 Effect of PGPR on population of *Aphis craccivora*

PGPR treated plants recorded significantly low population of *A. craccivora* than control at different intervals after release (Plate 3). Seed treatment of PGPR

was found to be significantly superior to soil drenching and foliar application in containing *A. craccivora*. Significant interaction was found to exist between PGPR treatment and method of application. The number of aphids recorded on PGPR treated cowpea seedlings at different intervals post release are presented in Table 3.

4.1.2.1 Five days after release

At five days after release least population of *A. craccivora* (13.06) was observed in *B. subtilis* (B_3) treated plants. This was on par with *S. marcescens* (B_5) (15.32). Maximum *A. craccivora* population (22.72) was recorded in *P. putida* (B_1) treated plants, which was on par with *Pseudomonas* sp. (B_2) (22.52).

Among the methods of application, seed treatment (M_1) (18.71) and soil drenching (M_2) (25.52) were found to be significantly different from each other irrespective of bacterial treatment applied.

Seed treatment with *B. subtilis* (B_3M_1) (11.09) harbored least population of *A. craccivora* and was on par with B_5M_1 (13.37) and B_4M_1 (13.46).

4.1.2.2 Seven days after release

Plants treated with *B. subtilis* (B_3) recorded minimum number of *A. craccivora* (23.27) and was on par with *S. marcescens* (B_5) (23.95) and *B. pumilus* (B_4) (27.53). *P. putida* treatment recorded maximum number of *A. craccivora* (38.61) which was on par with *Pseudomons* sp. (36.72).

Seed treatment (M_1) (22.84), soil drenching (M_2) (39.65) and foliar application (M_3) (42.06) significantly differed with respect to number of *A. craccivora*.

Minimum number of *A. craccivora* (10.04) was recorded in plants treated with *B. subtilis* at seed stage (B_3M_1) which was significantly superior to rest of the

Table 3. Population of *A. craccivora* on PGPR treated cowpea seedlings

PGPR	Mean number of aphids per plant										
	5 DAR			7 DAR				10 DAR			
	Seed treatment	Soil drenching	Mean	Seed treatment	Soil drenching	Foliar application	Mean	Seed treatment	Soil drenching	Foliar application	Mean
P. putida (B_1)	18.31 (4.39)	27.67 (5.35)	22.72 (4.87)	29.05 (5.48)	45.88 (6.85)	41.94 (6.55)	38.61 (6.29)	40.07 (6.41)	50.04 (7.14)	42.29 (6.58)	44.04 (6.71)
Pseudomonas sp. (B_2)	18.96 (4.47)	26.27 (5.22)	22.52 (4.85)	23.79 (4.98)	45.83 (6.84)	42.61 (6.60)	36.72 (6.14)	30.69 (5.63)	50.92 (7.20)	43.63 (6.68)	41.32 (6.51)
B. subtilis (B_3)	11.09 (3.48)	15.10 (4.01)	13.06 (3.75)	10.04 (3.32)	28.85 (5.46)	34.89 (5.99)	23.27 (4.93)	13.97 (3.87)	32.52 (5.79)	29.23 (5.49)	24.52 (5.05)
B. pumilus (B_4)	13.46 (3.80)	25.77 (5.17)	19.16 (4.49)	15.79 (4.09)	31.54 (5.70)	37.71 (6.22)	27.53 (5.34)	20.07 (4.59)	36.17 (6.09)	35.49 (6.04)	30.09 (5.58)
S. marcescens (B_5)	13.37 (3.79)	17.42 (4.29)	15.32 (4.04)	12.18 (3.63)	29.47 (5.52)	33.03 (5.83)	23.95 (4.99)	17.73 (4.33)	32.53 (5.79)	33.03 (5.83)	27.28 (5.32)
Control (Water) (B_6)	43.79 (6.69)	45.62 (6.83)	44.69 (6.76)	59.67 (7.79)	61.07 (7.88)	65.78 (8.17)	62.15 (7.95)	69.65 (8.41)	73.43 (8.63)	76.01 (8.78)	73.01 (8.60)
Mean	18.71 (4.44)	25.52 (5.15)		22.84 (4.88)	39.65 (6.38)	42.06 (6.56)		29.68 (5.58)	44.91 (6.78)	42.14 (6.57)	
CD values(0.05)	PGPR : 0.414			PGPR: 0.358				PGPR : 0.349			
	Method : 0.239			Method : 0.253				Method : 0.247			
	Interaction : 0.587			Interaction : 0.619				Interaction : 0.606			

Figures in parenthesis are $\sqrt{x+1}$ transformed values DAR: Days after release

B. subtilis seed treatment and control

S. marcescens seed treatment and control

B. pumilus seed treatment and control

Plate 3. Effect of PGPR on plant growth and aphid suppression

treatments. B_5M_1 recorded next low value (12.18), which was on par with B_4M_1 (15.79), B_2M_1 (23.79), B_3M_2 (28.85), B_1M_1 (29.05) and B_5M_2 (29.47).

4.1.2.3 Ten days after release

B. subtilis treatment (B_3) was significantly superior and supported minimum numbers of *A. craccivora* (24.52), followed by *S. marcescens* (B_5) (27.28) and *B. pumilus* (B_4) (30.09) which were on par with each other. Maximum number of aphids (44.04) were observed with *P. putida* (B_1) treated plants.

Seed treatment (M_1) had the least *A. craccivora* population (29.68) and was superior to other two methods of application. Foliar application (M_3) (42.14) and soil drenching (M_2) (44.91) were on par.

B. subtilis seed treatment B_3M_1 (13.97) was found to be significantly superior in resisting aphid population over the rest of the treatments. This was followed by B_5M_1 (17.73) and B_4M_1 (20.07) which were on par with each other.

4.1.3 Effect of PGPR on chlorophyll and epicuticular wax content

All the PGPR treatments gave superior results over control. Among method of application seed treatment was superior and interaction was not significant. Observations are presented in Table 4.

4.1.3.1 Chlorophyll a

Plants treated with *P. putida* (B_1) recorded maximum chlorophyll a content (0.36 mg/g). *Pseudomonas* sp. (B_2) (0.28 mg/g) was significantly different from all other treatments. Treatments *B. subtilis* (B_3) and *B. pumilus* (B_4) recorded same value (0.18 mg/g). *S. marcescens* (B_5) showed least chlorophyll content (0.16 mg/g) among treated plants.

Maximum chlorophyll a content (0.47 mg/g) was observed in foliar treatment. Seed treatment (M_1) recorded least chlorophyll content (0.16 mg/g) which was on par with soil drenching (M_2) (0.17 mg/g).

Table 4. Effect of PGPR treatment on Chlorophyll content of cowpea seedlings

PGPR	Chlorophyll a content (mg/g)				Chlorophyll b content (mg/g)				Total Chlorophyll content (mg/g)			
	Seed treatment	Soil drenching	Foliar application	Mean	Seed treatment	Soil drenching	Foliar application	Mean	Seed treatment	Soil drenching	Foliar application	Mean
P. putida (B_1)	0.71	0.19	0.19	0.36	0.27	0.08	0.07	0.14	1.00	0.25	0.26	0.50
Pseudomonas sp. (B_2)	0.59	0.17	0.09	0.28	0.32	0.07	0.13	0.18	0.93	0.25	0.23	0.47
B. subtilis (B_3)	0.21	0.19	0.15	0.18	0.10	0.41	0.07	0.19	0.27	0.19	0.10	0.19
B. pumilus (B_4)	0.23	0.16	0.15	0.18	0.11	0.09	0.09	0.10	0.27	0.16	0.11	0.18
S. marcescens (B_5)	0.15	0.15	0.18	0.16	0.07	0.07	0.11	0.08	0.11	0.11	0.19	0.13
Control (Water) ($B_{6)}$	0.89	0.19	0.17	0.42	0.60	0.39	0.08	0.36	1.41	0.26	0.26	0.65
Mean	0.16	0.17	0.47		0.09	0.19	0.25		0.19	0.21	0.66	
CD values(0.05)	PGPR : 0.075				PGPR : 0.176				PGPR : 0.103			
	Method : 0.053				Method : NS				Method : 0.073			
	Interaction : NS				Interaction : NS				Interaction : NS			

4.1.3.2 Chlorophyll b

All the treatments *viz*., *B. subtilis* (B_3) (0.19 mg/g), *Pseudomons* sp. (B_2) (0.18 mg/g), *P. putida* (B_1) (0.14 mg/g), *B. pumilus* (B_4) (0.10 mg/g) and *S. marcescens* (B_5) (0.08 mg/g) found to be on par with each other.

The chlorophyll b content did not vary with method of application.

4.1.3.3 Total chlorophyll content

P. putida (B_1) treated plants recorded maximum total chlorophyll content (0.5 mg/g) which was on par with *Pseudomonas* sp. (B_2) (0.47 mg/g). *S. marcescens* (B_5) recorded minimum value (0.13 mg/g), which was on par with *B. pumilus* (B_4) (0.18 mg/g) and *B. subtilis* (B_3) (0.19 mg/g).

Foliar application of PGPR resulted in maximum mean total chlorophyll content (0.66 mg/g). Seed treatment (M_1) yielded 0.19 mg/g of total chlorophyll content which was on par with soil drenching (M_2) (0.21 mg/g).

4.1.3.4 Epicuticular wax content

Epicuticular wax content did not vary with PGPR treatments. All the treatments were found to be on par with control.

4.2 BIOEFFICACY OF ENTOMOPATHOGENS ON SUCKING PESTS

Different entomopathogens used in this experiment is shown in plate 4.

4.2.1 On *A. craccivora*

The percentage mortality of the *A. croccivora* sprayed with different entomopathogens is presented in Table 5.

4.2.1.1 Two days after spray

Fusarium pallidoroseum proved to be significantly superior to all other treatments recording 25.06 percent mortality. *Beauveria bassiana* (11.83%) and

Table 5. Mortality of *Aphis craccivora* treated with different entomopathogens

		Mean per cent mortality		
Entomopathogens		2 DAS	4 DAS	7 DAS
T1	*Fusarium pallidoroseum*	25.06 (5.11)	62.87 (7.99)	70.97 (8.48)
T2	*Beauveria bassiana*	11.83 (3.58)	18.82 (4.45)	35.34 (6.03)
T3	*Metarhizium anisopliae*	8.57 (3.09)	11.53 (3.54)	23.22 (4.92)
T4	*Serratia marcescens*	4.62 (2.37)	7.29 (2.88)	11.34 (3.51)
T5	Control (Water spray)	0.71 (1.31)	1.21 (1.49)	1.79 (1.67)
	CD values (0.05)	0.611	0.414	0.551

Figures in parenthesis are $\sqrt{x+1}$ transformed values

DAS: Days after sowing

Metarhizium anisopliae (8.57%) were found statistically on par with each other. *Serratia marsescens* caused least percent mortality of 4.62%. All the treatments were significantly superior to control.

4.2.1.2 Four days after spray

All the treatments *viz.*, *F. pallidoroseum, B. bassiana, M. anisopliae* and *S. marcescens* were significantly different from each other and from control with mortality percent of 62.87, 18.82, 11.53 and 7.29 respectively.

4.2.1.3 Seven days after spray

F. pallidoroseum recorded the highest mortality (70.97%) which was significantly superior to rest of the treatments. This was followed by *B. bassiana* (35.34%), *M. anisopliae* (23.22%) and *S. marcescens* (11.34%). All the treatments differed significantly from each other and from control.

4.2.2 On *R. pedestris*

The percentage mortality of the *R. pedestris* sprayed with different entomopathogens is presented in Table 6 (Plate 5).

4.2.2.1 Five days after spray

B. bassiana and *S. marcescens* recorded the highest percent mortality (16.60%) which was superior to other treatments. *M. anisopliae* (11.52%) was significantly different from the rest. *F. pallidoroseum* recorded least percent mortality (2.65%). All the treatments except *F. pallidoroseum* were significantly superior over control.

4.2.2.2 Seven days after spray

S. marcescens showed highest percent mortality (28.66) which was on par with *B. bassiana* (26.22%). Next highest value was recorded by *M. anisopliae*

Table 6. Mortality of *Riptortus pedestris* treated with different entomopathogens

Entomopathogens		Mean per cent mortality				
		5 DAS	7 DAS	9 DAS	11 DAS	13 DAS
T1	*Beauveria bassiana*	16.60 (4.19)	26.22 (5.22)	41.18 (6.49)	50.43 (7.17)	73.03 (8.60)
T2	*Metarhizium anisopliae*	11.52 (3.54)	16.79 (4.22)	41.90 (6.55)	47.46 (6.96)	63.69 (8.043)
T3	*Fusarium pallidoroseum*	2.65 (1.91)	2.65 (1.91)	5.09 (2.47)	9.14 (3.18)	18.35 (4.39)
T4	*Serratia marcescens*	16.60 (4.19)	28.66 (5.45)	51.88 (7.27)	61.23 (7.89)	81.09 (9.06)
T5	Control (Water spray)	1.91 (1.71)	3.25 (2.06)	5.85 (2.62)	9.14 (3.18)	12.99 (3.74)
	CD values (0.05)	1.325	1.387	1.219	1.306	0.806

Figures in parenthesis are $\sqrt{x+1}$ transformed values

DAS: Days after sowing

R. pedestris infected with *B. bassiana*

R. pedestris infected with *M. anisopliae*

Plate 5. *R. pedestris* infected with entomopathogens

(16.79%). Least percent mortality was recorded with *F. pallidoroseum* (2.65). All the treatments except *F. pallidoroseum* were significantly superior over control.

4.2.2.3 Nine days after spray

S. marcescens caused highest percent mortality (51.88), which was on par with *M. anisopliae* (41.90) and *B. bassiana* (41.18). *F. pallidoroseum* recorded least value (5.09%) and was on par with control.

4.2.2.4 Eleven days after spray

S. marcescens recorded highest percent mortality (61.23), which was on par with *B. bassiana* (50.43) and *M. anisopliae* (47.46). *F. pallidoroseum* recorded least percent mortality (9.14) and was on par with control.

4.2.2.5 Thirteen days after spray

S. marcescens recorded highest percent mortality (81.09) and was on par with *B. bassiana* (73.03). Next highest percent mortality was recorded with *M. anisopliae* (63.69). *F. pallidoroseum* was having least mortality (18.35%) and was significantly different from all other treatments and control.

4.3 ASSESSMENT OF COMPATIBILITY OF PGPR AND ENTOMOPATHOGENS

The *in vitro* interaction was studied among different PGPRs, entomopathogens and between PGPR and entomopathogens by dual culturing them in different combinations. Existence of a zone of inhibition in dual culture is given in Tables 7, 8 and 9.

4.3.1 Between PGPR Strains

After 48 hours of streaking, there was no inhibition zone between the bacterial strains (Plate 6).

4.3.2 Between Entomopathogens

M. anisopliae showed strong antagonism (>5mm) against *F. pallidoroseum*. In all other cases only slight inhibition (<5mm) was observed (Table 7) (Plate 7).

4.3.3 Between PGPR and Entomopathogens

B. pumilus showed no antagonism against *F. pallidoroseum* and mycelium grow over it. Whereas *B. subtilis* showed very strong antagonism (>5mm) against *F. pallidoroseum*. *P. putida, Pseudomonas* sp. and *S. marcescens* showed slight inhibition against *F. pallidoroseum* where mycelial edge was touching them (Plate 8a).

P. putida and *Pseudomonas* sp. showed no antagonism against th *B. bassiana* and showed enhanced mycelial growth than the control. The antagonism by *B. subtilis* and *B. pumilus* against *B. bassiana* ranged from slight antagonism to an inhibition zone of less than 5 mm. *S. marcescens* showed no antagonism against *B. bassiana* (Plate 8b).

B. subtilis, P. putida and *Pseudomonas* sp. showed strong antagonism (>5mm), *B. pumilus* showed slight antagonism and *S. marcescens* showed no antagonism against *M. anisopliae* (Plate 8c).

4.4 EFFECT OF DUAL APPLICATION OF SELECTED PGPR AND ENTOMOPATHOGENS

Three PGPR and entomopathogens each selected based on the results of the preliminary experiments, were evaluated for plant growth promotion and pest suppression in pot culture experiment.

4.4.1 Effect of PGPRs on vegetative growth of Cowpea

4.4.1.1 Germination

Observations on days taken for germination are presented in Table 10.

Table 7. Antagonism among entomopathogens in dual culture

Entomopathogens	Inhibition zone		
	F. pallidoroseum	*B. bassiana*	*M. anisopliae*
F. pallidoroseum		++	++
B. bassiana	++		++
M. anisopliae	+++	++	

+++ Zone of inhibition >5 mm

++ Zone of inhibition <5 mm

+ Entomopathogen touches another entomopathogen

* Observations from five replications

Table 8: Antagonism of PGPR against different entomopathogens in dual culture

PGPRs	Entomopathogens (Inhibition zone)*		
	F. pallidoroseum	*B. bassiana*	*M. anisopliae*
B. subtilis	+++	++	+++
B. pumilus	_	++	+
P. putida	+	_	+++
Pseudomonas sp.	+	_	+++
S. marcescens	+	_	_

+++ Zone of inhibition >5 mm

++ Zone of inhibition <5 mm

+ Entomopathogen touches PGPR

- Entomopathogen grows over PGPR

*Observations from eight replications

Table 9. Antagonism of PGPR against different entomopathogens in dual culture

	Mean number of spores/ml *		
PGPRs	*B. bassiana*	*F. pallidoroseum*	*M. anisopliae*
B. subtilis	2.23×10^8	1.45×10^8	2.92×10^8
B. pumilus	2.47×10^8	2.60×10^8	3.38×10^8
P. putida	3.68×10^8	2.37×10^8	2.42×10^8
Pseudomonas sp.	3.75×10^8	2.38×10^8	2.66×10^8
S. marcescens	2.69×10^8	2.38×10^8	3.47×10^8
Control	2.75×10^8	2.68×10^8	3.52×10^8

* Observations from eight replications

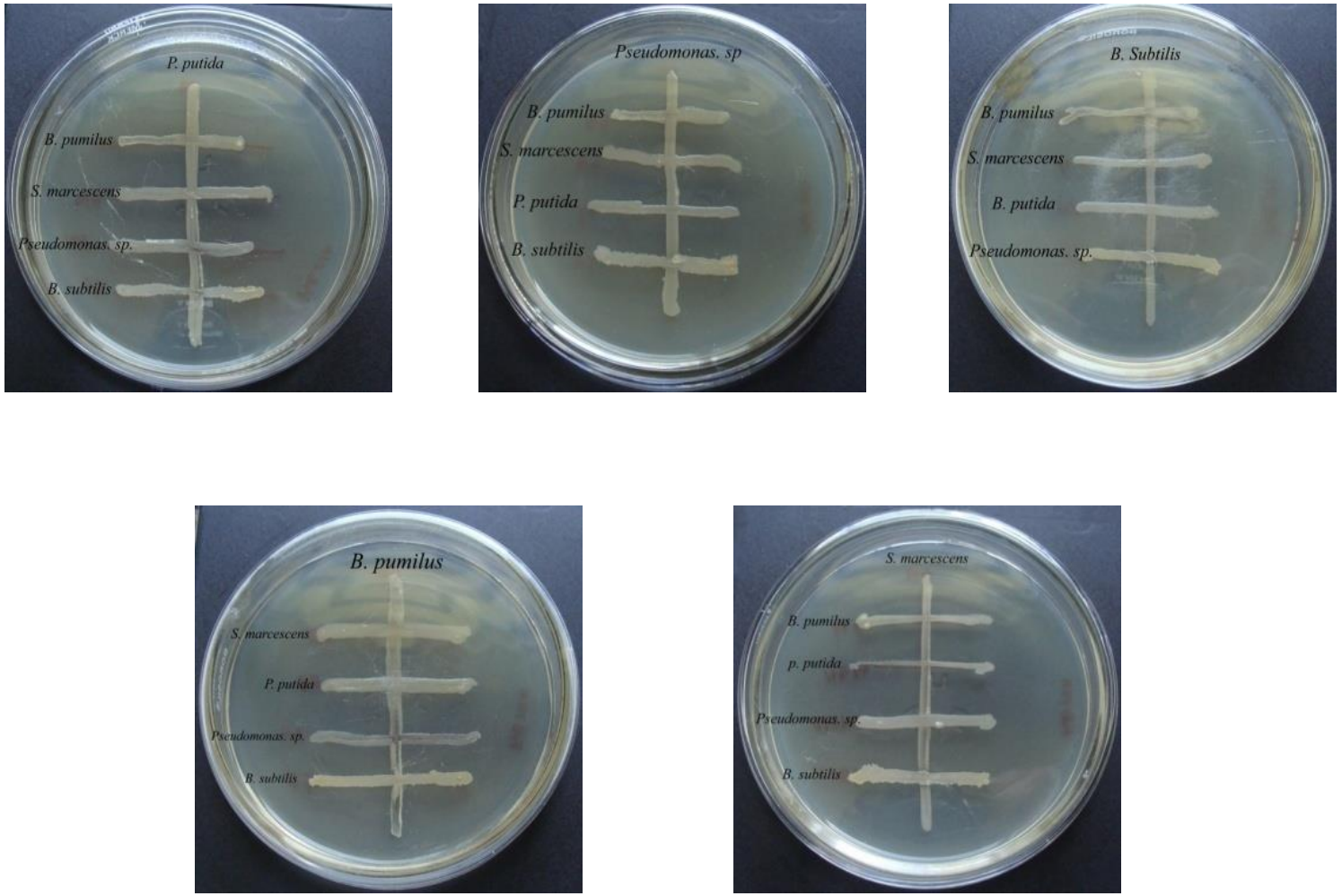
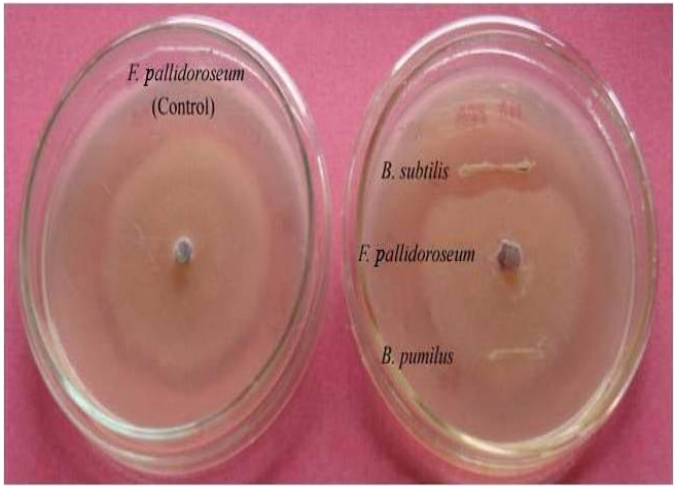


Plate 6. Assessment of compatibility among PGPR

Plate 7. Assessment of compatibility among entomopathogens



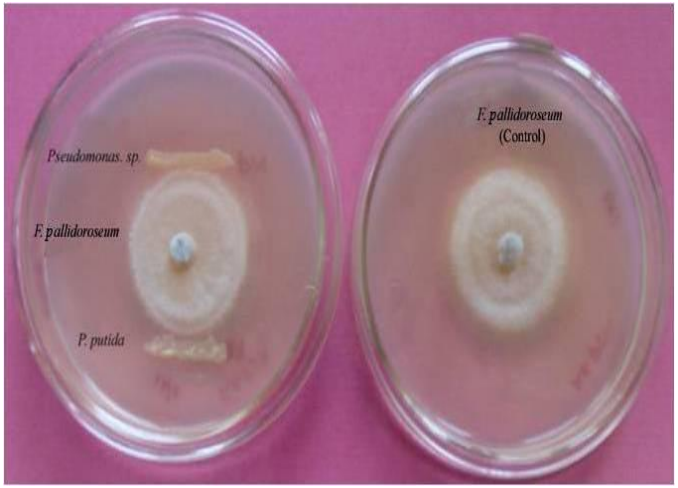


Plate 8a. Assessment of compatibility of PGPR and entomopathogens

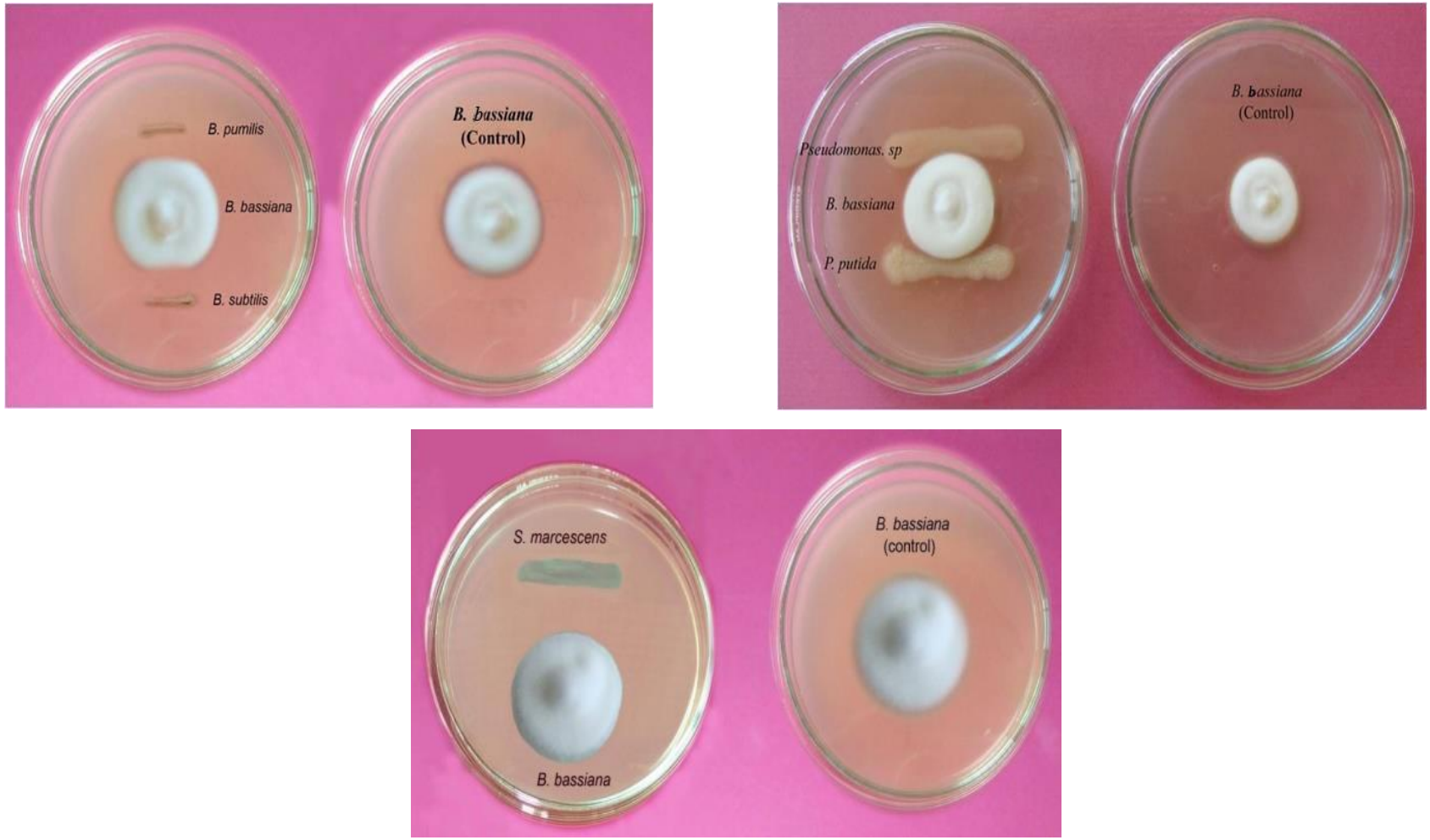


Plate 8b. Assessment of compatibility of PGPR and entomopathogens

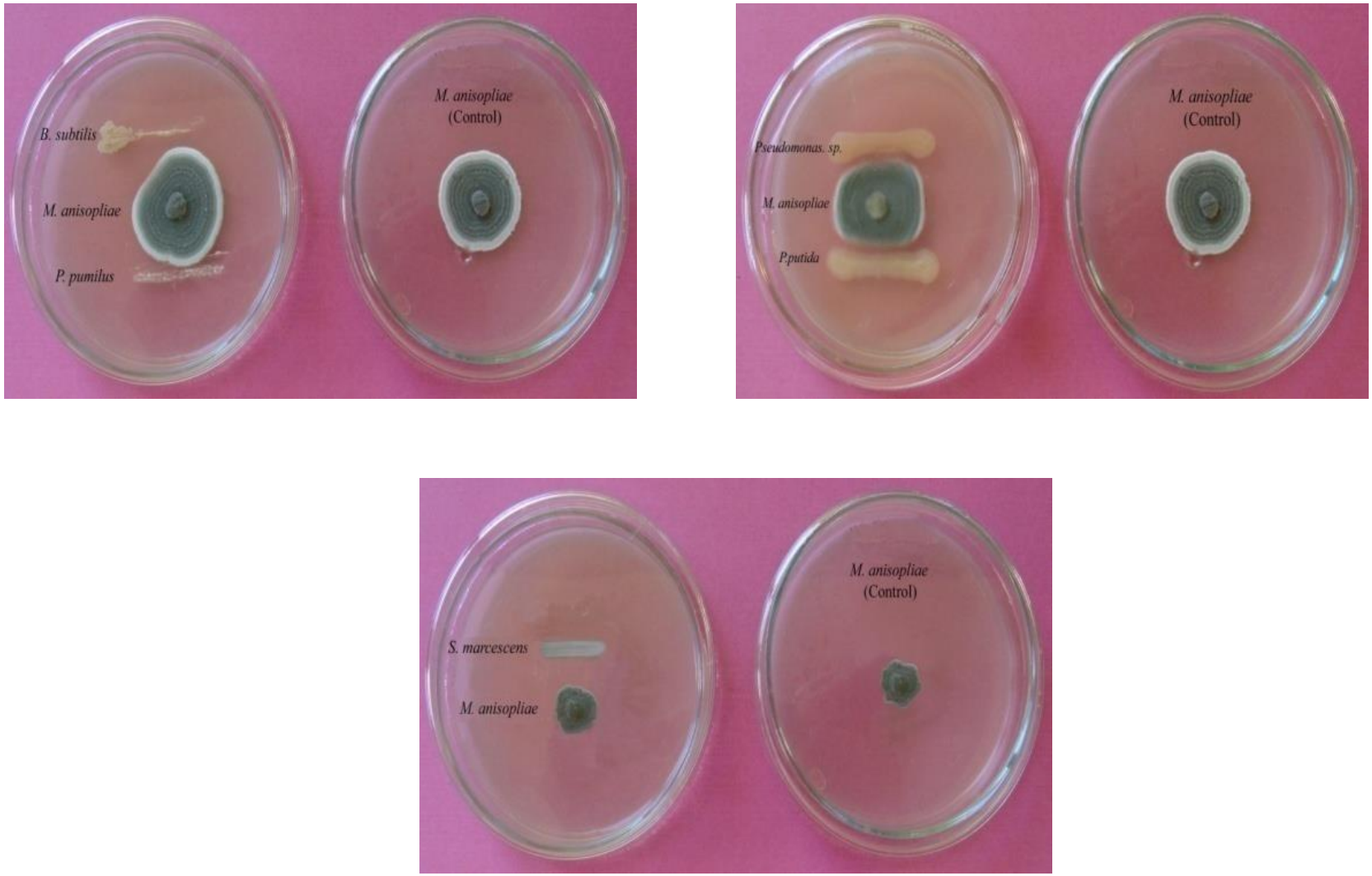


Plate 8c. Assessment of compatibility of PGPR and entomopathogens

No significant difference in germination was noted between treatments and control.

4.4.1.2 Plant height

Effect of PGPR on plant height at different intervals are given in Table 10.

Significant difference in plant height was observed in PGPR treated plants at different intervals after treatment except at 4 week after sowing.

4.4.1.2.1 One week after sowing

All PGPR treatments significantly increased plant height compared to the control. *B. subtilis* treated plants recorded highest plant length (5.02 cm), which was on par with *B. pumilus* (4.82 cm).

4.4.1.2.2 Two weeks after sowing

All the treatments significantly increased the plant height over control. Maximum plant height (12.13 cm) was observed in *B. pumilus* treated plants which was on par with *S. marcescens* (12.11cm) and *B. subtilis* (12.10 cm).

4.4.1.2.3 Four weeks after sowing

All the treatments were statistically on par with each other and with control.

4.4.1.2.4 Six weeks after sowing

B. subtilis treated plants recorded maximum plant height (33.53 cm), which was on par with *B. pumilus* (31.97 cm). *S. marcescens* recorded least plant height (31.47 cm). All the treatments were significantly superior over control.

4.4.1.2.5 Eight week after sowing

Plants treated with *B. subtilis* recorded maximum plant height (39.12 cm), which was on par with *B. pumilus* (38.08 cm) and *S. marcescens* (37.78 cm). All the treatments were significantly superior to control in increasing the plant height.

Table 10. Effect of PGPR on days taken for germination and plant height of cowpea

Treatments	Days for germination	Mean plant height (cm)				
		1 WAS	2 WAS	4 WAS	6 WAS	8 WAS
T_1 (*B. subtilis*)	3.33	5.02	12.10	22.93	33.53	39.12
T_2 (*S. marcescens*)	3.44	4.63	12.11	22.51	31.47	37.78
T_3 (*B. pumilus*)	3.35	4.82	12.13	22.73	31.97	38.08
T_4 (Control)	3.54	4.21	10.02	21.87	29.50	35.59
CD (0.05)	NS	0.197	0.469	NS	1.927	2.021

WAS : Week after sowing

4.4.1.3 Number of leaves

Effect of PGPR on number of leaves at different intervals are given in Table 11.

4.4.1.3.1 One week after sowing

B. subtilis treated plants recorded maximum number of leaves (4.31) which was on par with *B. pumilus* (4.3).

4.4.1.3.2 Two weeks after sowing

Both *B. subtilis* and *B. pumilus* treatments resulted in same number of leaves (7.38) and were significantly superior over *S. marcescens* (6.63) and control.

4.4.1.3.3 Three weeks after sowing

B. subtilis treatment significantly increased the number of leaves (12.00) compared to other treatments and control. *B. pumilus* was the next best treatment in increasing number of leaves (11.06) which was on par with *S. marcescens* (10.56). All the treatments were significantly different from control.

4.4.1.3.4 Four weeks after sowing

Plants treated with *B. subtilis* recorded maximum (14.87) number of leaves, which was on par with *B. pumilus* (14.13) and *S. marcescens* (14.13). All the treatments were significantly superior over control.

4.4.1.4 Root characteristics

Effect of PGPR on root characters like root length, number of root branches and number of root nodules are presented in Table 12 (Plate 7).

4.4.1.4.1 Root length

20 days after sowing, *B. subtilis* treated plants recorded the highest root length (11.52 cm) which was significantly superior over rest of the treatments and

Table 11. Effect of PGPR on number of leaves of cowpea

Treatments	Mean number of opened leaves			
	1 WAS	2 WAS	3 WAS	4 WAS
T_1 (*B. subtilis*)	4.31	7.38	12.00	14.88
T_2 (*S. marcescens*)	3.63	6.63	10.56	14.13
T_3 (*B. pumilus*)	4.3	7.38	11.06	14.13
T_4 (Control)	3.44	5.45	8.56	11.63
CD (0.05)	0.516	0.496	0.794	0.984

WAS : Week after sowing

Table 12. Effect of PGPR on root characteristics of cowpea

Treatments	Root length (cm)		Number of root branches		Number of root nodules	
	20 DAS	80 DAS	20 DAS	80 DAS	20 DAS	80 DAS
T_1 (*B. subtilis*)	11.52	12.44	9.08	14.08	5.94	9.85
T_2 (*S. marcescens*)	9.39	12.38	7.39	13.67	5.13	9.54
T_3 (*B. pumilus*)	9.33	12.32	8.94	13.52	5.71	9.81
T_4 (Control)	6.99	10.25	6.31	8.98	4.65	6.94
CD (0.05)	0.343	1.02	0.594	1.86	0.872	1.623

DAS : Days after sowing

Control & *B. subtilis* treated plant

Control & *S. marcescens* treated plant

Control & *B. pumilus* treated plant

Plate 9. Effect of PGPR on root characteristics of cowpea

control. Root length of plants treated with *S. marcescens* (9.39 cm) and *B. pumilus* (9.33 cm) were on par with each other and significantly different from control.

At 80 days after sowing, all the treatments *viz*., *B. subtilis* (12.44 cm), *S. marcescens* (12.38 cm) and *B. pumilus* (12.32 cm) though statistically on par with each other, significantly increased root length of plants compared to control.

4.4.1.4.2 Number of root branches

At 20 days after sowing, *B. subtilis* treatment resulted in maximum root branches (9.08) and which was on par with *B. pumilus* (8.94). *S. marcescens* treated plants had the least number of root branches (7.39) though it was significantly superior to control.

All the treatments *viz*., *B. subtilis*, *S. marcescens* and *B. pumilus* were found to significantly increase number of root branches at 80 days after sowing over control.

4.4.1.4.3 Number of root nodules

At 20 days after sowing *B. subtilis* treated plants recorded maximum number of root nodules (5.94) which was on par with *B. pumilus* (5.71) and *S. marcescens* (5.13). All the treatments were significantly different from control.

At 80 days after sowing, all the treatments were statistically on par with each other and significantly different from control.

4.4.2 Effect of PGPR treatment on yield parameters

Various yield characteristics *viz*., days for first flowering, number of pods per plant, pod length, number of seeds per pod, number of seeds per plant, 100 seed weight, dry weight of plant and dry weight of pods per plant of cowpea treated with PGPR and entomopathogens for the management of the sucking pests are shown in Table 13 to 17.

4.4.2.1 Days to first flowering

Plants treated with *B. subtilis* recorded minimum number of days (35.79) to first flowering which was on par with *B. pumilus* (35.95). *S. marcescens* treated plants recorded maximum days to first flowering and was superior over control (Table 13).

4.4.2.2 Number of pods per plant

With regards to number of pods per plant, all the PGPR treatments significantly increased number of pods per plant compared to control (Table 14). *B. subtilis* yielded maximum mean number of pods (16.00) which was significantly superior over other treatments. Among different entomopathogens when employed independently against sucking pests *S. marcescens* against *R. pedestris* yielded maximum number of pods per plant (14.00) which was on par with *F. pallidoroseum* employed against *A. craccivora* (13.67), *B. bassiana* against *R. pedestris* (13.50) and as well as chemical control check (13.29). Significant interaction was found to exist between PGPR and entomopathogens. *B. subtilis* applied in combination with *F. pallidoroseum* when employed against *A. craccivora* yielded the highest number of pods per plant. This was on par with *B. subtilis* and *S. marcescens* against *R. pedestris* (17.00), *B. subtilis* and *B. bassiana* against *R. pedestris* (16.83) and *B. subtilis* and chemical control (16.50).

4.4.2.3 Pod length

All the PGPR treatments were found to be significantly increased pod length superior over control (Table 14) (Plate 8). Plants treated with *B. subtilis* yielded longest pods (17.80 cm) which was on par with *B. pumilus* (17.72 cm). *S. marcescens* recorded with least pod length (16.83 cm). Significant interaction was found to exist between PGPR and entomopathogens. *B. pumilus* applied in combination with *F. pallidoroseum* against *A. craccivora* yielded maximum pod

Table 13. Effect of PGPR on days taken for first flowering in cowpea

Treatments	Days to first flowering
T_1 (*B. subtilis*)	35.79
T_2 (*S. marcescens*)	37.27
T_3 (*B. pumilus*)	35.95
T_4 (Water)	39.52
CD values	0.746

Table 14. Effect of dual application of PGPR and entomopathogens on number of pods/plant and pod length of cowpea

	Mean number of pods/plant					Mean pod length (cm)				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
P_1E_1	17.50	12.33	14.33	10.50	13.67	18.55	18.08	18.60	14.73	17.49
P_1E_2	16.17	12.33	13.17	8.17	12.46	18.57	17.97	17.42	14.53	17.12
P_1E_3	15.50	12.00	14.83	8.00	12.58	18.00	16.53	18.13	13.85	16.63
P_2E_1	14.17	11.83	14.83	10.50	12.83	17.42	16.25	18.00	13.50	16.29
P_2E_2	16.83	13.50	14.33	9.33	13.50	18.22	17.97	18.42	13.55	17.04
P_2E_3	17.00	13.50	14.33	11.17	14.00	18.32	16.33	17.68	13.78	16.53
Chemical check (Ci)	16.50	13.17	13.83	9.67	13.29	17.42	15.95	17.40	12.47	15.81
No entomopathogens	14.33	12.33	13.67	8.33	12.17	15.87	15.52	16.10	11.97	14.87
Mean	16.00	12.63	14.17	9.50		17.80	16.83	17.72	13.55	
CD values	First factor (PGPR Treatments): 0.726					First factor (PGPR Treatments): 0.499				
	Second factor (Entomopathogens): 1.027					Second factor (Entomopathogens) NS				
	Interaction : 2.055					Interaction : 1.410				

Ci: Quinalphos 0.03%
P_1: *Aphis craccivora*, P_2 : *Riptortus pedestris*
E_1: *Fusarium pallidoroseum*, E_2: *Beauveria bassiana*, E_3: *Serratia marcescens*
T_1 - *Bacillus subtilis* T_2 - *Serratia marcescens* T_3 - *Bacillus pumilus* T_4 - No PGPR

Control & *B. subtilis* treated plant pods

Control & *S. marcescens* treated plant pods

Control & *B. pumilus* treated plant pods

Plate 10. Effect of dual application of PGPR and entomopathogens on pod length of cowpea

length (18.60 cm), which was on par with *B. subtilis* and *B. bassiana* against *A. craccivora* (18.57 cm), *B. subtilis* and *F. pallidoroseum* against *A. craccivora* (18.55 cm), *B. subtilis* and *S. marcescens* against *R. pedestris* (18.32 cm), *B. subtilis* and *B. bassiana* against *R. pedestris* (18.22 cm), *B. subtilis* and *S. marcescens* against *A. craccivora* (18.00 cm) and *B. subtilis* and Quinalphos (17.42 cm).

4.4.2.4 Number of seeds per pod

Effect of PGPRs, entomopathogens and their interaction were found to be significant with respect to number of seeds per pod (Table 15). Plants treated with *B. subtilis* yielded maximum number of seeds per pod (17.79) which was superior over rest of the treatments and control. Entomopathogen *B. bassiana* when used against *A. craccivora* yielded maximum number of seeds per pod (16.24), which was on par with *S. marcescens* against *R. pedestris* (16.14), *F. pallidoroseum* against *A. craccivora* (16.11), *B. bassiana* against *R. pedestris* (15.73) and Quinalphos (15.69). *B. subtilis* applied in combination with *B. bassiana* against *R. pedestris* yielded maximum number of seeds per pod (18.27) which was on par with *B. subtilis* and *B. bassiana* against *A. craccivora* (18.23), *B. subtilis* and *S. marcescens* against *R. pedestris* (18.10), *B. subtilis* and Quinalphos (17.82) and *B. subtilis* and *F. pallidoroseum* against *A. craccivora* (17.73).

4.4.2.5 Number of seeds per plant

For number of seeds per plant, effect of PGPR and interactions between PGPR and entomopathogens were found to be significant (Table 15). Plants treated with *B. subtilis* yielded maximum number of seeds per plant (278.78) and was superior over rest of the treatments. Next best PGPR treatment was *B. pumilus* (226.64) followed by *S. marcescens* (204.94). *B. subtilis* applied in combination with *B. bassiana* against *R. pedestris* yielded maximum number of seeds per plant (311.50) and was on par with *B. subtilis* and *S. marcescens* against *R. pedestris* (301.83), *B. subtilis* and *F. pallidoroseum* against *A. craccivora* (289.00), *B. subtilis* and *B. bassiana* against *A. craccivora*

Table 15. Effect dual application of PGPR and entomopathogens on number of seeds/pod and number of seeds/plant in cowpea

	Mean number of seeds/pod					Mean number of seeds/plant				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
P_1E_1	17.73	16.25	17.10	13.35	16.11	289.00	189.33	273.50	134.83	221.67
P_1E_2	18.23	17.23	17.12	12.38	16.24	286.83	207.00	220.00	124.00	209.46
P_1E_3	17.35	16.75	16.58	9.63	15.08	266.58	196.17	239.67	135.33	209.44
P_2E_1	17.48	16.80	16.60	12.57	15.86	240.33	220.67	232.00	128.00	205.25
P_2E_2	18.27	16.75	17.28	10.60	15.73	311.50	201.33	240.67	131.67	221.29
P_2E_3	18.10	16.57	17.27	12.63	16.14	301.83	218.17	241.17	225.50	246.67
Chemical check (Ci)	17.82	16.72	17.00	11.25	15.69	286.50	215.83	227.50	131.17	215.25
No entomopathogens	17.33	16.07	16.28	7.98	14.42	247.67	191.00	138.67	124.33	175.42
Mean	17.79	16.64	16.90	11.30		278.78	204.94	226.65	141.85	
CD values	First factor (PGPR Treatments): 0.739					First factor (PGPR Treatments): 13.017				
	Second factor (Entomopathogens): 1.045					Second factor (Entomopathogens): NS				
	Interaction : 2.090					Interaction : 36.819				

Ci: Quinalphos 0.03%

P_1: *Aphis craccivora,* P_2 : *Riptortus pedestris*

E_1: *Fusarium pallidoroseum,* E_2: *Beauveria bassiana,* E_3: *Serratia marcescens*

T_1 - *Bacillus subtilis* T_2 - *Serratia marcescens* T_3 - *Bacillus pumilus* T_4 - No PGPR

(286.83) and *B. subtilis* and B.bassiana against A.craccivora (286.83) and B. subtilis and Quinalphos (286.50).

4.4.2.6 Hundred seed weight

With regards to 100 seed weight, effect of PGPR and interaction between PGPR and entomopathogen was found to be significant (Table 16). *B. subtilis* treatment yielded maximum weight of 100 seeds (16.01g) and on par with *B. pumilus* (15.69g). *B. subtilis* applied in combination with *B. bassiana* against *R. pedestris* recorded maximum weight of 100 seeds (16.97g) and was on par with *B. pumilus* and *F. pallidoroseum* used against *A. craccivora* (16.68g), *B. subtilis* and *S. marcescens* against *R. pedestris* (16.50g), *B. subtilis* and *B. bassiana* against *A. craccivora* (16.24g), *B. subtilis* and *F. pallidoroseum* against *A. craccivora* (16.03g) and *B. subtilis* + Quinalphos 0.05% (15.88g).

4.4.2.7 Dry weight of pods per plant

Effect of PGPR and interaction effect between PGPR and entomopathogen were found to be significant with respect to dry weight of pods per plant (Table 17). Plants treated with *B. subtilis* yielded maximum dry weight of pods per plant (42.51g) and was significantly superior over the rest of the treatments and control. *B. subtilis* applied in combination with *B. bassiana* against *R. pedestris* was having maximum dry weight of pods per plant (45.74g), which was on par with *B. subtilis* and *B. bassiana* against *A. craccivora* (43.88g), *B.subtilis* and *S. marcescens* against *R. pedestris* (43.16g), *B. subtilis* and *F. pallidoroseum* against *A. craccivora* (42.94g) and *B. subtilis* and Quinalphos (42.35g).

4.4.3 Effect of PGPR and entomopathogen on sucking pests

4.4.3.1 On A. craccivora

4.4.3.1.1 Effect of PGPR on occurrence of A. craccivora at early stage

Percentage of plants sowing presence of *A. craccivora* at 15 and 20 days after sowing in different treatments are presented in Table 18.

Table 16. Effect of dual application of PGPR and entomopathogens on 100 seed weight of cowpea

	Mean weight of 100 seeds (g)				
	T_1 (*Bacillus subtilis*)	T_2 (*Serratia marcescens*)	T_3 (*Bacillus pumilus*)	T_4 (No PGPR)	Mean
P_1E_1	16.03	13.17	16.68	12.73	14.65
P_1E_2	16.24	13.43	14.87	11.21	13.94
P_1E_3	15.90	13.62	16.20	10.22	13.99
P_2E_1	15.40	13.70	15.74	11.76	14.15
P_2E_2	16.97	12.89	15.64	12.11	14.40
P_2E_3	16.50	13.40	15.75	12.97	14.66
Chemical check (Ci)	15.88	13.51	15.86	11.71	14.24
No entomopathogens	15.17	12.74	15.61	10.97	13.62
Mean	16.01	13.31	15.69	11.71	
CD values	First factor (PGPR Treatments): 0.509				
	Second factor (Entomopathogens): NS				
	Interaction : 1.442				

Ci: Quinalphos 0.03%
P_1: *Aphis craccivora*, P_2 : *Riptortus pedestris*
E_1: *Fusarium pallidoroseum*, E_2: *Beauveria bassiana*, E_3: *Serratia marcescens*

Table 17. Effect of dual application of PGPR and entomopathogens on dry weight of pods/plant

	Dry weight of pods/plant (g)				
	T_1 (*Bacillus subtilis*)	T_2 (*Serratia marcescens*)	T_3 (*Bacillus pumilus*)	T_4 (No PGPR)	Mean
P_1E_1	42.94	32.02	37.82	21.28	33.52
P_1E_2	43.88	33.23	35.45	18.35	32.73
P_1E_3	41.18	32.84	39.63	15.41	32.27
P_2E_1	39.67	35.66	38.58	19.55	33.37
P_2E_2	45.74	32.38	37.74	20.12	33.99
P_2E_3	43.16	35.95	37.99	21.81	34.73
Chemical check (Ci)	42.35	35.45	36.45	18.15	33.1
No entomopathogens	41.13	32.48	36.85	15.85	31.58
Mean	42.51	33.75	37.57	18.82	
CD values	First factor (PGPR Treatments): 1.503				
	Second factor (Entomopathogens): NS				
	Interaction (between PGPR and entomopathogens): 4.253				

Ci: Quinalphos 0.03%

P_1: *Aphis craccivora*, P_2 : *Riptortus pedestris*

E_1: *Fusarium pallidoroseum*, E_2: *Beauveria bassiana*, E_3: *Serratia marcescens*

Table 18. Percentage of different PGPR treated plants infested with *A. craccivora*

Treatments	Per cent of plants showing presence of *A. craccivora*	
	15 DAS	20 DAS
T_1 (*B. subtilis*)	29.67 (32.99)	60.64 (51.12)
T_2 (*S. marcescens*)	40.00 (39.22)	77.85 (61.89)
T_3 (*B. pumilus*)	33.26 (35.20)	70.33 (56.97)
T_4 (Control)	53.49 (46.98)	96.33 (78.92)
CD (0.05)	NS	NS

Values are transformed to their angles

DAS : Days after sowing

PGPR treatments were not significant in resisting the *A. craccivora* infestation both at 15 and 20 days after sowing.

4.4.3.1.2 Effect of PGPR on natural incidence of A. craccivora

Effect of different PGPR treatment on population of *A. craccivora* counts are presented in Table 19.

At 25 and 30 DAS *B. subtilis* treated plants supported minimum number of *A. craccivora* (7.03 and 20.46 respectively) which was on par with *S. marcescens* (9.12 and 23.11) and *B. pumilus* (11.23 and 25.74) respectively. At 35 DAS minimum number of *A. craccivora* (35.65) was recorded in *B. subtilis* treated plants which was statistically different from rest of the treatments and control. At 40 and 45 DAS *B. subtilis* (48.69 and 63.18) and *B. pumilus* (54.44 and 70.09) treated plants showed on par results for aphid infestation.

4.4.3.1.3 Effect of dual application of PGPR and entomopathogens on A. craccivora population

Number of *A. craccivora* which survived on PGPR treated plants after the entomopathogen treatment at different intervals are given in Tables 20a and 20b.

Effect of PGPR, entomopathogen and their interaction found to be significant for *A. craccivora* population.

At two days after treatment

Least number of *A. craccivora* (53.72) survived on plants treated with *B. subtilis* which was on par with *B. pumilus* (56.49) and *S. marcescens* (58.83). Plants treated with Quinalphos showed least number of aphids (49.58) followed by *F. pallidoroseum* (66.16), *B. bassiana* (80.99) and *S. marcescens* (94.47) All the treatments were significantly different from each other and from control. Combined effect of *B. pumilus* with Quinalphos recorded least number of *A. craccivora* (32.44), which was on par with *B. subtilis* and Quinalphos (34.84),

Table 19. Effect of PGPR on *A. craccivora* population in cowpea

Treatments	Mean number of of *A. craccivora* per plant				
	25 DAS	30 DAS	35 DAS	40 DAS	45 DAS
T_1 (*B. subtilis*)	7.03 (2.83)	20.46 (4.63)	35.65 (6.05)	48.69 (7.05)	63.18 (8.01)
T_2 (*S. marcescens*)	9.12 (3.18)	23.11 (4.91)	40.14 (6.41)	55.84 (7.54)	71.82 (8.53)
T_3 (*B. pumilus*)	11.23 (3.50)	25.74 (5.17)	40.82 (6.47)	54.44 (7.45)	70.09 (8.43)
T_4 (Water spray)	46.79 (6.91)	76.94 (8.83)	115.99 (10.82)	152.41 (12.39)	187.76 (13.74)
CD values	0.956	0.568	0.653	0.699	0.604

Figures in parenthesis are $\sqrt{x+1}$ transformed values

DAS: Days after sowing

Table 20a. Effect of dual application of PGPR and entomopathogens on *A. craccivora* population

	Number of *A. craccivora* 2 DAT					Number of *A. craccivora* 4 DAT				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
P_1E_1	38.59 (6.29)	45.33 (6.81)	46.75 (6.91)	162.09 (12.77)	66.16 (8.19)	9.16 (3.19)	17.68 (4.32)	21.09 (4.70)	50.09 (7.15)	22.42 (4.84)
P_1E_2	55.49 (7.52)	59.62 (7.79)	54.51 (7.45)	180.37 (13.47)	80.99 (9.06)	42.42 (6.59)	48.75 (7.05)	35.59 (6.05)	160.30 (12.70)	64.58 (8.09)
P_1E_3	71.26 (8.50)	71.19 (8.49)	66.07 (8.19)	192.10 (13.89)	94.47 (9.77)	74.15 (8.67)	77.84 (8.88)	65.84 (8.18)	206.85 (14.42)	99.70 (10.04)
Chemical check (Ci)	34.84 (5.99)	39.82 (6.39)	32.44 (5.78)	104.91 (10.29)	49.58 (7.11)	9.67 (3.27)	13.54 (3.81)	2.83 (1.96)	33.57 (5.88)	12.91 (3.73)
No entomopathogens	74.51 (8.69)	83.61 (9.19)	90.67 (9.57)	209.82 (14.52)	109.15 (10.49)	83.38 (9.19)	96.75 (9.89)	113.14 (10.68)	264.15 (16.28)	131.48 (11.51)
Mean	53.72 (7.39)	58.83 (7.74)	56.49 (7.58)	167.71 (12.99)		37.19 (6.18)	45.12 (6.79)	38.85 (6.31)	126.37 (11.29)	
CD values	First factor (PGPR Treatments): 0.569					First factor (PGPR Treatments): 0.841				
	Second factor (Entomopathogens): 0.636					Second factor (Entomopathogens): 0.940				
	Interaction : 1.272					Interaction : 1.881				

Figures in parenthesis are $\sqrt{x+1}$ transformed values
DAT: Days after treatment
Ci: Quinalphos 0.03%
P_1: *Aphis craccivora*, E_1: *Fusarium pallidoroseum*, E_2: *Beauveria bassiana*, E_3: *Serratia marcescens*
T_1 - *Bacillus subtilis* T_2 - *Serratia marcescens* T_3 - *Bacillus pumilus* T_4 - No PGPR

Continued…

B. subtilis and *F. pallidoroseum* (38.59), *S. marcescens* and Quinalphos (39.82), *S. marcescens* and *F. pallidoroseum* (45.33) and *B. pumilus* and *F. pallidoroseum* (46.75).

At four days after treatment

B. subtilis treated plants showed least number of *A. craccivora* (37.19), which was on par with *B. pumilus* (38.85) and *S. marcescens* (45.12). Plants treated with Quinalphos recorded least number of *A. craccivora* (12.91) followed by *F. pallidoroseum* (22.42) and *B. bassiana* (64.58). In *S. marcescens* (99.70) treated plants, increase in number of *A. craccivora* observed from 2 days after sowing to four days after sowing. Combined effect of *B. pumilus* and Quinalphos recorded least (2.83) number of *A. craccivora*, which was on par with *B. subtilis* and *F. pallidoroseum* (9.16), *B.subtilis* and Quinalphos (9.67) and *S. marcescens* and Quinalphos (13.54).

At seven days after treatment

B. subtilis treated plants showed least (29.38) number of *A. craccivora*, which was on par with *B. pumilus* (30.45) and *S. marcescens* (35.61). Plants treated with *F. pallidoroseum* recorded with least (3.62) number of *A. craccivora* which was followed by Quinalphos (10.52) and *B. bassiana* (35.00). *S. marcescens* treated plants showed increase (105.05) in number of *A. craccivora* from four days after sowing to seven days after sowing. Combined effect of *B. subtilis* and *F. pallidoroseum* recorded no *A. craccivora* population which was on par with *B. pumilus* and Quinalphos (2.14), *S. marcescens* and *F. pallidoroseum* (3.62) and *B. pumilus* and *F. pallidoroseum* (3.90).

At ten days after treatment

Least number of *A. craccivora* (26.82) was recorded in plants treated with *B.pumilus* which was on par with *B. subtilis* (31.91) and *S. marcescens* (32.22). Plants treated with *F. pallidoroseum* recorded least number of *A. craccivora*

Table 20b. Effect of PGPR and entomopathogen on *A. craccivora* population

	Number of *A. craccivora* 7 DAT					Number of *A. craccivora* 10 DAT				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
P_1E_1	0 (1.00)	3.62 (2.15)	3.90 (2.22)	9.46 (3.23)	3.62 (2.15)	1.98 (1.73)	0.99 (1.41)	0 (1.00)	2.83 (1.96)	31.91 (1.52)
P_1E_2	27.73 (5.36)	24.16 (5.02)	20.24 (4.61)	80.72 (9.04)	35.00 (6.00)	9.39 (3.22)	8.75 (3.12)	6.74 (2.78)	34.96 (5.99)	13.29 (3.78)
P_1E_3	73.49 (8.63)	83.29 (9.18)	72.89 (8.59)	217.63 (14.79)	105.05 (10.29)	77.08 (8.84)	86.64 (9.36)	75.25 (8.73)	230.98 (15.23)	110.09 (10.54)
Chemical check (Ci)	9.16 (3.19)	12.07 (3.62)	2.14 (1.77)	24.00 (5.00)	10.52 (3.39)	23.83 (4.98)	16.72 (4.21)	6.69 (2.77)	31.94 (5.74)	18.59 (4.43)
No entomopathogens	86.98 (9.38)	104.96 (10.29)	116.63 (10.85)	279.85 (16.76)	138.69 (11.82)	97.29 (9.91)	113.77 (10.71)	121.81 (11.08)	306.08 (17.52)	150.49 (12.31)
Mean	29.38 (5.51)	35.61 (6.05)	30.45 (5.61)	94.34 (9.76)		31.91 (5.74)	32.22 (5.76)	26.82 (5.27)	85.30 (9.29)	
CD values	First factor (PGPR Treatments): 0.862					First factor (PGPR Treatments): 0.948				
	Second factor (Entomopathogens): 0.964					Second factor (Entomopathogens): 1.06				
	Interaction : 1.928					Interaction : 2.121				

Figures in parenthesis are $\sqrt{x+1}$ transformed values
DAT: Days after treatment
Ci: Quinalphos 0.03%
P_1: *Aphis craccivora*, E_1: *Fusarium pallidoroseum*, E_2: *Beauveria bassiana*, E_3: *Serratia marcescens*
T_1 - *Bacillus subtilis* T_2 - *Serratia marcescens* T_3 - *Bacillus pumilus* T_4 - No PGPR

(1.32) followed by *B. bassiana* (13.29).). *B. bassiana* was on par with Quinalphos (18.59). *S. marcescens* treatment did not control the *A. craccivora* population. Combined effect of *B. pumilus* and *F. pallidoroseum* recorded cent percent control of *A. craccivora*, which was on par with *S. marcescens* and *F. pallidoroseum* (0.99), *B. subtilis* and *F. pallidoroseum* (1.98), *B. pumilus* and Quinalphos (6.69), *B. pumilus* and *B. bassiana* (6.74) and *S. marcescens* and *B. bassiana* (8.75).

4.4.3.1.5 Effect of dual application of PGPR and entomopathogens on population of coccinellid predators

Population of cocconellids 50 days after sowing are presented in Table 21.

The number of coccinellids was found to be unaffected by PGPR and entomopathogen treatments.

4.4.3.2 On R. pedestris

4.4.3.2.1 Effect of PGPR on occurrence of R. pedestris

Percentage of plants infested by *R. pedestris* before and after release from field are presented in Table 22.

Both before and after augmentation, plants treated with *B. subtilis* were less infested by *R. pedestris* which was on par with *B. pumilus*.

4.4.3.2.2 Efficacy of PGPR on R. pedestris population at 55 days after sowing

Observations on number of nymphs and adults on PGPR treated plants are given in Table 23.

There was no significant difference in the population of *R. pedestris*.

Table 21. Effect of dual application of PGPR and entomopathogen on population of Coccinellids

	Number of coccinelid beetles at 50 DAS				
	T_1 (*Bacillus subtilis*)	T_2 (*Serratia marcescens*)	T_3 (*Bacillus pumilus*)	T_4 (No PGPR)	Mean
P_1E_1	5.99 (2.64)	4.16 (2.27)	4.94 (2.44)	4.39 (2.32)	3.85 (2.42)
P_1E_2	5.70 (2.59)	5.46 (2.54)	4.86 (2.42)	5.54 (2.56)	5.39 (2.53)
P_1E_3	5.71 (2.59)	4.91 (2.43)	5.47 (2.54)	4.99 (2.45)	5.27 (2.50)
Chemical check (Ci)	4.86 (2.42)	5.83 (2.61)	4.95 (2.44)	5.09 (2.47)	5.18 (2.49)
No entomopathogens	5.16 (2.48)	4.74 (2.39)	4.79 (2.41)	4.16 (2.27)	4.71 (2.39)
Mean	5.48 (2.55)	5.00 (2.45)	4.99 (2.45)	4.83 (2.41)	
CD values	First factor (PGPR Treatments): NS				
	Second factor (Entomopathogens): NS				
	Interaction: NS				

Figures in parenthesis are $\sqrt{x+1}$ transformed values
Ci: Quinalphos 0.03%
P_1: *Aphis craccivora*
E_1: *Fusarium pallidoroseum*, E_2: *Beauveria bassiana*, E_3: *Serratia marcescens*

Table 22. Percentage of different PGPR treated plants infested with *R. pedestris*

Treatments	Per cent of plants showing presence of *R. pedestris*	
	Before release	After release
T_1 (*B. subtilis*)	37.46 (37.72)	45.79 (42.57)
T_2 (*S. marcescens*)	60.56 (51.07)	70.91 (57.34)
T_3 (*B. pumilus*)	45.83 (42.59)	56.30 (48.59)
T_4 (Control)	77.92 (61.95)	95.89 (78.27)
CD (0.05)	11.632	11.482

Values are transformed to their angles

DAS : Days after sowing

Table 23. Effect of PGPR on population of *R. pedestris* at 55 days after sowing

Treatments	Mean number of *R. pedestris* per plant at 55 DAS	
	Nymphs	Adults
T_1 (*B. subtilis*)	1.16 (1.47)	2.42 (1.85)
T_2 (*S. marcescens*)	1.78 (1.67)	3.28 (2.07)
T_3 (*B. pumilus*)	1.69 (1.64)	3.04 (2.01)
T_4 (Water spray)	2.13 (1.77)	3.28 (2.07)
CD values	NS	NS

Figures in parenthesis are $\sqrt{x+1}$ transformed values

4.4.3.2.3 Effect of dual application of PGPR and entomopathogens on R. pedestris population

Observations on number of *R. pedestris* nymphs and adults survived after entomopathogen spray is given in Table 24 and 25.

Both nymphs and adults population found to be not affected by the entomopathogen treatments both at three and seven days after treatment.

Table 24. Effect of PGPR and entomopathogens on *R. pedestris* nymphs

	Number of *R. pedestris* nymphs at 3 DAT					Number of *R. pedestris* nymphs at 7 DAT				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
P_2E_1	1.12 (1.46)	1.64 (1.63)	1.69 (1.64)	2.37 (1.84)	1.69 (1.64)	0.55 (1.24)	1.49 (1.58)	1.78 (1.67)	1.99 (1.73)	1.43 (1.56)
P_2E_2	0.66 (1.29)	0.50 (1.22)	1.12 (1.46)	1.43 (1.56)	0.90 (1.38)	1.12 (1.46)	0.61 (1.27)	0.55 (1.24)	0.61 (1.27)	0.72 (1.31)
P_2E_3	0.98 (1.41)	0.66 (1.29)	0.81 (1.34)	1.31 (1.52)	0.93 (1.39)	0.98 (1.41)	1.04 (1.43)	1.26 (1.50)	0.91 (1.38)	1.04 (1.43)
Chemical check (Ci)	0.98 (1.41)	1.14 (1.46)	0.50 (1.22)	1.48 (1.58)	1.01 (1.42)	0.32 (1.15)	1.07 (1.44)	0.32 (1.15)	0.92 (1.39)	0.64 (1.28)
No entomopathogens	1.43 (1.56)	1.76 (1.66)	1.74 (1.65)	1.92 (1.71)	1.71 (1.65)	0.94 (1.39)	1.72 (1.65)	2.24 (1.79)	1.87 (1.69)	1.67 (1.63)
Mean	1.02 (1.42)	1.11 (1.45)	1.14 (1.46)	1.69 (1.64)		0.77 (1.33)	1.17 (1.47)	1.16 (1.47)	1.23 (1.49)	
CD values	First factor (PGPR Treatments): NS					First factor (PGPR Treatments): NS				
	Second factor (Entomopathogens): NS					Second factor (Entomopathogens): NS				
	Interaction : NS					Interaction : NS				

Figures in parenthesis are $\sqrt{x+1}$ transformed values
DAT: Days after treatment
Ci: Quinalphos 0.03%
P_2: *Riptortus pedestris*,E_1: *Fusarium pallidoroseum*, E_2: *Beauveria bassiana*, E_3: *Serratia marcescens*
T_1 - *Bacillus subtilis* T_2 - *Serratia marcescens* T_3 - *Bacillus pumilus* T_4 - No PGPR

Table 25. Effect of entomopathogen and PGPR on *R. pedestris* adults

	Number of *R. pedestris* adults at 3 DAT					Number of *R. pedestris* adults at 7 DAT				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
P_2E_1	2.15 (1.77)	3.34 (2.08)	3.07 (2.02)	2.70 (1.92)	2.80 (1.95)	0.83 (1.35)	2.03 (1.74)	1.64 (1.62)	1.99 (1.73)	1.59 (1.61)
P_2E_2	1.57 (1.60)	1.12 (1.46)	1.57 (1.60)	1.99 (1.73)	1.55 (1.59)	1.19 (1.48)	1.17 (1.47)	1.20 (1.48)	0.98 (1.41)	1.14 (1.46)
P_2E_3	1.87 (1.69)	1.99 (1.73)	2.45 (1.86)	2.02 (1.74)	2.08 (1.75)	1.59 (1.61)	1.16 (1.47)	0.86 (1.37)	2.08 (1.76)	1.40 (1.55)
Chemical check (Ci)	1.44 (1.56)	1.14 (1.46)	1.48 (1.57)	2.15 (1.77)	1.54 (1.59)	1.26 (1.50)	0.77 (1.33)	1.31 (1.52)	1.12 (1.46)	1.10 (1.45)
No entomopathogens	0.83 (1.35)	2.89 (1.97)	2.53 (1.88)	2.85 (1.96)	2.21 (1.79)	1.63 (1.62)	3.00 (2.00)	1.91 (1.71)	2.60 (1.89)	2.27 (1.81)
Mean	1.55 (1.59)	2.03 (1.74)	2.19 (1.79)	2.33 (1.83)		1.29 (1.51)	1.57 (1.60)	1.37 (1.54)	1.72 (1.65)	
CD values	First factor (PGPR Treatments): NS					First factor (PGPR Treatments): NS				
	Second factor (Entomopathogens): NS					Second factor (Entomopathogens): NS				
	Interaction : NS					Interaction : NS				

Ci: Quinalphos 0.03%
P_2 : *Riptortus pedestris*
E_1: *Fusarium pallidoroseum*, E_2: *Beauveria bassiana*, E_3: *Serratia marcescens*
T_1 - *Bacillus subtilis* T_2 - *Serratia marcescens* T_3 - *Bacillus pumilus* T_4 - No PGPR

Discussion

5. DISCUSSION

Plant Growth-Promoting Rhizobacteria (PGPR) enhances plant growth and yield by direct or indirect mechanisms. The direct mechanisms of action include nitrogen fixation, production of phytohormones and lowering of ethylene concentration. The indirect mechanisms include inducing systemic resistance towards invading pathogens and pests. In view of the effectiveness of PGPR, effort is being made to exploit their efficacy in managing pests under field condition. The manifestation of ill effects of over reliance on chemical pesticides calls for greater attention on ecofriendly pest management tactics. Use of entomopathogens, primarily bacteria, fungi and viruses are gaining importance because of their versatility, persistent action and environmental safety.

By combining PGPR capable of enhancing plant resistance with entomopathogens triggering diseases among insects, the effectiveness of both of these bioagents could be exploited to their full strength. The present study is an attempt in this direction, wherein five potential PGPR and four proven entomopathogens were evaluated for their efficacy in suppressing two prominent sucking pests of cowpea, the black pea aphid, *Aphis craccivora* and the pod bug, *Riptortus pedestris*.

Five PGPRs *viz*., *Pseudomonas putida*, *Pseudomonas* sp., *Bacillus subtilis, Bacillus pumilus* and *Serratia marcescens* were screened to test their efficacy in growth promotion and pest suppression in cowpea seedlings raised under glass house conditions. All PGPR treatments were found significantly superior to control in promoting plant growth and in reducing population of *A. craccivora*. Seed treatment with PGPR gave significantly superior results than other methods of application like soil drenching and foliar application.

PGPR treatments reduced the time taken for seed germination. Among the different treatments, cowpea seeds treated with *B. subtilis* germinated quickly in 3.03 days and increased the number of opened leaves to 3.79 at 15 days after sowing. At 15 and 25 days after sowing *B. subtilis* seed treated plants recorded maximum plant height (7.89 cm and 16.47 cm) also. *S. marcescens* and *B. pumilus* were found to be on par with *B. subtilis* for majority of characters studied (Fig. 1).

PGPR treated plants recorded significantly low *A. craccivora* population than control at different intervals. At five days after release *B. subtilis* seed treated plants recorded least population of *A. craccivora* (11.09) which was on par with *S. marcescens* and *B. pumilus* seed treatment. At seven and ten days after release *B. subtilis* seed treated plants harbored least population of *A. craccivora* (10.04 and 13.97 respectively), which was superior over rest of the treatments and control. *S. marcescens* and *B. pumilus* seed treatments were next best treatments in suppressing aphid population at seven and ten days after release. The result on *A. craccivora* population on PGPR treated plants indicate clearly that *A. craccivora* proliferate less on plants treated with PGPR than in control (Fig 2). Among the different PGPR, *B. subtilis*, *B. pumilus* and *S. marcescens* were identified as potential PGPR and selected for evaluation under pot culture experiment.

A large body of evidence suggests that PGPR enhances seed germination, plant growth and brings about pest suppression under green house and glass house conditions. Çakmakçi et al. (2006) found that *Bacillus* species, such as OSU-142, RC07 and M-13, *Paenibacillus polymyxa* RC05 and *Pseudomonas putida* RC06 increased the leaf, root and sugar yield in sugar beet under green house conditions. *P. putida* and *P. fluorescens* promoted 50 per cent increase in plant growth of spinach in green house experiment (Urashima and Hori, 2003). Hanafi et al. (2007) observed that *Bemicia tabaci* proliferated less on tomato plants treated with *B. subtilis* and notedthat the inoculation of plants with *B. subtilis* confers to them some type of resistance or avoidance behavior which resulted in

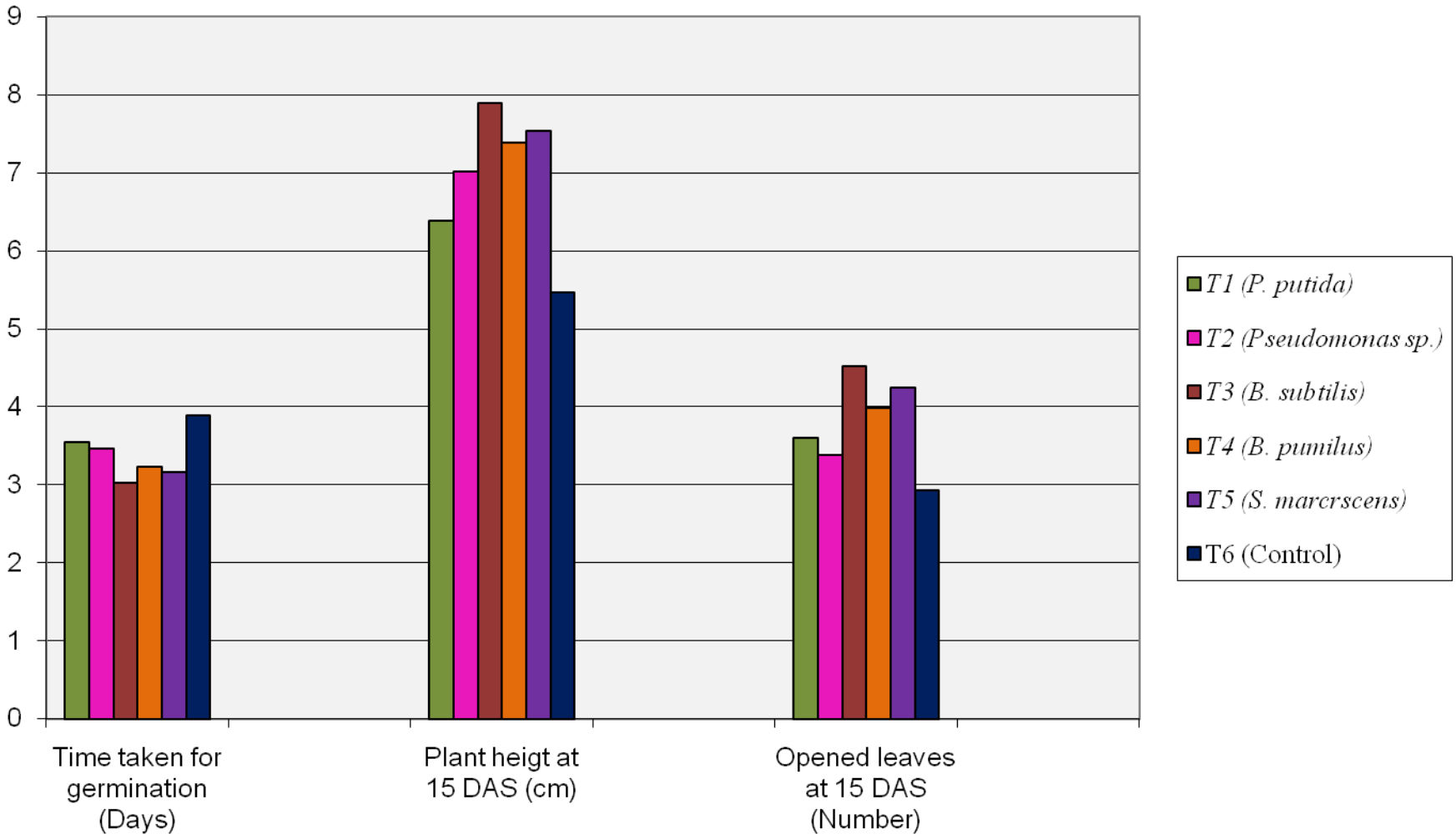


Fig. 1 Effect of PGPR seed treatment on growth characteristics of cowpea

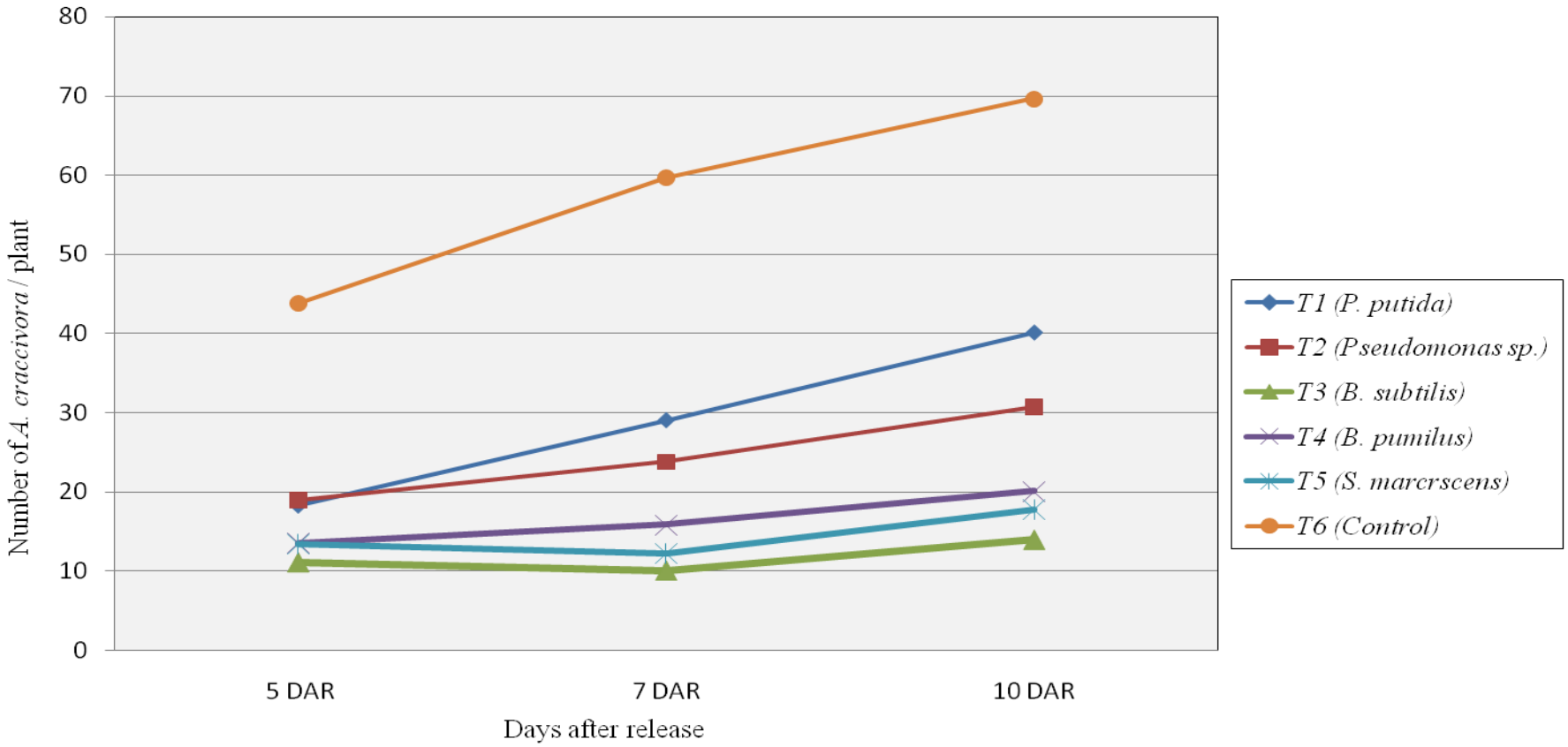


Fig. 2 Effect of PGPR seed treatment on *A. craccivora* population

less proliferation of *B. tabaci*. Saravanakumar et al. (2008) demonstrated that application of fluorescent pseudomonas significantly reduced leaf folder attack in rice plants compared to untreated control. This was due to induction of enzymes, PPO and LOX in plants accounting to defence reactions against insect pests.

All the four entomopathogens *viz*., *B. bassiana*, *M. anisopliae*, *F. pallidoroseum* and *S. marcescens* tested against *A. craccivora* under laboratory condition were found to be pathogenic but their virulence varied greatly. *F. pallidoroseum* proved to be very effective against *A. craccivora* as shown by consistently increasing mortality of aphids with increase in exposure period *viz*., 25.06, 62.87 and 70.97 per cent mortality after two, four and seven days post treatment. *B. bassiana* and *M. anisopliae* were moderately effective with 18.82 to35.34 per cent and 11.53 to 23.22 per cent mortality at four and seven days post treatment respectively. *S. marcescens* proved to be least effective among the entomopathogens tested by recording only 11.34 per cent mortality seven days post treatment. Similar results were recorded in previous research work of Mathai et al, (2002) which proved high virulence of *F. pallidoroseum* on *A. craccivora* in Kerala. Laboratory bioassay of different fungal isolates of *B. bassiana, M. anisopliae* and *V. lecanii* showed mortality ranging from 16.70 to 60.45, 20.00 to 60.00 and 20.00 to 74.00 per cent, respectively against cowpea aphid, *A. craccivora* (Nirmala et al., 2006). However, Ekesi et al. (2000) while working in Agricultural Research Farm, Zari, Nigeria on the bioassay of fungal pathogens on *A. craccivora*, recorded 58 to 91 and 66 to 100 per cent mortality of aphids exposed to *B. bassiana* and *M. anisopliae*, respectively at seven days post treatment. This difference from the results of present study may be due to variation in the pathogenicity of different isolates of the same fungus.

All entomopathogens tested against the cowpea pod bug, *R. pedestris* were found to be effective and consistent in bringing mortality of *R. pedestris* with prolonged time of exposure. *S. marcescens* proved to be more pathogenic to *R. pedestris* with highest mean mortality of 81.09 per cent 13 days post treatment.

However, *F. pallidoroseum* showed its ineffectiveness in infesting *R. pedestris* as compared to *A. craccivora* with least mortality of 18.35 per cent 13 days post treatment. Similar results were recorded by Hu et al. (1996) working on the pathogenicity of *B. bassiana* to the coreid bug *R. linearis* under laboratory conditions, where all the life stages of the bug was shown to be susceptible to *B. bassiana*. At different concentrations, *B. bassiana* and *M. anisopliae* caused mortality in *Clavigralla tomentosicollis* ranging from 58 to 97 per cent and 53 to 100 per cent, respectively at seven days post treatment (Ekesi, 1999). Based on the results of bioefficacy tests, three entomopathogens *viz*., *F. pallidoroseum, B. bassiana* and *S. marcescens* were selected for evaluation under pot culture experiment.

All PGPR were found compatible with each other as no inhibition was observed in their dual culture. Saravanakumar et al. (2008) found different isolates of fluorescent pseudomonads compatible with each other through dual culture technique.

When entomopathogens were dual cultured *M. anisopliae* inhibited growth of *F. pallidoroseum* strongly. In all other combinations only slight inhibition was observed.

Some PGPR strains tested were found to antagonize growth of entomopathogens *in vitro*., as evidenced by inhibition of *F. pallidoroseum* and *M. anisopliae* by *B. subtilis* and *M. anisopliae* by *Pseudomonas* sp. and *P. putida*. The sporulation of these fungi near the inhibition zone were also comparatively low. The inhibition of entomopathogenic fungi by PGPR is comparable to suppression of growth of phytopathogenic fungi. Antagonism of *B. subtilis, B. pumilus* and *P. putida* to *Rhizoctonia solani* causing foliar blight of amaranthus has been reported (Nair, 2006). The production of antifungal substances involved in inhibition of fungal proliferation was also documented in several rhizobacteria (Landa et al., 1997). Majority of PGPR tested either did not exert any influence on mycelial growth of entomopathogens or enhanced its

growth, making it possible to combine different PGPR and entomopathogens to develop appropriate microbial consortia to induce growth promotion and pest tolerance in cowpea.

Combined application of PGPR and entomopathogens attempted in pot culture experiment, to suppress sucking pests, enhance growth and yield of cowpea gave promising results.

Though the effect of PGPR on seed germination was found to be statistically not significant, seedling vigor was significantly improved by PGPR treatments as shown by increased plant height, number of leaves, number of root branches, root length and number of root nodules. At one, six and eight weeks after sowing plants treated with *B. subtilis* recorded the maximum plant height. At two week after sowing *B. pumilus* treatment gave superior results (Fig. 3). With respect to number of leaves all treatments were found to be superior to control at different intervals. At first and second week after sowing, *B. subtilis* treated plants recorded maximum number of leaves (4.31 and 7.38) which was on par with *B. pumilus*. At three weeks after sowing plants treated with *B. subtilis* treatment recorded maximum number of leaves (12.00) which was superior over rest of the treatments (Fig 4).

At 20 days after sowing maximum root length of 11.52 cm was observed in plants treated with *B. subtilis* which was superior over rest of the treatments. Maximum root branches (9.08 and 14.08 respectively) were also observed in plants treated with *B. subtilis* at 20 and 80 days after sowing. However at 20 days after sowing, *B. subtilis* was on par with *B. pumilus* and at 80 days after sowing it was on par with both *S. marcescens* and *B. pumilus*. The same trend was observed with respect to number of root nodules also.

PGPR treatment significantly improved reproductive parameters in cowpea when employed in combination with entomopathogens to tackle sucking pests.Plants treated with *B. subtilis* recorded maximum value for number of pods per

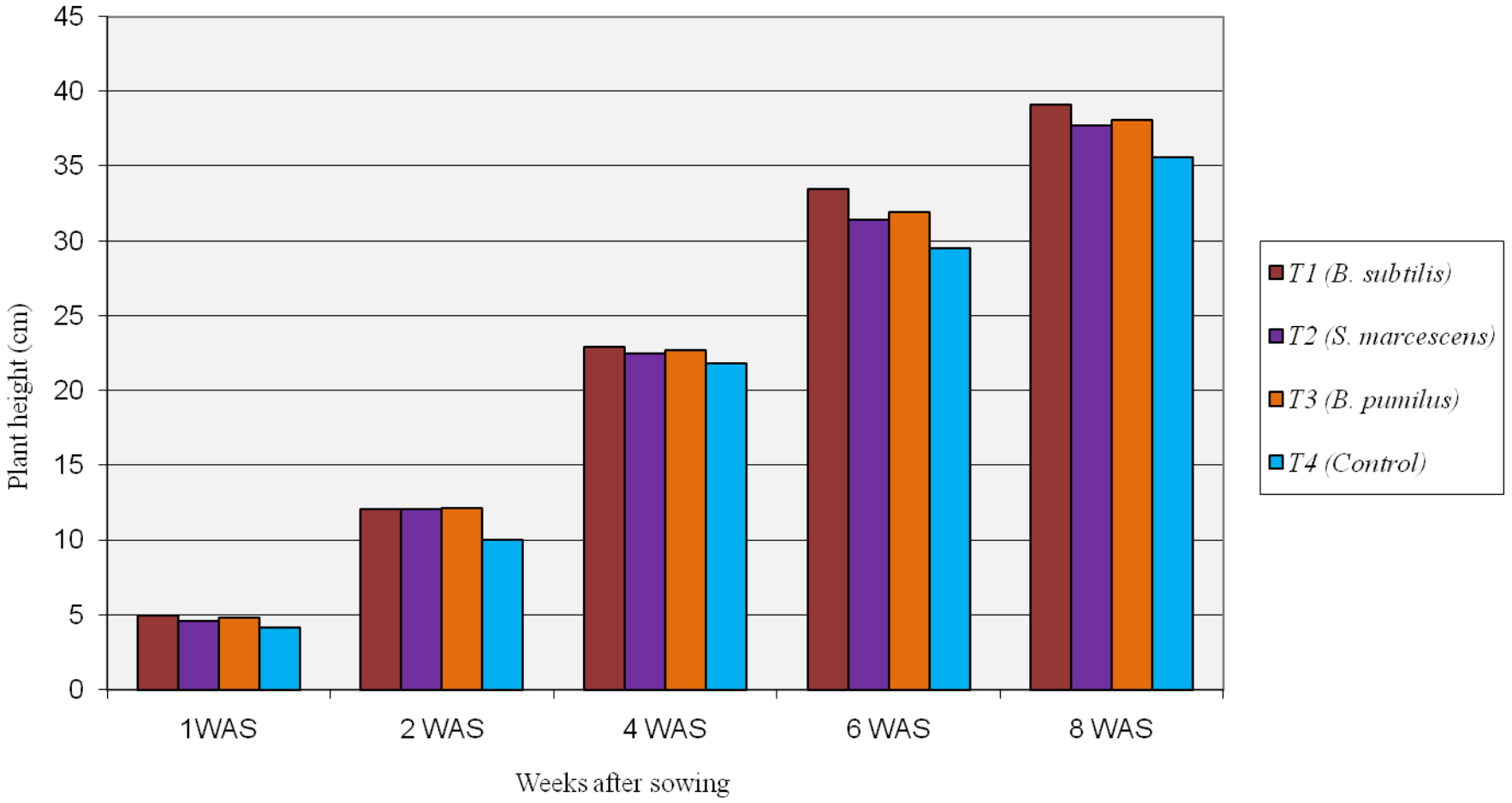


Fig. 3 Effect of PGPR on plant height of cowpea in pot culture experiment

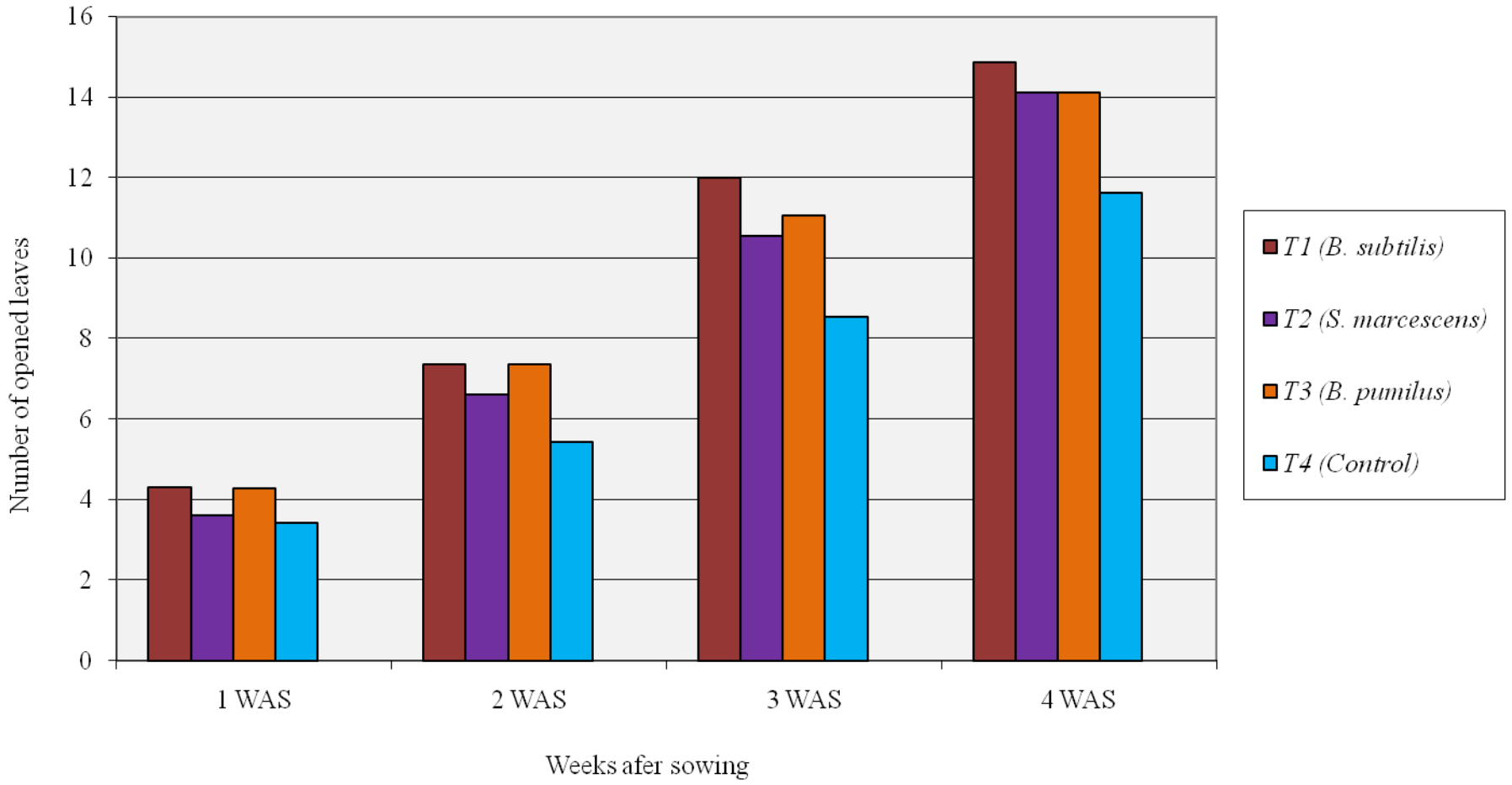


Fig. 4 Effect of PGPR on number of leaves of cowpea in pot culture experiment

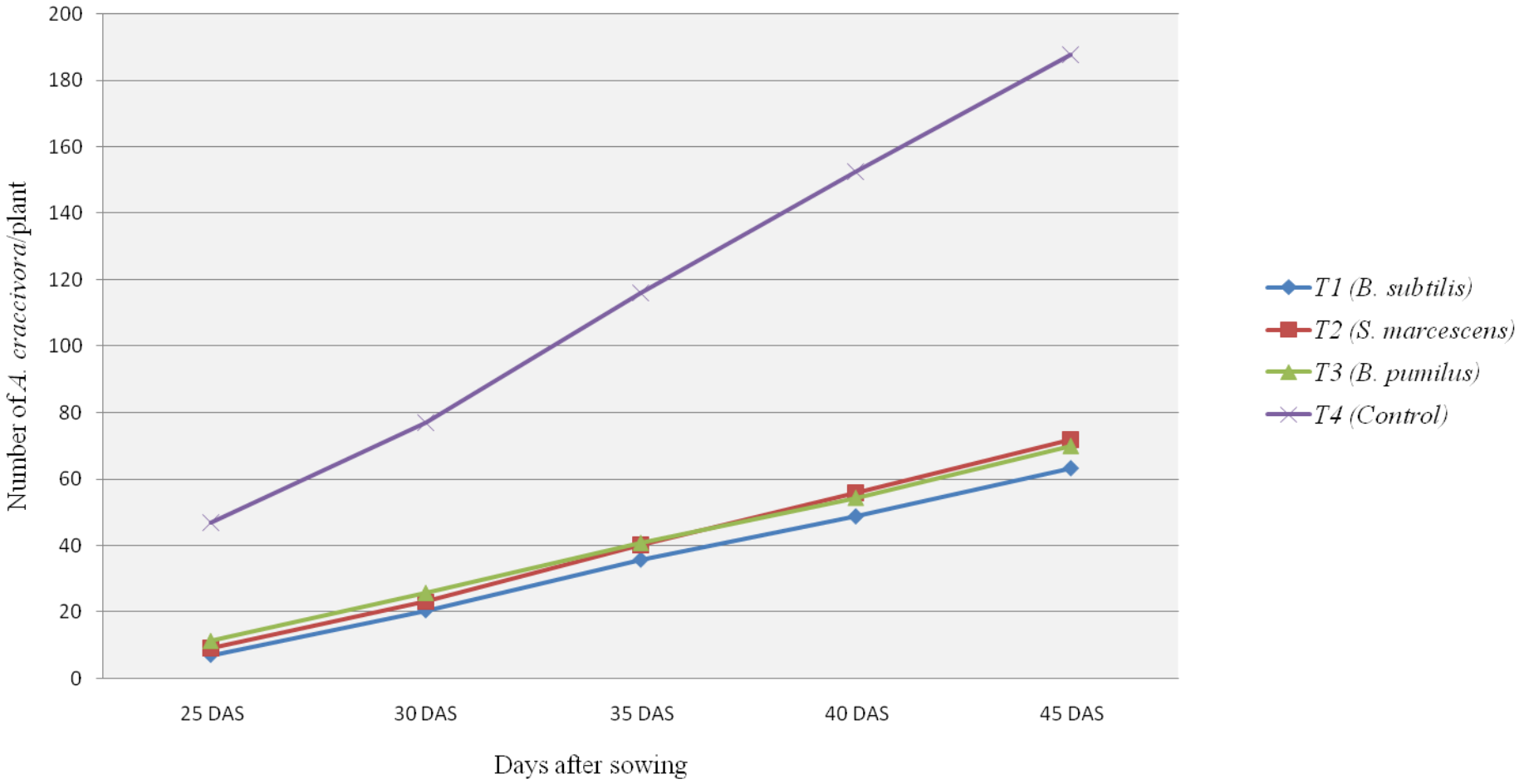


Fig. 5 Effect of PGPR on natural incidence of *A. craccivora* population

B. subtilis applied in combination with *F. pallidoroseum* was found to be the best treatment in controlling *A. craccivora* which was found to on par with *B. pumilus* and *F. pallidoroseum* combination and *S. marcescens* and *F. pallidoroseum* combination (Fig. 6).

PGPR mediated enhancement of plant characters, yield attributes and pest suppression was observed by several researchers in various crop plants. Zehnder et al. (1997) while studying the induced systemic resistance in cucumber against cucumber beetles by *P. putida*, *S. marcescens*, *Flavomonas oryzihabitans* and *B. pumilus* observed higher mean main runner length per plant, mean leaf number/plant and mean fruit weight per plant in addition to reduced cucumber beetle population. Reduced cucurbitacin content in PGPR treated plants was correlated with resistance against beetles. Through root dip application method, *P. aeruginosa* and *P. fluorescens* provided 26 to 49 per cent and 32 to 42 per cent reduction in root knot nematode penetration, in tomato. Through soil drenching method these PGPR recorded 29 to 53 per cent and 38 to 58 per cent reduction in nematode penetration, respectively. Reduced penetration of nematodes was attributed to systemic resistance by PGPR treatment (Siddiqui and Shaukat, 2002). Different *P. fluorescens* isolates significantly enhanced the pod yield (18-26 %), number of pods and 100 kernel mass in peanut treated through seed inoculation and contributed to systemic induced resistance to root rot disease (Dey et al., 2004). In the host preference study, Tomczyk (2006) observed reduced host acceptance, reduced fecundity of spider mites and enhanced plant growth parameters in cucumber plants treated with *P. fluorescens*. This was due to antixenosis mechanism connected with volatile substance emission from *P. fluorescens* treated plants. In a study to assess the induced systemic resistance of banana plantlets against bunchy top virus, Kavino et al. (2007) recorded improved vegetative growth, protein and phenol content, reduced banana aphid population and reduced bunchy top disease in banana plants treated with *P. fluorescens* and *B. subtilis* through root colonization and foliar application. Seed treatment of *B. subtilis* to mung bean

	Number of pods/plant				Pod length (cm)				Number of seeds/pod				Number of seeds/plant				Dry weight of pods/plant (g)				100 seeds weight				Aphid population/plant			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
P_1E_1	■				●		■		●				●				●				●		●		■	●	●	
P_1E_2					●				●				●				●				●							
P_1E_3																												
P_2E_1																												
P_2E_2	●				●				■				■				■				■							
P_2E_3	●				●				●				●				●				●							
Ci					●				●				●				●				●						●	
Control																												

■ Treatment which gave best results ● Treatments which were on par with the best and statistically superior over control

P_1: *Aphis craccivora*, P_2 : *Riptortus pedestris* E_1: *Fusarium pallidoroseum*, E_2: *Beauveria bassiana*, E_3: *Serratia marcescens* Ci: Quinalphos 0.03% T_1: *Bacillus subtilis* T_2 : *Serratia marcescens* T_3: *Bacillus pumilus* T_4: No PGPR

Fig. 6. Abstract observation on yield parameters and aphid population/plant treated with PGPR and entomopathogens

induced resistance against *A. craccivora* by enhancing phenol and peroxidase concentrations (Bhattacharya et al., 2008). Herman et al. (2008) studied the effect of *B. subtilis* and *B. amyloliquefaciens* on growth and yield enhancement and suppression of the green peach aphid, *Myzus persicae* on bell pepper plants. *Bacillus* treatment increased bell pepper yield by reducing *M. persicae* colonization.

Results of study reveals growth promotion and yield increase in cowpea treated with PGPR particularly *B. subtilis*, *S. marcescens* and *B. pumilus*. The tolerance to sucking pest, *A. craccivora* was significantly improved by these PGPR treatments. Entomopathogen when employed was found successful in reducing number of insect pest colonize PGPR treated plants. This will pave way for development of appropriate microbial consortia for cowpea consisting of PGPR, enhancing growth, yield and pest tolerance and entomopathogens causing mortality among major sucking pests.

Summary

6. SUMMARY

The study entitled “Management of major sucking pests in cowpea *Vigna unguiculata* (L.) Walp. with entomopathogens and plant defense inducing rhizobacteria” was carried out at the Department of Entomology, College of Agriculture, Vellayani during 2007-2009. The main objectives of the experiment was to identify the potential PGPR in cowpea and to evaluate their efficacy in enhancing plant resistance against the major sucking pests, to test bioefficacy of entomopathogens against sucking pests, to study the interaction and compatibility of PGPR and entomopathogens and to develop and evaluate dual application of PGPR and entomopathogens to contain sucking pests.

The salient findings of present investigation are as follows.

- PGPR *viz*., *Pseudomonas putida*, *Pseudomonas* sp., *Bacillus subtilis, Bacillus pumilus* and *Serratia marcescens* treatments significantly enhanced seedling vigor in cowpea.

- Population build up of *A. craccivora* was slow in PGPR treated plants compared to control.

- Seed treatment with PGPR gave superior results over soil drenching and foliar application.

- *B. subtilis*, *B. pumilus* and *S. marcescens* were identified as potential PGPR suited for growth promotion and aphid suppression.

- *F. pallidoroseum* proved to be very effective against *A. craccivora* showing consistently increasing mortality of aphids with increase in exposure time. *B. bassiana* and *M. anisopliae* were found to be moderately effective.

- *S. marcescens* was found to be pathogenic to *R. pedestris* exhibiting high mean mortality of 81.09 per cent 13 days post treatment. This was followed by *B. bassiana* (73.03%) and *M. anisopliae* (63.69%).

- All the PGPR tested were found compatible with each other as no inhibition was observed in dual culture.

- *M. anisopliae* inhibited growth of *F. pallidoroseum* strongly. In all other entomopathogen combinations only slight inhibition was observed.

- In PGPR-entomopathogen dual culture plate assay *P. putida* and *Pseudomonas* sp. showed enhanced growth of *B. bassiana*.

- *B. subtilis* was found to be the best PGPR treatment in enhancing vegetative growth and yield of cowpea in pot culture experiment.

- *F. pallidoroseum* gave maximum per cent mortality of *A. craccivora*. *B. subtilis* applied in combination with *F. pallidoroseum* was effective in suppressing aphids and also recorded superior results for yield characters.

- Population of *R. pedestris* was not affected by PGPR and entomopathogen treatment.

- Dual application of *B. subtilis* and *S. marcescens* when employed against *R. pedestris* resulted in significantly higher yield.

- Combined infestation of *A. craccivora* and *R. pedestris* could be managed by seed treatment with *B. subtilis* followed by application of *Fusarium pallidoroseum* and *S. marcescens*.

The results of the study helps in development of appropriate microbial consortia for cowpea consisting of PGPR, enhancing growth, yield and pest tolerance and entomopathogens causing mortality among major sucking pests.

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Management of major sucking pests in cowpea *Vigna unguiculata* (L.) Walp. with entomopathogens and plant defense inducing rhizobacteria

KAVITHA, S. J.

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DEPARTMENT OF AGRICULURAL ENTOMOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM- 695 522

ABSTRACT

A study was conducted at the Department of Entomology, College of Agriculture, Vellayani during 2007-2009 to major sucking pests in cowpea *Vigna unguiculata* (L.) Walp. with entomopathogens and plant defense inducing rhizobacteria.

All PGPR screened *viz*., *Pseudomonas putida*, *Pseudomonas* sp., *Bacillus subtilis, Bacillus pumilus* and *Serratia marcescens* significantly enhanced seedling vigor in cowpea. Seed treatment with PGPR gave superior results over soil drenching and foliar application. Population build up of *A. craccivora* was slow in PGPR treated plants compared to control. *B. subtilis*, *B. pumilus* and *S. marcescens* were identified as potential PGPR suited for growth promotion and aphid suppression in screening experiment in glass house.

Fusarium pallidoroseum and *S. marcescens* proved very effective entomopathogen against *A. craccivora* and *R. pedestris* respectively showing consistently higher mortality with increase in exposure time. *B. bassiana* and *M. anisopliae* were found moderately effective.

In dual culture plate assay, all PGPR were compatible with each other, Among the entomopathogens, *M. anisopliae* inhibited growth of *F. pallidoroseum* strongly. In combination of PGPR and entomopathogens, *B. subtilis*, *Pseudomonas* sp. and *P. putida* strongly inhibited the growth of M. anisopliae.

In pot culture studies, *B. subtilis* was the best PGPR treatment in enhancing the biometric characters and yield of cowpea. *F. pallidoroseum* gave maximum per cent mortality of *A. craccivora*. *B. subtilis* applied in combination with *F. pallidoroseum* was effective in suppressing aphids and increasing the yield. Dual application of *B. subtilis* and *S. marcescens* against *R. pedestris* resulted in

significantly higher yield. Combined infestation of *A. craccivora* and *R. pedestris* could be managed by seed treatment with *B. subtilis* followed by application of *F. pallidoroseum* and *S. marcescens*.