Management of Epilachna Beetle, *Henosepilachna vigintioctopunctata* (Fab.) with Phylloplane and Pathogenic Microorganisms

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by

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DECLARATION

I, hereby declare that this thesis entitled **"Management of epilachna beetle, *Henosepilachna vigintioctopunctata* (Fab.) with phylloplane and pathogenic microorganisms"** is a bonafide record of research work done by me during the course of research and 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.

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CERTIFICATE

Certified that this thesis entitled **"Management of epilachna beetle, *Henosepilachna vigintioctopunctata* (Fab.) with phylloplane and pathogenic microorganisms"** is a record of bonafide research work done independently by Ms. Aswathy Viswanathan (2013-11-155) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Aswathy Viswanathan

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Percentage
@	At the rate of
^{0C}	DegreebCelsius
CD	Critical difference
cfu	Colony forming units
et al.	And other co workers
h	Hours
sec	Seconds
min	Minutes
ha	Hectares
T ha-1	Tons per hectare
q ha-1	Quintal per hectare
1	Litre
ml	Milliliter
µ1	Microliter
cm	centimeter
Kg	Kilogram
^{g}	Gram
nm	Nano meter
M	Molar
mM	Milli molar
rpm	Rotation per minute

Sp. Or spp.	Species (Singular and Plural)
SC	Suspension concentrate
JA	Jasmonic acid
POP	Poly phenol oxidase
OD	Optical density

Introduction

INTRODUCTION

Brinjal, *Solanum melongena* L. belongs to the family solanaceae and is one of the common and well-accepted vegetables grown in the tropics and sub tropics (Sarker *et al.*, 2006). India is the second leading producer of brinjal in the world contributing one quarter of the world production and almost 9 per cent of the total vegetable production of the country. As a crop native to tropics it is well adapted to high rainfall and high temperature conditions of hot humid tropics (Hanson *et al.*, 2006). As this environment is congenial for the proliferation of pests, brinjal is heavily infested by a number of insect and non insect pests like epilachna beetle, fruit and shoot borer, whitefly, aphid, leaf hopper, leaf roller, mealy bug, red spider mite etc. of which epilachna beetle is the most destructive one.

The 28 spotted epilachna beetle, *Henosepilachna vigintioctopunctata* (Fab.) (Coleoptera: Coccinellidae) is a notorious polyphagous leaf eating pest of solanaceous and cucurbitaceous vegetable crops causing considerable damage to the host (Rahaman *et al*., 2008). The beetle and its larvae feed on the epidermal tissues of leaves, flowers and fruits of brinjal (Sharma and Saxena, 2012). The grubs and adults with chewing mouth parts scrape the chlorophyll from the epidermal layers of the leaves resulting in typical ladder-like windows. In severe infestations, several windows coalesce together leading to skeletonization. Extensive feeding by the pest results in the reduction in total photosynthetic area causing decline in the fruit yield up to 60 per cent (Mall *et al.*, 1992).

To mitigate the heavy crop loss, farmers often resort to use chemical pesticides in large quantity, under the adage “if little is good, a lot more will be better” (Aktar *et al.,* 2009). Inspite of dumping large volumes of insecticides, the crop loss increases due to various reasons like development of insecticide resistance, pest resurgence and replacement of target pests with new ones. The over use of

pesticides has also lead to the development of resistance in epilachna beetle (Jeyasankar *et al*., 2014) necessitating to investigate on alternate control strategies.

A better alternative to chemical pesticides, with maximum insect pest control efficiency and minimum side effects to the environment and human health is the need of the hour. Microbial control of pests and diseases affecting cultivated plants has gained much attention in the past two decades as a way of reducing the use of chemical pesticides. Many pathogenic microorganisms are reported to cause diseases in adults and grubs of epilachna beetle (Rajendran and Gopalan, 1999; Markandeya *et al.*, 2001).

In addition many plant-associated beneficial microorganisms that thrive on the nutrients obtained from plants without causing any side effects in the host are also reported to be having pest control potential. These microorganisms are called by different names depending on the sites they inhabit. Some of them thrive at the root surface or in the surrounding soil and are known as rhizosphere microorganisms. Other that can live endophytically, invading the inner parts of plants are called endophytic microorganisms and those dwelling on the surface of the host plant epiphytically are referred as phylloplane microorganisms. Chitinolytic phylloplane bacteria are reported to reduce feeding and cause mortality of foliage feeding insects (Otsu *et al.*, 2004).

Being an exposed leaf surface feeder epilachna is susceptible to both entomopathogenic and phylloplane microorganisms. In this context the present work entitled "Management of epilachna beetle, *Henosepilachna vigintioctopunctata* (Fab.) with phylloplane and pathogenic microorganisms was undertaken.

Objectives of the Study

- Monitoring of epilachna beetle and its natural enemies
- Isolation of disease causing microorganisms from epilachna beetle

- Isolation of phylloplane microorganisms from brinjal
- Screening of pathogenicity of the isolated microorganisms to epilachna beetle
- Characterization of isolated microorganisms
- Testing the efficacy of isolated microorganisms against epilachna grubs in the laboratory
- Pot culture evaluation of efficacy of the isolated microorganisms against epilachna grubs

Review of Literature

2. REVIEW OF LITERATURE

Vegetables are the fresh and edible part of herbaceous plants. They play a vital role in maintaining human health and are unanimously accepted as healthy food. These protective food rich in valuable nutritional factors like vitamins, minerals and fibre are essential in preventing most of the lifestyle diseases including cardiovascular diseases, obesity and even cancer (Southon, 2000; Wargovich, 2000; Herrera *et al.*, 2009). They provide phytochemicals that function as antioxidants, phytoestrogens and anti-inflammatory agents (Slavin and Lloyd, 2012). The fresh taste and corresponding health benefits make vegetables the most favoured food item. Brinjal is one such vegetable that is relished by people around the world especially Indians.

Brinjal is heavily infested by a number of insect pests among which epilachna beetle forms the somber one next to brinjal shoot and fruit borer *Leucinodes orbonalis* (Guen.). Other insects like *Antoba olevaceae*, whitefly, *Bemisia tabaci* (Genn.), leafhopper, *Amarasca devastans* (Distant), aphid, *Aphis gossypii* (Glover.), mealy bug, *Centroccocus insolitus* (Guen.), lace wing bug, *Urentius hystricellus* (Richt.) etc. also attack brinjal.

2.1 EPILACHNA BEETLE (*Henosepilachna vigintioctopunctata*) IN BRINJAL

The twenty eight spotted leaf beetle or epilachna beetle, *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae) is a notorious polyphagous pest extensively found all over India and other countries. The pest is seen infesting brinjal and other economically important crops belonging to solanaceous and cucurbitaceous groups. Lately the pest is also seen attacking leguminous crops especially cowpea (Anam *et al*., 2006; Rahaman *et al*., 2008, Halder and Srinivasan, 2011) causing considerable economic damage.

They attack many valuable medicinal plants including ashwagandha, *Withania somnifera* (Solanacae) causing dire reduction in foliage, seed as well as root production (Kumar *et al.*, 2009).

The insect is capable of causing about 35 to 75 per cent leaf injury in the plants that they colonise (Srivastava and Katiyar, 1972). Both adult and larval stages feed on the epidermal tissues of leaves as well as flowers and fruits by scrapping the chlorophyll content causing extensive damage to the crop right from the nursery stage to maturity (Imura and Ninomiya, 1978; Reddy, 1997; Gosh and Senapati, 2001 and Varma and Anandhi, 2008). The attack of epilachna beetle thus significantly hinders the growth and development of the crop accompanied by striking yield reduction (Maurice *et al.*, 2013).

Rajagopal and Trivedi, (1989) reported that extensive feeding caused by these insects can result in a damage of up to 80 per cent. A reduction in the fruit yield of brinjal by up to 60 per cent is reported by Mall *et al*. (1992). In spite of being a serious pest, only limited measures are under for its control which purely depends on conventional methods involving the use of synthetic insecticides. The frequent and indiscriminate application of chemical pesticides in vegetable fields has resulted in widespread development of resistance and presence of toxic residues in food, besides causing environmental and health hazards (Subramanyam and Hagstrum, 1995; Kranthi *et al*., 2002). The case with epilachna beetle is no different. This serious pest of various economically important vegetable crops has developed resistance in almost all commercially available chemical pesticides (Jeyasankar *et al*., 2014). This necessitates the adoption of alternate pest management strategies.

2.1.1 Effect of Weather Parameters on the Population Build up of Epilachna Beetle

Reports reveal that the incidence of *H. vigintioctopunctata* was high during temperature range of 24 - 31 °C along with a relative humidity of 58 -75% in the field (Ramzan *et al.*, 1990). Raghuraman and Veeravel (1999), reported that the population of epilachna beetle in brinjal was highest in February (24.2 insects per plant) and March (27.4 insects per plant), whereas Ghosh and Senapati (2001) noted highest population during mid September. Reports of Muthukumar and Kalyansundaram (2003) indicated that the peak population density of epilachna beetle was during March – April and declined thereafter.

Reports furnished by Venkatesha (2006) indicated that the adult and grub population were high in mid August and later declined by the end of August and reached zero level in October during the year 2004 – 2005. They also showed that during peak period of infestation maximum temperature, minimum temperature and relative humidity were 27.5±0.88 °C, 19.58±0.49 °C and 75.55±13.37% respectively.

Studies on the incidence of *H. vigintioctopunctata* on brinjal in Madhya Pradesh during 2004-05 and 2005-06 showed that the incidence of the beetle started by first week of November with an average population of 2.85 beetles per plant and reached its peak by the third week of February in 2004-2005 whereas it attained its peak by the third week of November in 2005-2006. The beetle incidence showed negative correlation with maximum and minimum temperature and positive correlation with all the other weather parameters. The study also revealed a highest activity of parasitoids during February and the parasitoid population were positively correlated to maximum relative humidity, rain, wind velocity and sunshine hours and negatively correlated to minimum and maximum temperature (Varma and Anandhi, 2008).

Reports of Haseeb *et al.* (2009) showed that the number of beetle population in brinjal gradually decreased from February to April reaching the minimum during the third week of March to first week of April while the highest population was observed during third week of February. The population epilachna beetle showed positive correlation with relative humidity and negative correlation with maximum and minimum temperature.

Omprakash and Raju (2014b), reported that epilachna beetle population during the year 2011 reached its peak by mid October attaining a mean population of 6.98 insects per plant and thereafter showed a declining trend reaching zero level by December. The results indicted a positive significant and non significant correlation with maximum and minimum temperature respectively while the association of pest population showed a negative correlation with relative humidity and rainfall.

2.2 MICROBIAL CONTROL OF EPILACHNA BEETLE

The use of microorganisms that specifically kills insect pests has gained popularity in the present era. These organisms collectively known as entomopathogenic microorganisms include entomopathogenic fungi, nematodes, bacteria and viruses. Among the various categories, entomopathogenic fungus is exploited commercially for the control of arthropod pests followed by bacteria. Microbial control agents offer alternatives to chemical pest control as they are more selective. Being an exposed leaf surface feeder, epilachna beetle is susceptible to both fungal and bacterial infections. Many pathogenic microorganisms are reported to cause diseases in adults and grubs of epilachna beetle (Rajendran and Gopalan, 1999; Markandeya *et al.*, 2001).

2.2.1 Fungal entomopathogens

Group of fungi that kill the insect by attacking and infecting the host insect is termed as entomopathogenic fungi (Singkaravanit *et al.,* 2010). Several species of

fungi are potent bio control agents of arthropods as well as other phytopathogenic microorganisms. Generally the main barrier that restricts the control of insect pest is the hard integument that they possess.

The insect cuticle which is composed of chitin and enzymes associated with lipids and phenolic compounds prevent the entry of invading microorganisms. The combined action of mechanical pressure of penetrating hyphae and hydrolytic enzymes such as chitinase, protease and lipases enable the fungal entomopathogens to easily penetrate the insect cuticle, making them the most appropriate microbial control agent for all insects especially sucking pests (Ignoffo, 1978). They are important regulating factors of insect population and many are used as biocontrol agents of insect pests (Lancey *et al.,* 2001).

Roughly 700 species of fungi under 90 genera are known to be entomopathogens of most of the insect taxa including Lepidoptera, Coleoptera, Diptera, Hemiptera, Orthoptera and Isoptera (Charnley,1989). Several fugal entomopathogens such as *Beauveria, Metarhizium, Lecanicillium, Paecilomyces, Hirsutella, Nomuraea, Aspergillus, Coelomomyces* etc. are being investigated for their pest control potential.

2.2.1.1 Beauveria bassiana (Balsamo) Vuillemin

Klochko (1969) reported the effectiveness of *B. bassiana* and *B. tenella* in managing epilachna beetle, *Epilachna vigintioctopunctata.* According to him these fungi isolated from infected insects could cause a mortality of 98 per cent of the grubs in 13 days.

Padmaja and Kaur (1998) reported that virulent isolates of *B. bassiana* was effective against the second instar larvae of *E. vigintioctopunctata* with the LD_{50} values ranging from 1.33 to 4.8 in different isolates. They also proved that larval stages of *E. vigintioctopunctata* were more susceptible than pre-pupal and adult

stages. Rajendran and Gopalan (1999), reported that direct spraying of white muscardine fungus, *B. bassiana* could kill 58.1% first instar larvae and 35.2% pre-pupal stage larvae while the adults were not susceptible to the fungus with the maximum mortality being 10.3% in the case of newly emerged adults. The fungus caused 54.6% hatchability of one-day old eggs of the spotted beetle.

Jiji *et al.* (2008) observed a mean mortality of 63.33 per cent on epilachna beetle grubs due to *B. bassiana* infection. Studies conducted by Devi *et al.* (2008) revealed that various strains of *B. bassiana* were infective to epilachna beetle (*H. vigintioctopunctata*) causing a mortality that ranges from 70 to 100 per cent depending upon the stain.

Vishwakarma *et al.* (2011) reported significant reduction in the population of epilachna beetle (74.91%) when treated with *B. bassiana* @ 3.0 g l^{-1} of water. He also noted a crop yield of 315.36 q ha^{-1} in the treated plants.

According to Ghosh and Chakraborty (2012), the microbial pesticide *B. bassiana* provided only 39.56% suppression of the epilachna beetle population when compared to treatment with chemical insecticide (cartap hydrochloride 50 SP).

Joseph, (2014) reported that the adults and grubs of *H. vigintioctopunctata* recorded a mean per cent mortality of 35.52 and 64.48 respectively after 7 days of treatment with *B. bassiana* @ 10^8 spores ml^{-1}.

2.2.1.2 Metarhizium anisopliae (Metschnikoff) Sorokin

M. anisopliae is a mitosporic haploid fungus which has a wider host range of important agricultural pests and therefore, it holds great potential for use as biological control agent (Butt *et al.,* 2001; Nahar *et al.,* 2004).

Rajendran (2002), reported *M. anisopliae* at both doses of 10^8 and 10^{10} conidia ml^{-1} caused high mortality of first and second instar grubs (100%) seven days following application, while third and fourth instar grubs recorded mortality exceeding 70% during the same time interval at the same doses. It was also reported by him that the green muscardine fungus was not as potent against adults causing only 17.4% mortality in newly emerged adults.

Aqueous formulation of *M. anisopliae* @ 5×10^{12} conidia was effective against the adult *H. vigintioctopunctata* (Swaminathan *et al*., 2010). Reports of Vishwakarma *et al.* (2011) showed that *M. anisopliae* applied at 3 g l^{-1} water caused a mortality of 70.71 per cent in grubs of *H. vigintioctopunctata.*

H. vigintioctopunctata adults treated with *M. anisopliae* @ 10^8 spores ml^{-1} showed a mortality of 17.66 per cent whereas the grubs exhibited 42.21 per cent mortality with the same treatment 7 days after treatment (Joseph, 2014).

2.2.1.3 Lecanicillium lecanii Zare and Gams

The larvae and pupae of *H. vigintioctopunctata* was found susceptible to fungal suspension of *Verticillium lecanii* at a concentration of 1.6×10^7 or 4.8×10^7 spores ml^{-1} (Santharam *et al.*, 1978). Ghatak and Mondal (2008), recorded a reduction of epilachna beetle population up to 60.99 per cent when treated with *V. lecanii* at a concentration of 2 g ml^{-1}.

2.2.1.4 Fusarium sp.

Larvae of *H. vigintioctopunctata* were susceptible to *Fusarium moniliforme* var. *subglutinans* (Wollenw and Reinking) and showed 100% mortality after 5 days in the third and fourth instar (Beevi and Jacob. 1982). An infection of 20 per cent was observed in the egg mass of *H. vigintioctopunctata* treated with *Fusarium solani* (Mart.) Sacc (Bhagat and Munshi, 1999).

2.2.1.5 Alternaria alternate (Fr.) Keissl.

Sharma *et al.* (2012) observed that grubs of *H. vigintioctopunctata* fed on leaves infested with leaf spot pathogen, *A. alternata* showed high larval and pupal mortality associated with delay in the development of larva into pupa and pupa into adult. Furthermore, an overall reduction in adult emergence was observed in such insects.

2.2.2 Bacterial entomopathogens

By the discovery of potential entomopathogenic bacteria, especially associated to species belonging to the genus Bacillus the paradigm of biocontrol changed upside down (Glare and O'Callaghan, 2000). The majority of bacterial pathogens of insects and related taxa occur in the family Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae, and Micrococcaceae

2.2.2.1 Bacillus thuringiensis Berliner

Markandeya *et al.* (2001) reported that feeding of leaf dipped in 1% *B. thuringiensis* formulation (Biotox) rendered leaf area protection of 56.39% from grubs and 80.62% from adult insects of *H. vigintioctopunctata.* The treatment also caused 39.03% mortality of grubs and 5.22% mortality of adults.

B. thuringiensis Ba9808 showed very high toxicity to the second instar larvae and adults of *H. vigintioctopunctata* and caused a mortality of 55.0% and 26.7%, respectively. Ba9808 caused feeding inhibition and reduction of pupation percentage to fourth instar larvae. When the adults were fed with Ba9808, their egg production significantly decreased (Hong *et al*., 2002).

Ping *et al.* (2008) observed that *B. thuringiensis* strain WZ-9 was found to be highly toxic to the second instar larvae of *H. vigintioctomaculata* and caused a mortality of 100% at 72 h with LC_{50} value of 2.95×10^{7} cell ml^{-1}. Reports show that Bt WZ-9 strain, containing a single Cry7Ab3 toxin, had effective insecticidal activity against larvae of *H. vigintioctopunctata* (Song *et al.,* 2012; Song *et al.,* 2013).

2.3 OTHER BACTERIAL ENTOMOPATHOGENS IN PEST MANAGEMENT

2.3.1 *Pseudomonas* sp.

Pseudomonas fluorescens (Flügge) strains were reported to be capable of killing or causing morphological defects to widely used laboratory insect (Pimenta *et al*., 2003).

Sezen *et al.* (2004) reported that *P. fluorescens* caused a mortality of 70% and 56% in the larvae and adults of *Agelastica ani* L. respectively within 7 days after treatment while *Pseudomonas chlororaphis* (Guignard and Sauvageau) brought about 37 and 30% mortality of larvae and adults respectively.

Sezen *et al.* (2007) observed that *Pseudomonas* sp. isolated from *Melolontha melolontha* larvae were effective in causing 50% mortality of the insect when treated at a concentration of 1.8×10^{9} bacterial cells ml^{-1}.

2.3.2 *Serratia marcescens* Bizio

Trought *et al.* (1982), documented that amber disease in *C. zealandica* (Coleoptera: Scarabaeidae) was caused by the entomopathogenic strains of *Serratia* sp. Reports of Lauzon *et al.* (2003) reveal that non-pigmenting strains of *S. marcescens* was pathogenic to apple maggot flies, *Rhagoletis pomonella* causing rapid mortality within 24 hours after treatment. According to Patel *et al.* (2011), the red colour pigment prodigiosin produced by *S. marcescens* NMCC46 had potent mosquito larvicidal activity causing 50 per cent mortality within the first 24 h.

Chattopathayay *et al.* (2012), noticed considerable reduction in feed consumption by lepidopteran insects (*Helicoverpa armigera*, Hübner and *Spodoptera litura*, Fab.) fed on diet supplemented with *Serratia* sp. and observed maximum mortality of 94.3 per cent and 92.7 per cent in *H. armigera* and *S. litura* respectively at 72 h after treatment.

2.3.3 *Bacillus megaterium* de Bary

Bacillus megaterium is reported to be pathogenic to eggs of European corn borer *Ostrinia nubilalis* (Hübner) (Lynch *et al.*, 1976) exposing the role of *B. megaterium* as a good biocontrol agent.

2.4 PLANT ASSOCIATED BACTERIA

Bacteria that live on the nutrients obtained from plants are referred to as plant-associated bacteria. They may be beneficial or harmful depending on the effects they exert on the plant. Many bacteria form beneficial associations with plants in their natural environments. These bacteria are called by different names depending on the sites they inhabit. Some of them thrive at the root surface or in the surrounding soil, taking advantage of carbon and energy-sources in root exudates. Such bacteria are known as rhizosphere bacteria. Other that can live endophytically, invading the inner parts of plant hosts without causing symptoms of disease are called endophytic bacteria and those dwelling on the surface of the host plant epiphytically are referred as phylloplane bacteria.

2.4.1 Rhizosphere bacteria

Bacteria that thrive in the rhizosphere are known as rhizosphere bacteria. Almost 2 – 5 per cent of bacteria associated with the rhizosphere exert a beneficial effect on plant growth and these bacteria are termed as plant growth promoting rhizobacteria (PGPR). PGPR can be defined as “beneficial free-living bacteria,

which improve plant health and increase crop yield (Kloepper and Schroth, 1981), hence they are also known as yield increasing bacteria. PGPR improve plant growth directly by facilitating the uptake of nutrients and indirectly by protecting the plant from harmful pathogens and insect pests. These organisms induce systemic resistance in plant and produce secondary metabolites which help in warding off disease causing pathogens and insect pests, thus acting as a good biocontrol agent. *Bacillus* and *Pseudomonas spp.* are predominant PGPRs that are exploited commercially.

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

Bong and Sikorowski (1991) reported that *Pseudomonas maltophila affects* the growth of larval stage of *Helicoverpa zea,* the corn earworm, leading to more than 60% reduction in adult emergence. An experiment conducted by Zehnder *et al.* (1996) showed that the feeding of diabroticine cucumber beetles on PGPR treated cucumber plants was comparatively low. This reduction in feeding was due to the reduction in cucurbitacin, which is an essential phytostimulant for the beetles.

Stout *et al.* (2002) reported a delay in population growth and population size of cotton aphids, *Aphis gossypii,* on PGPR (*Bacillus* spp.) treated cucumber. Hanafi *et al.* (2007) found out that *Bamisia tabaci* proliferate less on tomato plants that have been inoculated with *Bacillus subtilis*. Reports show that 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)

Plant growth promoting fluorescent *Pseudomonas* strains Pf1, TDK 1 and PY 15 significantly reduced leaf folder damage in rice plants. Natural enemy population in the plots treated with PGPR was also found to be greater than the chemical and

untreated control. This was because of the higher activity of PPO and lipoxygenase in plants treated with PGPR mixture (Saravankumar *et al.*, 2008).

2.4.2 Endophytic bacteria

Bacterial endophytes are ubiquitous colonizers of the inner plant tissues where they do not normally cause any substantial morphological changes and disease symptoms. They can colonize any organ of the host plant (roots, shoots, leaves, seeds and ovules) (Sturz *et al.*, 2000)

Certain endophytes like *Bacillus pumilus* are capable of influencing the growth and development of insect pests and thus act as biocontrol agents. *Bacillus subtilis* strains EPCO 102, EPCO 16 and *Pseudomonas fluorescens* Pf1 reduced the aphid infestation in cotton. *P. fluorescens* also control colorado potato beetle (*Leptinotarsa decemlineata*) (Costanzo *et al.*, 1998).

Melvin and Muthukumaran (2008) observed under the pot culture condition that tomato leaves treated with combined foliar application of jasmonic acid (JA) and *Pseudomonas aeruginosa* caused maximum larval mortality of *S. litura.* Pupation rate, adult emergence and adult longevity were reduced to the minimum in case of leaves treated with jasmonic acid and *P. aeruginosa.* JA treatment strongly affected the activity of proteinase inhibitor, moderately affected the polyphenol oxidase (PPO) activity and to a lesser extend the lipoxygenase activity.

Harish *et al.* (2009) observed a significant increase in the enzymatic activity of PR proteins and defense enzymes, as compared to control, in the plants inoculated with endophytic bacteria and challenged with viruliferous aphids. They concluded that there exist a differential accumulation of PR proteins and defense-related enzymes, when there is tritropic interaction between endophytic bacteria, virus, and insect.

2.4.3 Phylloplane bacteria

Phylloplane is a natural habitat on leaf surface which supports heterogeneous population comprising both pathogen and non-pathogenic microorganisms. Phylloplane bacteria stably colonize the leaf surface occupying sites like trichomes, stomata and epidermal cell wall junctions are potential carriers for conveying molecules to smother insect defoliators (Andrews, 1992). These bacteria are mostly commensalistic but some are capable of producing extracellular chitinase which will help in degrading the peritrophic membrane of chewing insects. The extracellular chitinase producing phylloplane bacteria can be used in biological control of insect pests.

Occurrence of *Serratia entomophila*, highly potent entomopathogenic bacteria are repoted from phyllosphere region of tomato and banana (Akutsu *et al*., 1993; Riveros *et al*., 2002). The bacteria was reported to cause 90 per cent mortality in *H. armigera* (Chattopadhyay and Sen, 2013).

Otsu, *et al.* (2003) reported that the chitinase secreted by *Alcaligenes paradoxus* strain KPM-012A isolated from tomato leaves and vitally entrapped in sodium alginate gel beads was potent enough to degrade the chitinous peritrophic membrane of phytophagous ladybird beetles *Epilachna vigintioctopunctata* and thereby caused the death of insect.

An entomopathogenic bacterium was isolated from tomato leaves and used as a microbial agent to control larvae of phytophagous ladybird beetles *E. vigintioctopunctata* by Otsu *et al.* (2004). The isolate identified as *P. fluorescens* KPM-018P produced extracellular chitinase and caused prompt death of the insects on sprayed leaves. It was noted that the enzymatic activity of chitinase and protease in infected larvae increased considerably in parallel with rapid multiplication of

KPM-018. Thus they showed that, this method is effective for decreasing the population of larvae and adult insect pests in the subsequent generation.

2.5 CHITINASE ACTIVITY OF ENTOMOPATHOGENIC BACTERIA AND PHYLLOPLANE BACTERIA

Since insect exoskeleton contains chitin as a major structural component, chitinolytic bacteria stand out as potential agents for biological control of the insect pests. The most notable chitinase producers among the Gram negative bacteria are *Aeromonas, Chromobacterium, Photobacterium, Pseudomonas, Serratia, Vibrio*, and the gliding bacteria *Chitinophaga, Cytophaga,* and *Lasobacter.* Among the Gram positive bacteria, chitinase producers are widespread among the actinomycetes, e.g., the species of *Arthrobacter, Nocardia*, and *Streptomyces*, and in the spore-forming genera Bacillus and Clostridium (Thamthiankul *et al*., 2001).

Okay *et al*. (2013), reported that extracellular enzyme chitinase obtained from novel isolate *Serratia marcescens* MO-1, isolated from *Poecilimon tauricola* has immense potential in biocontrol. Aggarwal *et al.* (2015) isolated chitinase secreting strain of *S. marcescens* SEN from *Pieris brassicae* L. and observed that endochitinase activity was predominant in these bacteria. The study also showed the insecticidal activity of *S. marcescens* SEN against *S. litura.*

Otsu *et al.* (2003) observed that *Alcaligenes paradoxus* KPM-012A isolated from tomato leaves was capable of secreting chitinase potent enough to degrade the chitinous peritrophic membrane of *E. vigintioctopunctata. P. fluorescens* KMP-018P*,* a stable phylloplane colonizer produced extracellular chitinase which when ingested caused rapid death of *H. vigintioctopunctata* grubs to eventually suppress their population (Otsu *et al.*, 2004).

Materials and Methods

3. MATERIALS AND METHODS

The experiment on the "Management of epilachna beetle, *Henosepilachna vigintioctopunctata* (Fab.) with phylloplane and pathogenic microorganisms" was carried out at Department of Entomology, College of Agriculture, Vellayani during 2013 – 2015.

The detailed materials and methods followed during the course of work are mentioned below.

3.1 SEASONAL INCIDENCE OF EPILACHNA BEETLE IN BRINJAL AND MONITORING OF DISEASE OCCURANCE

Population of epilachna beetle, *H. vigintioctopunctata* in brinjal and occurrence of diseases in different crop stages were monitored for an year from July 2014 to June 2015 in the Instructional Farm, College of Agriculture, Vellayani. Three isolated brinjal growing plots of 200 m^2 each were identified. The following observations were recorded at fortnightly intervals from ten randomly selected plants in each plot taking six leaves (two each from upper, middle and lower strata) from each plant.

- Number of egg masses, grubs and adults
- Number of insects exhibiting disease symptoms
- Number of insects parasitized

The weather parameters *viz.* maximum and minimum temperature, relative humidity and rainfall during the experimental period were recorded from the meteorological observatory of Department of Meteorology, College of Agriculture, Vellayani.

3.1.1 Correlation of Epilachna Beetle Population with Weather Parameters

As a measure to evaluate the degree of influence of abiotic factors on population build up of epilachna beetle in brinjal, the population data collected were tabulated and the correlation coefficient of mean number of the insects per plant with weather parameters was worked out.

3.1.2 Correlation of Parasitization of Epilachna with Weather Parameters

The mean number of epilachna grubs and egg masses parasitized were correlated with weather parameters to assess the association of abiotic factors on extent of parasitization.

3.2 ISOLATION AND MAINTENACE OF MICROORGANISMS AND TEST INSECTS

Grubs and adults of epilachna beetle were inspected for disease symptoms in the field. Those insects showing disease symptoms were collected for isolation of disease causing organism.

3.2.1 Diagnosis of Microbial Infection

A preliminary diagnosis was done based on the signs and symptoms present on the cadaver. Insect infected with change in colour and consistency and those from which body fluid seems exuding were diagnosed as bacterial infection . The cadavers with mycelial growth on the surface were suspected to be killed due to fungal infection.

3.2.2 Isolation and Maintenance of Disease Causing Microorganisms

The suspected specimens were brought to laboratory and disease causing agents were isolated from the cadavers, as detailed below.

3.2.2.1 Bacterial Pathogens

The insect cadavers were surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) solution for one minute and washed in three changes of sterile distilled water. The cadavers were then triturated in sterile distilled water and the suspension was used as inoculum. The organism was brought to pure culture in nutrient agar (NA) plates by streak plate method after repeated streak purification on NA medium. Pure cultures thus obtained were transferred to NA slants and stored at 4°C in a refrigerator. The cultures were also preserved in sterile glycerol by mixing 750 µl bacterial suspension with 250 µl sterile glycerol and stored at – 80°C in a deep freezer.

3.2.2.2 Fungal Pathogens

The epilachna grubs assumed to have been killed of mycosis were surface sterilized with 0.1 per cent $HgCl_2$ for one minute and washed in sterile distilled water thrice. The surface sterilized cadavers were placed individually in potato dextrose agar (PDA) slants and incubated at room temperature. Hyphal tip culture was repeatedly done to acquire pure culture of the fungus. The pure fungal cultures thus obtained were stored in PDA slants at 4°C in a refrigerator.

3.2.2.3 Isolation and Maintenance of Microorganisms from Phylloplane of Brinjal

The isolation of microorganisms from phylloplane was carried out by adopting the technique devised by Otsu *et al.* (2003) with slight modifications. Fully developed leaves were collected randomly from two month old brinjal plants. The upper surface of the detached leaves were pressed and left for two minutes on M9 minimal agar medium (12.8 g $Na_2HPO_4.7H_2O$, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 4 g glucose and 20 g agar in 1000 ml of water) to isolate bacteria and Rose Bengal Agar (RBA) medium for fungi respectively. The bacterial and fungal colonies growing on the media were brought to pure culture by streak purification and single

spore isolation in NA and PDA plates respectively. Purified cultures were transferred to NA or PDA slants and stored under refrigerated condition. The bacterial cultures were also preserved as glycerol stock as described in 3.2.2.1.

3.2.2.4 Commercial Entomopathogenic Microorganisms Included as Treatments and their Maintenance

Along with the entomopathogenic and phylloplane microorganisms isolated during the course of the present study, other commercial entomopathogens conventionally employed in pest control were also included as treatments.

The initial cultures of emtomopathogenic fungi were obtained from the Biocontrol Laboratory of Department of Agricultural Entomology, College of Agriculture, Vellayani, and subcultured on PDA medium at 25 °C.

Virulence of fungi was restored by passing them periodically through healthy epilachna grubs and re-isolating them in fresh culture media. This was achieved by spraying the test insect with spore suspension of fungi. The spore suspension was prepared by pouring 5 ml sterile distilled water on to heavily sporulated two week old culture plates. The treated insect showing symptoms of mycosis were surface sterilized with 0.1per cent $HgCl_2$ followed by three washes in sterile distilled water, transferred to fresh media and incubated at room temperature. When visible mycelial growth appeared, it was subcultured and maintained in PDA plates and slants for further studies.

The commercial formulations of bacterial entomopathogens were purchased from agricultural input stores and used at the recommended dose.

3.2.3 Maintenance of Test Insects

Adults of *H. vigintioctopunctata* were collected from the field. The collected insects were reared on brinjal leaves in polypet jars with their mouth covered using

muslin cloth. Ten adults were maintained in each jar. The egg masses obtained were transferred individually to fresh jars along with food for the emerging grubs. The healthy disease free insects thus obtained were further maintained in the laboratory and used as stock culture. The different stages obtained from the cohorts of same age were used in the experiment.

3.3 SCREENING OF ISOLATED ENTOMOPATHOGENS AND PHYLLOPLANE MICROORGANISMS FOR PATHOGENICITY TO EPILACHNA BEETLE

A preliminary screening of the isolated entomopathogens and phylloplane microorganism was done to confirm pathogenicity to epilachna grubs.

3.3.1 Screening of Bacterial Isolates

The isolated bacterial pathogens were fed to the second instar grubs by adopting leaf disc feeding method. Discs of three centimeter diameter were cut out from thoroughly washed brinjal leaves using steel cork borer. The leaf discs were dipped in bacterial suspension containing required colony forming units (10^8 cfu ml^{-1}) for five minutes. The leaf discs were dried under room temperature and each larva was fed individually on the inoculated disc contained in glass vials. Larvae which had completely ingested the treated leaf disc were transferred into troughs in groups of ten and supplied with fresh leaves and observed for development of disease.

3.3.2 Screening of Fungal Isolates

The spore suspension of fungi isolated from diseased insect and phylloplane of brinjal was sprayed over the test insects. The larvae were transferred into troughs in groups of ten and supplied with fresh leaves and observed for development of disease.

3.3.3 Proving Koch's Postulate

The dead insects obtained from the experiments 3.3.1 and 3.3.2 were surface sterilized with 0.1% $HgCl_2$ for one minute and washed in three changes of sterile distilled water and placed individually in sterile Petri dishes containing NA or PDA for re-isolation of bacteria and fungi respectively. The microorganisms which could be re-isolated from the host were selected for further studies.

3.4 CHARACTERIZATION OF SELECTED MICROORGANISMS

3.4.1 Colony Morphology

Selected microorganisms were streak purified on NA plates to get single isolated colonies and colony morphology of selected bacteria were studied.

3.4.2 Gram staining

The Gram Staining was carried out in four basic steps that include applying a primary stain (crystal violet) to a heat-fixed bacterial smear, followed by the addition of a mordant (Gram's Iodine), rapid decolorization with alcohol or acetone and lastly, counterstaining with safranin. All the stains except safranin were retained over the smear for one minute and washed off before the application of next stain. The later was kept for 30 seconds and excess stain was removed by washing. The stained smear was observed under oil immersion (100X) objective.

3.4.3 Chitinase Activity

The chitinase activities of selected bacterial isolates were assessed by both visual and quantitative methods. This was achieved by growing the microorganisms in minimal media containing colloidal chitin as the sole carbon source.

3.4.3.1 Preparation of Colloidal Chitin from Crude Chitin Powder

Preparation of colloidal chitin was done based on the method adopted by Hsu and Lockwood (1975) with slight modification. Forty grams of ground crude chitin purchased from MATSYAFED (Neendakara, Kerala) was dissolved in 200 ml of cold concentrated Hydrochloric acid (HCl) by intermittently stirring for 30 to 50 minutes. After filtration through glass wool the resulting mixture was added drop wise into 2000 ml of ice cold water (5 to 10 °C) with constant stirring using a magnetic stirrer. The white gelatinous precipitate thus obtained was separated by filtering through Whatman No. 1 filter paper. The precipitate was washed by re-suspending it in one liter of tap water followed by filtration. The process was repeated 5 times until the pH of the suspension was near neutral.

3.4.3.2 Detection of Chitinase Activity

The selected bacteria were screened for the production of chitinase, adopting plate assay method devised by Sowmya *et al.* (2012). Bacterial strains were spot inoculated on minimal soft agar medium (1.5% agar) containing 0.1% colloidal chitin as the sole carbon source. Congo red solution (0.1%) was poured over the inoculated plates after an incubation period of seven days and observed for clearance zone. Formation of clear zone around the bacterial growth indicated chitinase activity.

3.4.3.3 Quantification of Chitinase Activity

3.4.3.3.1 Preparation of Enzyme Sample for Chitinase Assay

100 ml of liquid medium containing three per cent chitin was taken in 250 ml flask and sterilized by autoclaving. A loopful of bacterial cells from an actively growing culture was transferred into it and incubated for 48 h at 28 °C (Avelizapa *et al.*, 1999; Avelizapa *et al.*, 2001). The growth was centrifuged at 8000 rpm for ten minutes and the supernatant collected was used for further analysis.

3.4.3.3.2 Chitinase Assay with Chitin-Azure

1 ml of enzyme sample and 1 ml of 0.2 M sodium phosphate buffer (pH 7) was mixed with 5 mg chitin-azure and incubated in a water bath at 50 °C for 3h. The samples were given a brief spin at 7000 rpm and absorbance of the samples was read at 560 nm. The amount of enzyme that produced an increase of 0.01 in absorbance was equivalent to 1 unit of chitinase (Ramírez *et al.*, 2004).

3.4.4 Molecular Characterization

Molecular characterization of bacterial isolates were done by 16S rRNA cataloging using universal primers with the help of microbial identification service at the Department of Microbiology, College of Horticulture, Vellanikkara.

3.4.4.1 Genomic DNA Isolation

Genomic DNA was isolated from the tissues using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions.

Loop full bacterial culture was transferred to one ml sterile distilled water taken in a microcentrifuge tube. 180 µl of T1 buffer and 25 µl of proteinase K was added and incubated at 56 °C in a water bath until it was completely lysed. After lysis, 5 µl of RNase A (100 mg / ml) was added and incubated at room temperature for 5 minutes. 200 µl of B3 buffer was added and incubated at 70 °C for ten minutes. 210 µl of 100% ethanol was added and mixed thoroughly by vortexing. The mixture was pipetted into NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11000 × g for one minute. The NucleoSpin® Tissue column was transferred to a new 2 ml tube and washed with 500 µl of BW buffer. Wash step was repeated using 600 µl of b5 buffer. After washing the NucleoSpin® Tissue column was placed in a clean 1.5 ml tube and DNA was eluted out using 50 µl of BE buffer.

3.4.4.2 Agarose Gel Electrophoresis for DNA Quality and Quantity Check

The quality of the DNA isolated was checked using agarose gel electrophoresis. 1 µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH – 8.0) was added to 5 µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µl / ml ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were observed in a UV Transilluminator GeNei®.

3.4.4.3 PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X PCR buffer (100 mM Tris HCl, pH – 8.3, 500 mM KCl), 0.2 mM each dNTP's (dATP, dGTP, dCTP and dTTP), 2.5 mM $MgCl_2$, 1 unit of AmpliTaq Gold DNA polymerase enzyme, 0.1 mg / ml BSA, 4% DMSO, 5 pM of forward and reverse primers and FTA disc as template.

Primers used :

Target	Primer Name	Direction	Sequence (5' → 3')
16S rRNA	16S-RS-F	Forward	CAGGCCTAACACATGCAAGTC
	16S-RS-R	Reverse	GGGCGGWGTGTACAAGGC

The amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied biosystems).

PCR amplification profile :

16S rRNA

95 °C	-	5.00 min	
95 °C	-	30 Sec	35 cycles
60 °C	-	40 Sec	
72 °C	-	60 Sec	
72 °C	-	7.00 min	
4 °C	-	∞	

3.4.4.4 Agarose Gel Electrophoresis of PCR Products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg / ml ethidium bromide. 1 µl of 6X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was performed at 75 V power supply with 0.5 TBE as electrophoresis buffer foe about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was a 2-log DNA ladder. The gels were visualized in a UV Transilluminator Genei®.

The PCR products obtained were send for 16S rRNA sequencing at SciGenom, Kakkanad, Kochi.

3.4.4.5 Sequence Analysis

The nucleotide sequence of 16S rRNA was compared with the sequence available in the database using the BLAST tool offered by National Centre for

Biotechnology Information (NCBI). BLASTn provided by NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was carried for homology search.

3.5 LABORATORY SCREENING OF MICROBIAL CONTROL AGENTS AGAINST EPILACHNA BEETLE

The selected entomopathogenic bacteria isolated from dead insects, phylloplane bacteria and commercial microbial control agents were screened against grubs and adults of *H. vigintioctopunctata* at dose mentioned in Table 1.

3.5.1 Preparation of Microbial Suspensions

The bacterial cell suspension and fungal filtrate having required cfu were prepared by serial dilution and plating method. A loopful of required bacterial culture was added to 1 ml sterile distilled water and vortexed thoroughly. These stocks were serially diluted to get concentrations of 10^{-1}, 10^{-2}, 10^{-3}, up to 10^{-8}. Simultaneously the Optical Density (OD) of the serially diluted suspension were read in a UV – Visible spectrophotometer. From the serially diluted suspension 0.1 ml was poured on to NA plates and spread uniformly using a "L" rod and cfu was determined.

The fungal cultures with required cfu were prepared by serial dilution and plating method as described earlier. The fungal broth cultures were serially diluted in sterile distilled water to get concentrations of 10^{-1}, 10^{-2}, 10^{-3} until a concentration of 10^{-8} was obtained. 0.1 ml of serially diluted suspension was uniformly spread on PDA plates to estimate the cfu.

$$\text{Number of cfu ml}^{-1} = \frac{\text{Number of colonies}}{\text{Volume plated} \times \text{dilution}}$$

Table 1. List of treatments and media used for maintenance of entomopathogenic microorganisms

No.	Treatments	Dose	Source	Category	Media
1	*Metarhizium anisopliae* (Ma4)	10^8 cfu ml^{-1}	NBAII, Bangalore	Fungus	PDA
2	*Beauveria bassiana* (Bb5)	10^8 cfu ml^{-1}	NBAII, Bangalore	Fungus	PDA
3	*Lecanicillium lecanii* (Ll8)	10^8 cfu ml^{-1}	NBAII, Bangalore	Fungus	PDA
4	*Bacillus thuringiensis*	0.25 %	Abtech	Bacteria	
5	*Pseudomonas fluorescens*	2 %	Deepa Farm inputs and private Ltd.	Bacteria	
6	*Microbacterium* sp.	10^8 cfu ml^{-1}	Isolated from epilachna	Bacteria	NA
7	*Serratia marcescens*	10^8 cfu ml^{-1}	Isolated from epilachna	Bacteria	NA
8	*Pseudomonas aeruginosa*	10^8 cfu ml^{-1}	Isolated from epilachna	Bacteria	King's B (KB)
9	*P. fluorescens*	10^8 cfu ml^{-1}	Isolated from brinjal phylloplane	Bacteria	NA
10	*C. flaccumfaciens*	10^8 cfu ml^{-1}	Isolated from brinjal phylloplane	Bacteria	
11	*Bacillus megaterium*	10^8 cfu ml^{-1}	Isolated from brinjal phylloplane	Bacteria	NA
12	Chlorantraniliprole 18.5 SC	0.006 %		Chemical check	

The dilution which developed colonies equivalent to 10^8 was selected for treatment. In case of bacteria, suspension with an OD value corresponding to that producing 10^8 cfu ml^{-1} was selected for further treatment.

3.5.1.1 Treatment of Microbial Agents on Target Insects

Brinjal leaf discs of 3 cm diameter cut with a sterile cork borer were dipped in bacterial suspension (10^8 cfu $ml^{-1)}$ and dried by placing in a Laminar Air Flow chamber. Second instar grubs and adults of *H. vigintioctopunctata* of same age were starved for 12 h and allowed to feed individually on the treated leaf discs kept in sterile glass vials until complete feeding. Ten insects each receiving same treatment were transferred to fresh leaves taken in sterile Petri plates. Three replications each containing ten insects were maintained for each treatment. The insects fed on leaf disc dipped in sterile water served as untreated check.

Five ml spore suspension each of *M. anisopliae*, *B. bassiana* and *L. lecanii* at 10^8 cfu ml^{-1} were sprayed on test insects (second instar grubs and adults) separately using an atomizer. After 20 minutes, treated insects were transferred on to fresh brinjal leaves placed in sterile Petri plates. Three replications each containing ten insects were maintained for each treatments. Untreated check was maintained by spraying test insects with sterile water.

The dead insects were removed at regular intervals and observed for symptoms of infection.

3.5.2 Observations

The treated insects were examined regularly for their mortality. Observations on mortality and leaf area damaged were recorded at one, three, five and seven days after treatment (DAT). Dead insects were transferred to Petri plates containing moist tissue paper and observed for symptom development. Pathogenicity was further

confirmed by Koch's postulates. The per cent mortality was worked out using the formula.

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

The leaf area damaged by the insects was recorded first at 24 h. The leaf was plotted on a graph paper and area was estimated in cm^2. The larvae were then transferred to fresh leaves and the process was repeated at 3, 5 and 7 DAT. The per cent leaf area damaged was worked out using the following formula.

$$\text{Per cent leaf area damaged} = \frac{\text{Total leaf area} - \text{Leaf area left undamaged}}{\text{Total leaf area}} \times 100$$

3.6 POT CULTURE EVALUATION OF MICROORGANISMS

A pot culture experiment was conducted to evaluate the efficacy of selected entomopathogenic and phylloplane microorganisms along with commercial microbial agents against *H. vigintioctopunctata.*

Design : CRD

Treatments : 13

Replication : 3

3.6.1 Raising of Brinjal

Seedlings of brinjal (variety: Surya) were grown in pro-trays and maintained for four weeks. Twenty seven day old seedlings were transferred to grow bags filled uniformly with 1:2:1 potting mixture (sand : soil : cow dung). The crop was maintained following the KAU package of practice recommendations (2011).

3.6.2 Evaluation of Selected Entomopathogenic and Phylloplane Microorganisms

A consistent population of *H. vigintioctopunctata* was maintained in the crops raised for treatment application deliberately avoiding plant protection measures. Pre-treatment count of the insects were recorded from each plant.

Spore suspensions of fungal entomopathogens and cell suspension of bacterial pathogens at 10^8 cfu ml^{-1} were prepared following the method described in 3.5.1 and was sprayed on to the plants covering the entire plant one month after transplanting. Chlorantraniliprole 18.5 SC 0.006 % was sprayed as chemical check and application of sterile water served as untreated control.

3.6.3 Observations

3.6.3.1 Mortality

The per cent mortality at 1, 3, 5 and 7 DAT were recorded from 39 observational plants.

3.6.3.2 Leaf Area Damage

The leaf area damage caused by the insects was also recorded at time intervals of 1, 3, 5 and 7 DAT by taking observations from six leaves (2 each from upper, middle and lower strata) per plant. The leaves were plotted on a graph paper and area was estimated in cm^2. The per cent leaf area damaged was worked out using the following formula.

$$\text{Per cent leaf area damaged} = \frac{\text{Total leaf area} - \text{Leaf area left undamaged}}{\text{Total leaf area}} \times 100$$

3.6.3.3 Population of Epilachna

Post treatment count of beetles from plants sprayed with different treatments was recorded to estimate the population of the target insect after treatment. Number of epilachna beetles was recorded from six leaves (two each from upper, middle and lower strata) per plant at 1, 3, 5 and 7 DAT.

3.6.3.4 Other Pests

Pests other than *H. vigintioctopunctata* were also monitored during the study. Observations on the number of non target pests in treated plants were recorded from 6 leaves (two each from upper, middle and lower strata) per plant.

3.7 STATISTICAL ANALYSIS

Data of each experiment were analyzed applying suitable methods of analysis (Panse and Sukhatme, 1967). Data on mortality and mean population were analyzed by one way analysis of variance after square root transformation, whereas that of leaf area damage after arc sin transformation.

Results

4. RESULT

4.1 SEASONAL INCIDENCE OF EPILACHNA BEETLE, *H. vigintioctopunctata* IN BRINJAL AND MONITORING OF DISEASE OCCURENCE

The results of the studies conducted from July 2014 to June 2015 on the seasonal incidence of epilachna beetle and extend of parasitization in relation to weather parameters are presented below. The occurrence of disease on epilachna beetle was also studied.

4.1.1 Population of Epilachna Beetle in Brinjal

The population of epilachna beetle in brinjal during 2014 – 2015 are presented in Table 2.

The adult population remained low during July 2014 (0.25 adults per plant) to January 2015 (0.65 adults per plant) and exhibited an increasing trend from then on extending up to first fortnight of April 2015. The population further dropped steadily reaching a very low level in June 2015 (1.57 adults per plant). The mean number of adult population was at its peak during the first fortnight of April 2015 followed by the second fortnight of April 2015 and second fortnight of March 2015 (4.74 and 4.20 adults per plant respectively).

The grub population gradually increased from the first fortnight of November 2014 (3.49 grubs per plant) though a slight drop was noticed in the first and second fortnight of December and first fortnight of January 2015 and remained high till the second fortnight of April. The grub population was highest during second fortnight of April 2015 (6.56 grubs per plant). This was followed by the first fortnight of April with a mean population of 6.39 grubs per plant. The grub population was least during July (0.60 grubs per plant) and August 2014 (1.92 grubs per plant).

Table 2. Seasonal incidence of Epilachna, *H. vigintioctopunctata* in brinjal

Year	Mean number / plant*						
		Egg mass		Grub		Adult	
	Fortnight / Month	1	2	1	2	1	2
2014	July	0.12	0.17	0.36	0.60	0.17	0.25
	August	0.18	0.12	1.5	1.92	0.27	0.2
	September	0.16	0.18	2.4	2.98	0.34	0.38
	October	0.27	0.24	2.78	2.97	0.54	0.54
	November	0.29	0.23	3.49	3.91	0.33	0.44
	December	0.24	0.37	3.33	3.06	0.46	0.56
2015	January	0.44	0.39	3.69	4.7	0.65	0.65
	February	0.34	0.36	4.56	4.9	1.24	1.12
	March	0.41	0.47	5.07	5.21	3.27	4.17
	April	0.48	0.60	6.39	6.59	4.74	4.2
	May	0.55	0.38	5.8	4.44	3.55	3.22
	June	0.34	0.34	3.24	3.35	1.92	1.57

* Mean of 2 observations taken from 10 plants each

Mean number of egg mass per plant remained low from July 2014 (0.12 egg masses per plant) to first fortnight of December 2014 (0.24 egg masses per plant). Increased number of egg masses were observed since then till second fortnight of May. The highest mean number of 0.60 egg mass per plant was seen during the second fortnight of April 2015 this was followed by the first fortnight of may 2015 (0.55 egg mass per plant).

The lowest mean population of adults as well as egg masses were recorded during the first fortnight of July (0.17 adults and 0.12 egg mass per plant respectively) and second fortnight of August 2014 (0.2 adults and 0.12 egg mass per plant respectively).

4.1.1.1 Correlation of Epilachna Beetle Population with Weather Parameters

The correlation between the incidence of epilachna beetle in brinjal and weather parameters was studied to explain the fluctuation in population, the results of which are presented in Table 3.

A significant positive correlation with maximum temperature (0.84 and 0.83 respectively) and significant negative correlation with relative humidity (-0.41 and -0.42 respectively) were observed with respect to number of egg masses and population of grubs. However, egg mass and grub population had negative correlation with minimum temperature (-0.02, and -0.05 respectively) and rainfall (-0.27 and -0.22 respectively). Adult population showed significant positive correlation with maximum and minimum temperature and negative correlation with relative humidity and rainfall. The correlation was significant with respect to maximum temperature at 5% level.

Table 3. Correlation coefficient of Epilachna, *H. vigintioctopunctata* population with weather parameters

Weather parameters	Mean No. / plant		
	Egg masses	Grubs	Adults
Maximum temperature	+0.84*	+0.83*	+0.91*
Minimum temperature	-0.02	-0.05	+0.39
Relative humidity	-0.41*	-0.42*	-0.19
Rainfall	-0.27	-0.22	-0.02

* At 5 % level of significance

4.1.2 Extend of Parasitization

Egg masses and pupae were frequently found parasitized whereas larval and adult parasitization was negligible. The mean number of epilachna beetle egg and pupae parasitized are presented in Table 4.

The field was devoid of parasitized egg masses and pupae from July 2014 to November 2014 except the slight parasitization of egg masses observed during first fortnight of July and second fortnight of August. The mean number of egg mass parasitized showed an increasing trend from the second fortnight of November and reached its peak by second fortnight of February (0.36 egg mass per plant). The population was comparatively high during the month of February and March 2015 which later declined. No parasitization was observed in pupae of epilachna during July 2014 to December 2014 except second fortnight of October. From January 2015, the number of parasitized pupae increased steadily reaching its maximum during second fortnight of February and first fortnight of March (0.53 and 0.52 pupae per plant) respectively and declined thereafter.

4.1.2.1 Correlation of Parasitization of Epilachna Beetle with Weather Parameters

The correlation coefficient of mean number of egg mass and pupae parasitized with weather parameters are presented in Table 5.

The number of parasitized pupae showed significant positive correlation with maximum temperature (+0.83) and significant negative correlation with relative humidity (-0.59). All the other weather parameters analyzed exhibited non-significant negative correlation with the number of parasitized pupae. Significant negative correlation with minimum temperature (-0.60), relative humidity (-0.51) and rainfall (-0.44) were observed with regard to number of parasitized pupae. Positive

Table 4. Extend of parasitization of Epilachna, *H. vigintioctopunctata*

Year	Mean number of insect parasitized / plant *				
	Fortnight / Month	Egg mass		Pupa	
		1	2	1	2
2014	July	0.05	0	0	0
	August	0	0.05	0	0
	September	0	0	0	0
	October	0	0.1	0	0.1
	November	0.1	0.15	0	0
	December	0.17	0.16	0	0
2015	January	0.2	0.27	0.06	0.33
	February	0.25	0.36	0.39	0.53
	March	0.29	0.3	0.52	0.47
	April	0.22	0.2	0.39	0.34
	May	0.23	0.16	0.34	0.26
	June	0.14	0.18	0.19	0.18

* Mean population of 2 observations taken from 10 plants

Table 5. Correlation of parasitization of Epilachna, *H. vigintioctopunctata* with weather parameters

Weather parameters	Mean No. / plant	
	Parasitized egg masses	Parasitized Pupa
Maximum temperature	+0.09	+0.83*
Minimum temperature	-0.60*	-0.09
Relative humidity	-0.51*	-0.59*
Rainfall	-0.44*	-0.29

* At 5 % level of significance

correlation were also observed between parasitized egg mass population and maximum temperature (+0.09).

Since the number of dead and disease insect was negligible due to the erratic occurrence of disease in natural conditions, the data obtained were not tabulated and correlated with weather parameters.

4.2 ISOLATION OF PATHOGENIC AND PHYLLOPLANE MICROORGANISMS

During the field survey twelve microorganisms were isolated from diseased epilachna grubs collected from the field. Out of which eleven were bacteria and the remaining one was a fungus. By leaf impression method six bacteria inhabiting brinjal phylloplane were also isolated. Thus, total of eighteen microorganisms were obtained during the entire study. The isolated microorganisms were sequentially numbered in the order in which they were isolated from isolate 1 to isolate 18. Isolates 1, 3 and 9 were the isolates obtained from diseased epilachna grubs collected from the field and 13, 16 and 18 were those isolates obtained from the phylloplane of brinjal.

4.3 PRELIMINARY SCREENING OF ISOLATED MICROORGANISMS FOR PATHOGENCITY TO EPILACHNA GRUBS

Out of the eighteen microorganisms isolated, bacterial isolates 1, 3, 9, 13, 16 and 18 were infective to epilachna grubs. The pathogenicity of these bacterial isolates were confirmed by proving Koch's postulates (Table 6).

4.3.1 Disease Symptoms

The symptoms exhibited by grubs treated with different bacterial isolates are described below (Plate 1).

Table 6. Preliminary screening of microorganisms isolated from the brinjal ecosystem for infectivity to grubs of Epilachna, *H. vigintioctopunctata*

Isolate No.	Organism	Source	Per cent mortality *	Koch's postulates
Isolate 1	Bacteria	Epilachna grub	29.45 (5.52)[e]	+
Isolate 2	Bacteria	Epilachna grub	0 (1)[f]	-
Isolate 3	Bacteria	Epilachna grub	96.61 (9.88)[a]	+
Isolate 4	Bacteria	Epilachna grub	0 (1)[f]	-
Isolate 5	Bacteria	Epilachna grub	0 (1)[f]	-
Isolate 6	Bacteria	Epilachna grub	0 (1)[f]	-
Isolate 7	Bacteria	Epilachna grub	0 (1)[f]	-
Isolate 8	Bacteria	Epilachna grub	0 (1)[f]	-
Isolate 9	Bacteria	Epilachna grub	40 (6.40)[d]	+
Isolate 10	Bacteria	Epilachna grub	0 (1)[f]	-
Isolate 11	Bacteria	Epilachna grub	0 (1)[f]	-
Isolate 12	Fungus	epilachna grub	0 (1)[f]	-
Isolate 13	Bacteria	Phylloplane of brinjal	63.25 (8.02)[b]	+
Isolate 14	Bacteria	Phylloplane of brinjal	0 (1)[f]	-
Isolate 15	Bacteria	Phylloplane of brinjal	0 (1)[f]	-
Isolate 16	Bacteria	Phylloplane of brinjal	29.45 (5.52)[e]	+
Isolate 17	Bacteria	Phylloplane of brinjal	0 (1)[f]	-
Isolate 18	Bacteria	Phylloplane of brinjal	33.18 (5.85)[e]	+
CD	-	-	0.52	

*Mean of 10 insects

Values in the parentheses are $\sqrt{x+1}$ transformed

Isolate 1

Isolate 3

Isolate 9

Isolate 13

Isolate 16

Isolate 18

Plate 1. Disease symptoms on grubs treated with different pathogenic and phylloplane bacteria

4.3.1.1 Isolate 1

The treated grubs were very active initially and later displayed lethargic movement. A mortality of five per cent was observed 24 h after treatment. The dead insect swelled and changed colour from yellow to brown.

4.3.1.2 Isolate 3

The grubs of epilachna released on leaf treated with isolate 3 were active on the first day of treatment with on hindrance in feeding. 25.93 per cent mortality was obtained after 24 h of treatment. The dead insects exhibited changes in colour and consistency. The infected grubs initially turned orange red, later attaining a brown shade few hours after death and was accompanied by putrefied smell. The body wall of the insect ruptured instantly even with a light pressure resulting in exudation of orange red body fluid.

4.3.1.3 Isolate 9

The grubs treated with isolate 9 exhibited normal mobility with little inhibition to feeding. Later the movement of the grubs was impaired and the food intake was reduced to a great extend. This isolate was able to induce a mortality of 23.12 per cent within 24 h of treatment. The dead insects turned brown with sticky body fluid exuding from the cadaver. The body content of the infected grubs turned watery, the cadaver shrinked and emitted fishy odour.

4.3.1.4 Isolate 13

Grubs fed with isolate 13 showed a mortality of 2.14 per cent in 24 h of treatment which increased to 36.51 per cent by 48 h. The symptoms developed in the infected grubs were similar to that described in 4.3.1.3. Cessation of feeding was

noted after 48 h of treatment. The leaf damage was comparatively low in the treatment.

4.3.1.5 Isolate 16

This isolate was able to cause a mortality of 12.98 per cent in the treated grubs. Though the remaining live insects were sluggish, they continued to feed at normal rate without any hindrance. The infected grubs turned brown with sticky fluid oozing from the cadaver along with putrefied smell.

4.3.1.6 Isolate 18

The infected grubs lost their vigour and movement. Though the mortality caused by the isolate was negligible at 24 h (2.14 per cent) it increased subsequently to 33.8 per cent at 72 h after treatment. An initial colour change from yellow to brown was noted at the tip of the abdomen which later progressed upwards within few hours.

4.4 CHARACTERIZATION OF SELECTED MICROORGANIMS

4.4.1 Colony Morphology

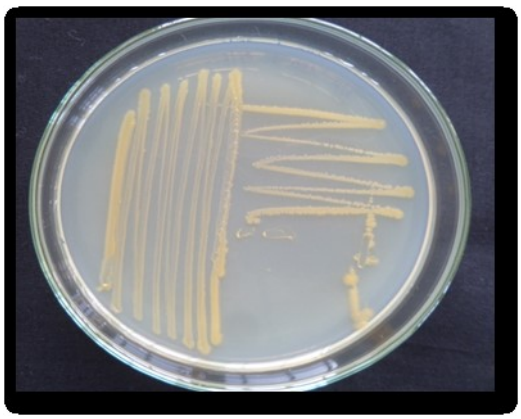
The microorganisms isolated were subjected to morphological characterization based on colony morphology by growing them on NA media. The colony characters of the isolates are presented in Table 7 (Plate 2).

4.4.1.1 Isolate 1

Bacterial isolate 1 obtained from the phylloplane of brinjal appeared bright yellow in colour. The isolated colonies were smooth and shiny in texture and exhibited slow growth on nutrient agar under room temperature.

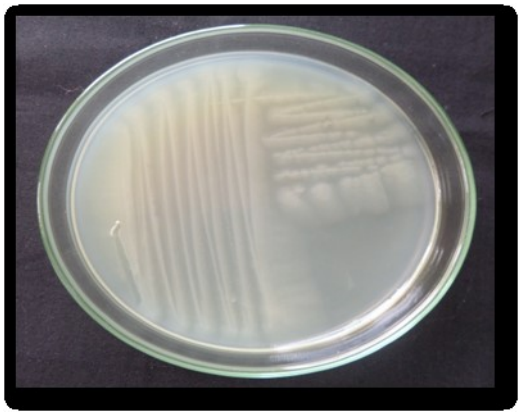
Table 7. Colony morphology of entomopathogenic bacteria

Selected isolates	Colony morphology	Gram staining
Isolate 1	Yellow coloured, smooth shiny colony Slow growing on NA plates	+
Isolate 3	Bright red coloured, smooth shiny colony Fast growing	-
Isolate 9	Light greenish - yellow coloured, smooth shiny colony with spreading growth and fluorescens	-
Isolate 13	Light yellow coloured smooth colony, with slight fluorescens on KB plate	-
Isolate 16	Bright yellow coloured, smooth shiny colony With sticky nature and slow growing	+
Isolate 18	Cream coloured rough, plague like colony	+

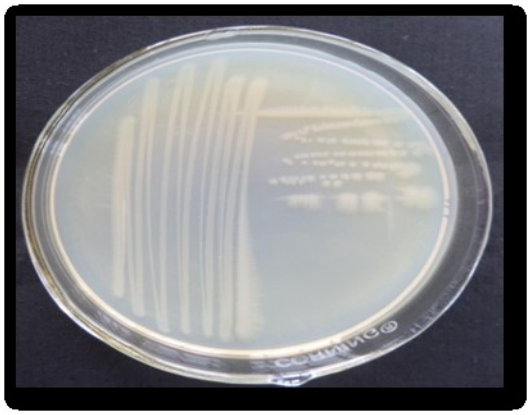


Isolate 1

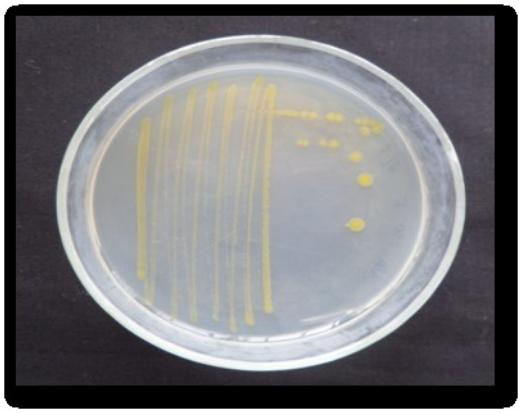
Isolate 3



Isolate 9



Isolate 13



Isolate 16

Isolate 18

Plate 2. Colony morphology of pathogenic and phylloplane bacteria

4.4.1.2 Isolate 3

The isolate appeared crimson red in colour when cultured on nutrient agar. The bacteria grew rapidly on the media and the colonies had smooth and shiny appearance.

4.4.1.3 Isolate 9

Pale yellow coloured colonies were observed when isolate 9 was grown on nutrient agar which later turned light green after two days. The bacteria were transferred to King's B medium and found that it grew rapidly in a spreading manner on the media. The colonies were fluorescent when observed under UV Transilluminator GeNei® (Plate 3) and thus it was tentatively identified as *Pseudomonas* sp.

4.4.1.4 Isolate 13

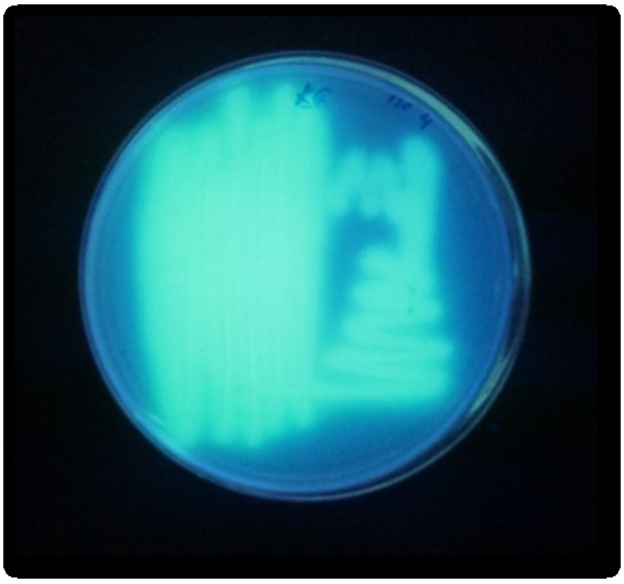
The bacterial isolate 13 displayed pale yellow colour on nutrient agar. When transferred to King's B media the growth was rapid showing slight fluorescence. It differed from isolate 2 in having a less spreading growth on King's B media and no change in colour from yellow to green. The isolate also showed fluorescence under UV Transilluminator GeNei®.

4.4.1.5 Isolate 16

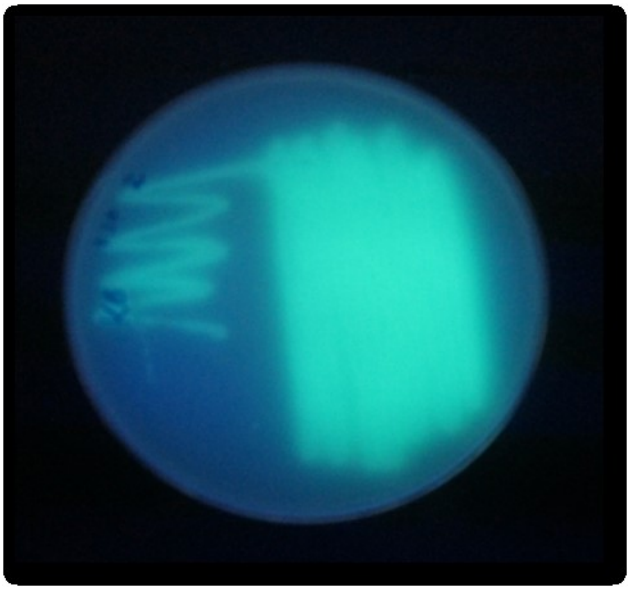
Isolate 16 produced bright yellow coloured colonies when grown on nutrient agar media. The colony was shiny and sticky and grew at a slow rate on the medium.

4.4.1.6 Isolate 18

Colonies of bacterial isolate 18 had an arborescent growth pattern on nutrient agar media. Colonies were creamy white in colour and grew rapidly on nutrient agar.



Isolate 9



Isolate 13

Plate 3. Fluorescence exhibited by pathogenic and phylloplane bacteria viewed under UV Transilluminator

Endospore staining of smear prepared from one week old culture with malachite green divulged the presence of endospores when examined with oil immersion objective (100X). Thus the bacterium was timidly identified as *Bacillus* sp.

4.4.2 Gram Staining

The Gram staining technique results revealed that isolate 1 was Gram positive. When subjected to Gram staining, isolate 3 developed a pink colour indicating that it was a gram negative bacterium. The results of Gram staining shows that isolate 9 was gram negative. Pink colour obtained during Gram staining technique expose that isolate 13 is gram negative. Purple colour was obtained when the isolate was examined for Gram staining indicating that isolate 16 was a gram positive bacterium. Gram staining of isolate 18 revealed purple coloured colonies hence proved as gram positive (Plate 4).

4.4.3 Chitinase Activity

4.4.3.1 Detection of Chitinase Activity

The selected microorganisms were grown on media containing colloidal chitin as the sole carbon source and incubated for ten days. Chitinase activity was confirmed by the presence of clear transparent zone around the colony that usually appear as a result of digestion of chitin present in the media by the chitinase enzyme produced by the growing bacteria.

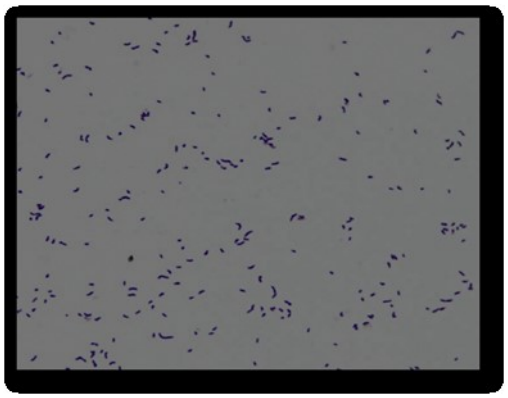
Isolates 3, 9, 13 and 18 produced halo around them which varied in diameter (Plate 5). Isolates 1 and 16 did not produce any halo.

Isolate 18 produced the largest halo with a diameter of 1.8 cm. The halo produced by isolate 9 measured 1.3 cm followed by isolate 13 (0.7 am) and isolate 3 (0.4 cm) (Table 8).

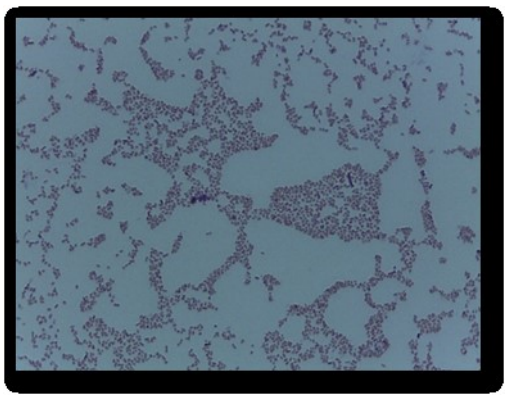
Table 8. Chitinase activity of pathogenic and phylloplane bacteria

Microorganism	Halo diameter (cm) *
Isolate 1	0
Isolate 3	0.4 cm
Isolate 9	1.3 cm
Isolate 13	0.7 cm
Isolate 16	0
Isolate 18	1.8 cm

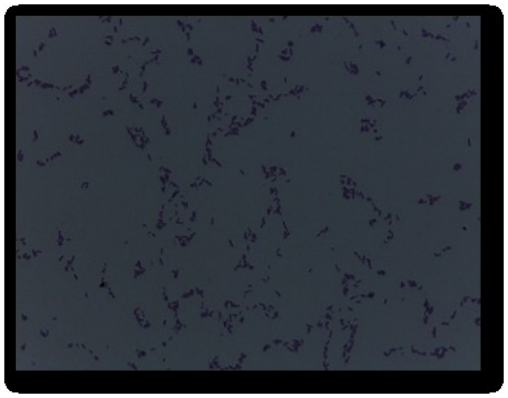
* Mean of 3 observations



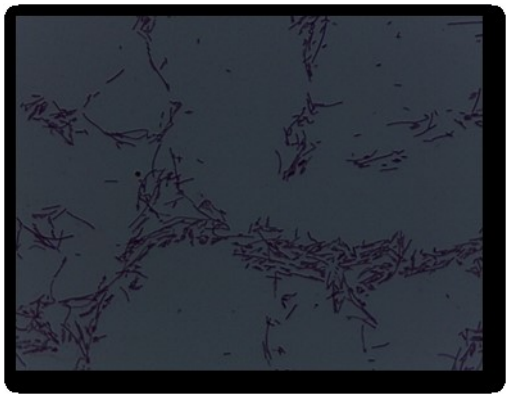
Isolate 1



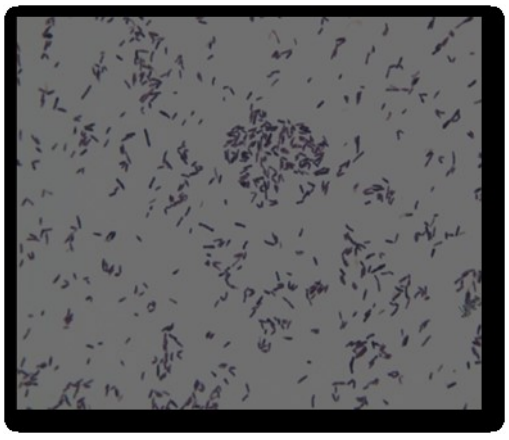
Isolate 3



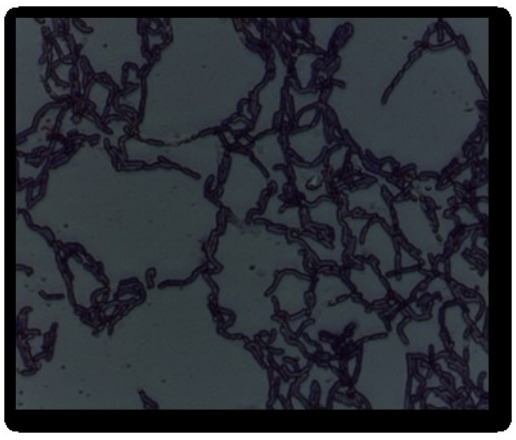
Isolate 9



Isolate 13

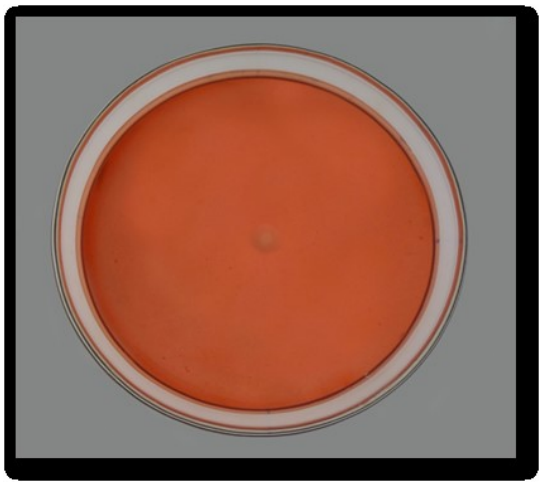


Isolate 16

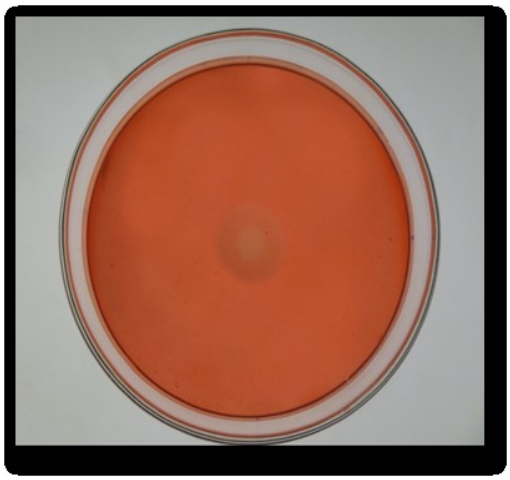


Isolate 18

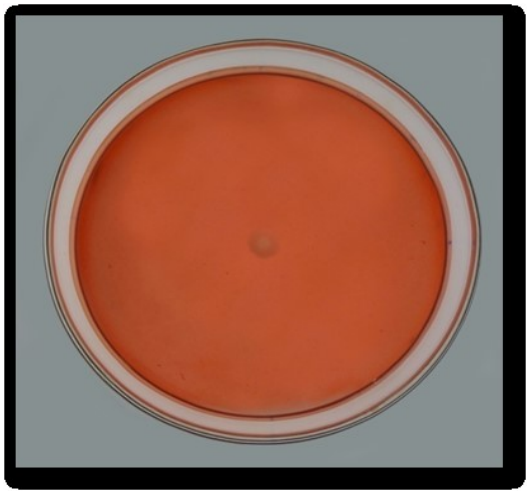
Plate 4. Gram staining of pathogenic and phylloplane microorganisms



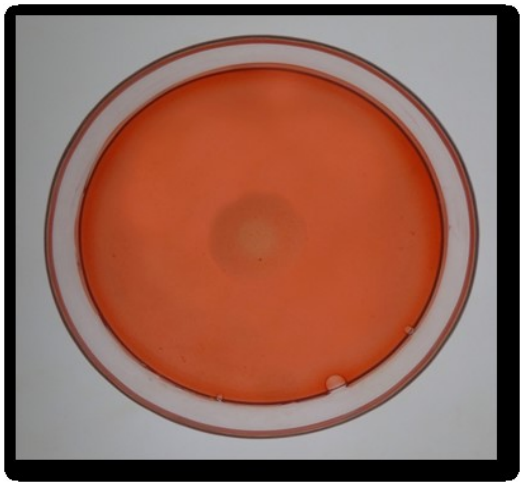
Isolate 3



Isolate 9



Isolate 13



Isolate 18

Plate 5. Chitinase activity of pathogenic and phylloplane bacteria

4.4.3.2 Quantification of Chitinase Activity

The highest chitinase activity was observed in isolate 9 (8.2 units). Isolates 18, 13 and 3 recorded a chitinase activity of 4.8, 1.7 and 1.4 units respectively (Table 9).

4.4.4 Molecular Characterization

16s rRNA sequence of isolates 1, 3, 9, 13, 16 and 18 obtained are presented in Table 10. The BLAST details of the most matching sequence are presented in Table 11.

Based on homology of sequence of 16S rRNA isolate 1, 3, 9, 13, 16 and 18 were identified as *Microbacterium* sp., *Serratia marcescens*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Curtobacterium flaccumfaciens* and *Bacillus megaterium* respectively (Appendix III a – f).

4.5 LABORATORY SCREENING OF ISOLATED MICROBIAL AGENTS

4.5.1 Pathogenicity to Grubs of Epilachna Beetle

The results of experiment on the efficacy of the five commercially available entomopathogens and six bacterial isolates obtained in the present study, along with the chemical check (Chlorantraniliprole 18.5 SC), on epilachna beetle under laboratory condition is presented in Table 12.

The results 24 h after treatment revealed that *S. marcescens* and *Microbacterium* sp. with a mortality of 26.45 and 20 per cent respectively were significantly superior to other treatments. *P. aeruginosa* and *C. flaccumfaciens* proved pathogenic to epilachna grubs with a mortality of 16.31 per cent each. Phylloplane isolate, *P. fluorescens* caused a mortality of 7.79%. All other microbial treatments failed to produce mortality in epilachna grubs 24 h after treatment.

Table 9. Quantification of Chitinase activity of pathogenic and phylloplane bacteria

Microorganism	Chitinase activity (Assay with chitin-Azure)
Isolate 3	1.4 unit
Isolate 9	8.2 unit
Isolate 13	1.7 unit
Isolate 18	4.8 unit

Table 10. 16S rRNA sequence of isolated pathogenic and phylloplane bacteria obtained with universal primer

ISOLATES	SEQUENCE
1	TCCCATGGCGCATGCTACTATGCAGTCGACGGTGAAGCCAAGCTTGCTTGGTGGATCAG TGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTGGACTCTGGGATAAGCGCTGG AAACGGCGTCTAATACTGGATATGAGCCTCTTCCGCATGGTGGGGGTTGGAAAGATTTT TTGGTCTGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGAGATAAAGGCTCAAAAAG GCTCGAACCGTAGCGGCTGAGAGGGGGACCGCCAACTGGACTGAGACAGGCCAGATC CTACCGGAGGCAGCATGGGGAATTTGCACAATGGGCGAAGCCTGATGCAGCACCCCGC GTGAGGGATGACGCTCGGGTGTAAACCTCTTTAGCAGGGAAGAAGCGAAGTGACGGTA CTGCAGAAAAAGCGCGGCTAACTACGTGCAGCAGCCGCGGTAATACGTAGGGCGCAA GCGTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCTGTGA AATCCCGAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGG GGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGAT GGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTGGGGAGCA AACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGA CCATTCCACGGTTTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGG CCGCAAGGCTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGCGGAGCATGC GGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCAGAACGGC CAGAATGTCACTCTTTGGACACTGTGAACAGGTGTGCATGCTGTCGTCAGCTCGTGTCG TGAGATGTTGGTAGTCCCGCAACGACGCACCCTCGTCTATGTTGCAGCACGTAATGGTG CAACTCATGGCATACTGCCGGATCAACTCGAGAGGTGGGATGACGTCAATCATCATGC CCGTAATCCTGACCTCCAGCATTACAATGCGTAAAGCGCTGCATACCGTCAGGTGGAG CAATCCAAAAAAGCCGGTCCCAGTTCGGATTGAGGTCTGCAACTCGACCTCATGAAGT CGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGTCTTGT ACACACCGCCCGTCAAGTCATGAAAGTCGGTAACACCTGAAGCCGGTGGCCCAACCCT TGTGGAGGGAGCCGTCGAAGGTGGGATCGGTAATAGACTAAGTCGAATCAAGTTATTT GGCTC
3	CGGCATGGGTAGCTATACATGCAGTCGAGCGGTAGCACGGGGGAGCTTGCTCCCTGGG TGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAA CTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTC GGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAATGGCT CACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCACACACACTGGACTGAAC CGTCAATCTACGAGGCACCAGTGGGAATTTGACAATGGGCAGCTGATGCAGCATGCGC TTTGAAGAAGCCTTCGGGTGTAAGCACTTCAGCGAGGAGGAAGGTGGTGAGCTAATAC CCTCATCAATTGACGTACTAGCAGAAGAGCACCGGCTAACTCGTGCCAGCAGCGCGGT ATACGGAGGGTGCAGCGTTATCGGAATACTGGGCGTAAGCGCACGCAGGCGGTTTGTT AAGTCAGATGTGAAATCCCCGGGCTCAACTGGGAACTGCATTTGAAACTGGCAAGCTA GAGTCTCGTAGAGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGA GGAATACCGGTGGCGAACGCGGCCCCTGGACGAAGACTGACGCTCACGTGCGAAAGC GTGGGGAGCAAACAGGATTAGATACCTGCTAGTCCACGCTGTAAACGATGTCGATTTG GAGGTTGTGCCCTTGAGGCGTGCTTCCGGAGCTAACGCGTTAATCGACCGCCTGGGGA GTACGGCCGCAAGGTTAAAACTCAAATGAATTGACCGGGGCCCGCACAAGCGGTGGAG CATGTAGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGACT TACCAGAGATGGATTCGTGCCTTCGGAACTCTGAAACAGGTGCTGCATGCCTGTCGTCA GCTCGTGTTGGAATGTATTAAGTCCCGCACGAGCGCAACCCTATCCTTTGTGCAGCGGT CGGCCGAACTCAAGAAACTGCATGATACTGAGAGTGGGATACTAGTCCATCCCTTAAG AATAGCGCTACACACGTGCTACAACACAATATACAAAGAGAAGCGACCTCGCGAGAGC AAGCGGACCTCATAAAGTACGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATG AAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCC TTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAA CCTTACGGGAGGGCGCTTACCACTTTGTGATCATGACTGGGGTGAAGTCGTAGCATGAT CACCCCCC

Table 10 continued

9	TATGGACAGGTCTCTTGTTTCGACTTCCCCCAGTCATGAATCACTCCGTGGTA ACCGTCCCCCTTGCGGTTAGACTAGCTACTTCTGGAGCAACCCACTCCCATG GTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGACATTCT GATTCACGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCG ATCCGGACTACGATCGGTTTTATGGGATTAGCTCCACCTCGCGGCTTGGCAA CCCTTTGTACCGACCATTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGCCAT GATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCCTT AGAGTGCCCACCCGAGGTGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTAC GGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCAC CTGTGTCTGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCAGCAT GTCAAGGCCAGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCC ACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTA CTCCCCAGGCGGTCGACTTATCGCGTTTAGTCTGCGCCACTAAGATCTCAAA GGATCCCAACGGTTAGTCGACCTTCGTTAACGGCGTGGACTACCAAGGGTAT CCTAATCCTGTTTTGCTCCCCCAACGCTTTCGCACCCTGCCATGCCGTAGTCT ACCATGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGA CGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGA AACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCG GACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAA AGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCAC ACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT ATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCCCGTGTGTGAAGAAG GTCTTCGGATTGTAAAGCAC
13	GTCAATGGGAGCTGCTTCTCTTGAGAGCGGCGGACGGGTGAGTAATGCCTAG GAATCTGCCTGGTAGTGGGGGATAACGCTCGGAAACGGACGCTAATACCGC ATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATG AGCCTAGGTCGGATTAGCTAGTTGGTGAGGAAAACGCTAACCAAGGCGACG ATCCGTAACTGTTGAGGATGATCAGTCCTGGACTGAGACACGTACAGACTCG AGGCAGCCAGAGTGGGGAACATGACATGCCGAAGCCTGATCCAGCATGCCG CGTGTGGGAAGAAGGTCTTCGGATGTAAGCACTTAAGTGGGAGGAAGGGCA TTACTAATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAAC TCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTA CTGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCG GGCTCAACCTGGGAACTGCATTCAAAACTGTCGAGCTAGAGTATGGTAGAGG GTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACAC CAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGC GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG TCAACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAA GTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGAC GGGGGCCCGCACAAGCGGTGGAGCATGTGGGTTAATTCGAAGCAACGCGAA GAACCTTACCAGGCCTTGACATCCAATGACTTTCCAGAGATGGATGGTGCCT TCGGAACATTGAAACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGA TGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAACAC GTCAAAGGGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTG GGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTA CAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCACA AAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGA ATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTT GTACACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGAAGTAGCTAGT CTAACCTTCGGGGGGACGGTTACCACGGTGTGATTCATGACTGGGGTGAAGT CGTAGCAAGAGGCTTGCCTT

Table 10 continued

16	AGCCTTAGGTCTCATTGATACGACTTAGTCTATCACCGATCCCACCTTCGACG GCTCCCTCCACAAGGGTTAGGCCACCGGCTTCGGGTGTTACCGACTTTCATG ACTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGC TGATCTGCGATTACTAGCGACTCCGACTTCATGAGGTCGAGTTGCAGACCTC AATCCGAACTGAGACCGGCTTTTTGGGATTCGCTCCACCTTACGGTATCGCA GCCCTTTGTACCGGCCATTGTAGCATGCGTGAAGCCCAAGACATAAGGGGCA TGATGATTTGACGTCATCCCCACCTTCCTCCGAGTTGACCCCGGCAGTCTCCT ATGAGTCCCCGCCATAACGCGCTGGCAACATAGAACGAGGGTTGCGCTCGTT GCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACC ACCTGTACACCGACCACAAGGGGGCGACCATCTCTGGCCGTTTCCGGTGTAT GTCAAGCCTTGGTAAGGTTCTTCGCGTTGCATCGAATTAATCCGCATGCTCCG CCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAGCCTTGCGGCCGTAC TCCCCAGGCGGGGCGCTTAATGCGTTAGCTACGACACAGAAACCGTGGAAA GGTCCCTACATCTAGCGCCCAACGTTTACGGCGTGGACTACCAGGGTATCTA ATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTACGGCCCACAGA TCTGCCTTCGCCATCGGTGTTTCCTCCTGATATCTGCGCATTCCACCGCTACA CCAGAATTCCCATCTCCCCTACCGCACTCTAGTCTGCCCGTACCCACTGCGAG CCCGAGGGTGAGCCTCGGGATTTCGCAGCAGACGCGACAAACCCGCCTACG AGCTCTTTACGCCCAATATTCCAGCACAACGCTTGCACCCTACGTATTAACCG CCGCCTGCTGGCACGTAAGTTAGCCGGTGCTCTTTCTGCTAGTGCGGTGCGC AGCTACCATGCAGTCGACGATGAAGCCCAGCTTGCTGGGTGGATTAGTGGCG AACGGGTGAGTAACACGTGAGTAACCTGCCCCTGACTCTGGGATAAGCGTTG GAAACGACGTCTAATACTGGATATGACTACTGGTCGCATGGCCTGGTGGTG GAAAGATTTTTTGGTTGGGGATGGACTCGCGGCCTATCAGCTTGTTGGTGAG GTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGG CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGG GAATATTGCACAATGGGCGAAAGCCTGATGCAGCAACGCCGCGTGAGGGAT GACGGCCTTCGGGTTGTAAACCTCTTTTAGTAGGGAAGAAGCGAAAGTGACG GTACCTGCAGAAAAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATA CGTAGGGTGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG GTTTGTCGCGTCTGCTGTGAAATCCCGAGGCTCAACCTCGGGCTTGCAGTGG GTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCG GTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTG GGCCGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGA TACCCTGGTAGTCCACGCCGTAACGTTGGGCGCTAGATGTAGGGGACCTTTC CACGGTTTCTGTGTCGTAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACG GCCGCTAGGCTTAATACTCAAAGGAATTGAATGGGGCCCGGCACCAGCGGG GGGAGCATGCGTATAATTTCGATGCTCGCGAAGAATCCTTTACCTAGGCCTG GACAT

Table 10 continued

18	GGCAATTGCGGCAGCTATAATGCAGTCGAGCGAACTGATTAGAAGCTTGCTTC TATGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAG ACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTC ATGGGAGATGATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGG TGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGAGGCAGAGAC CACAGGAGAGGGTAATCGGCCAAACTGGAACTAGAAAGGCCCAGATCCTACG GAAGGCAGCAGTAGGATCTTCCGCAATGGACGAAGTCGACGGAGCACGCGCT GAGTGATGAAGCTTCGGTCGTAAACTCTGTTGTTAGGAAGACAAGTACGAGAG TAACTGCTGTACTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCC AGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTA AAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCACGGCTCAACCGT GGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAAAGCGGAATT CCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAG GCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAAC AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAG AGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGG AGTACGGTCGCAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCG GTGAGCATGTGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACA TCTCTGACACTCTAGAGAAAGAGCGTTCCCCTTCGGGGACGAGTGACAGTGGT GCATGATTGTCGTCAGCTCGTGTCTGAAATGTGGTAAGTCCCGCAACGAGCGC ACCCTGATCTAGTGCAGCATTAGTGGGCACTCTAGGTGACTGCGGTGACACCG GAGAGGTGGGGATGACTCAATCATCATGCCCTTAGGACCTGCTACAGTCTCAA TGATGGTACAGGACTGCAGACCGCGAGGTCAAGCCAATCCCATAAAACCATTC TCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTA ATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGC CCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGAGTAACCGTAAG GAGCTAGCCGCCTAAGGTGGGACAGATGATGGGTGAAGTCGAGCATGTGCTC GGCG

Table 11. BLAST search details of the sequences producing most significant alignment of the pathogenic and phylloplane bacteria

Isolate No.	Description	Max score	Total score	Query cover	E value	Identity	Accession no.
1	*Microbacterium sp.* CGR1. Complete genome	1934	3869	97%	0.0	92%	NC 015125.1
3	*Serratia marcescens* SM39 DNA. Complete genome	1934	3869	97%	0.0	93%	NC 015125.1
9	*Pseudomonas aeruginosa* VRFPA04. Complete genome	1306	15928	97%	0.0	98%	NZ CP008739.1
13	*Pseudomonas fluorescens* NCIMB 11764. Complete genome	2313	13950	98%	0.0	96%	NZ CP010945.1
16	*Curtobacterium flaccumfaciens* UCD-AKU	1746	3287	98%	0.0	98%	NZ APJN01000083.1
18	*Bacillus megaterium* strain Q3. Complete genome	2202	28547	97%	0.0	95%	NZ CP010586.1

Table 12. Mortality of Epilachna, *H. vigintioctopunctata* grubs treated with different pathogenic and phylloplane microorganisms

Treatments		Per cent mortality *			
		1DAT	3 DAT	5 DAT	7 DAT
T1	*M. anisopliae* (10^8 cfu ml^{-1})	0 (1)^{e}	16.31 (4.16) ^{e}	40 (6.40) ^{de}	66.58 (8.22) ^{b}
T2	*B. bassiana* (10^8 cfu ml^{-1})	0 (1)^{e}	7.79 (2.96) ^{f}	39.59 (6.37) ^{de}	63.25 (8.02) ^{b}
T3	*L. lecanii* (10^8 cfu ml^{-1})	0 (1)^{e}	0 (1) ^{g}	0 (1) ^{f}	0 (1) ^{e}
T4	*B. thuringiensis* (0.25 %)	0 (1)^{e}	26.45 (5.24) ^{de}	40 (6.40) ^{de}	46.55 (6.89) ^{c}
T5	*P. fluorescens* (Deepa pseudo®) (2 %)	0 (1)^{e}	30 (5.57) ^{cd}	43.21 (6.65) ^{cd}	50 (7.14) ^{c}
T6	*Microbacterium* sp. (10^8 cfu ml^{-1})	20 (4.58) ^{bc}	23.12 (4.91) ^{de}	33.18 (5.85) ^{e}	33.18 (5.85) ^{d}
T7	*S. marcescens* (10^8 cfu ml^{-1})	26.45 (5.23) ^{b}	86.60 (9.36) ^{ab}	93.28 (9.71) ^{a}	93.28 (9.71) ^{a}
T8	*P. aeruginosa* (10^8 cfu ml^{-1})	16.31 (4.16) ^{c}	66.35 (8.20) ^{b}	73.01 (8.60) ^{b}	73.01 (8.60) ^{b}
T9	*P. fluorescens*(10^8 cfu ml^{-1})	7.79 (2.96) ^{d}	43.21 (6.65) ^{c}	50 (7.14) ^{c}	63.25 (8.02) ^{b}
T10	*C. flaccumfaciens* (10^8 cfu ml^{-1})	16.31 (4.16) ^{c}	33.18 (5.85) ^{cd}	43.21 (6.65) ^{cd}	50 (7.14) ^{c}
T11	*B. megaterium* (10^8 cfu ml^{-1})	0 (1)^{e}	25.93 (5.19) ^{de}	36.51 (6.12) ^{de}	36.51 (6.12)^{d}
T12	Chemical control (Chlorantraniliprole 18.5 SC, 0.006 %)	76.59 (8.80) ^{a}	100 (10.05) ^{a}	100 (10.05) ^{a}	100 (10.05) ^{a}
T13	Untreated control	0 (1)^{e}	0 (1)^{g}	0 (1)^{g}	0 (1)^{f}
	CD (0.05)	1.34	1.19	0.74	0.67

*Mean of 3 replications comprising 10 grubs each

Values in the parentheses are √x+1 transformed; figures followed by same letter in a column do not differ significantly

Chemical check (Chlorantraniliprole 18.5 SC) was found to be significantly superior over all the other treatments at 24 h after treatment.

At third day after treatment chemical control (Chlorantraniliprole 18.5 SC) was found to be statistically superior over other treatments causing cent per cent mortality of epilachna grubs. *S. marcescens* caused a mortality of 86.60 per cent on 3 DAT and was found to be on par with chemical control. *P. aeruginosa* resulted in 66.35 per cent mortality at three days of treatment and was on par with that of *S. marcescens*. Phylloplane isolated *P. fluorescens* proved to cause a mortality of 43.21 per cent which increased to 50 per cent 5 DAT. The mortality obtained in *C. flaccumfaciens* was found to be 33.18 and 43.21 per cent at 3 and 5 DAT respectively which was on par with the effect of conventional bacterial entomopathogen *P. fluorescens* (Deepa pseudo®) (30% at 3 DAT and 43.21 at 5 DAT) commercially used in pest control. A mortality of 26.45 per cent obtained in *B. thuringiensis* treatment, 25.93 in *B. megaterium* and 23.13 in *Microbacterium* sp. were found to be on par at 3 DAT. The mortality observed in case of *M. anisopliae* (16.31 %) and *B. bassiana* (7.79%) treatments were very less up to 3 DAT.

Five days after exposure, the highest per cent mortality (100%) was observed with chemical control (Chlorantraniliprole 18.5 SC) which was on par with the mortality percentage of 93.28 obtained with *S. marcescens*. *P. aeruginosa* was successful in causing a mortality of 73.01 per cent in epilachna grubs and was the next best treatment to *S. marcescens*. At the same timeframe *M. anisopliae*, *B. thuringiensis*, *B. bassiana* and *B. megaterium* were found to be on par recording a mortality per cent of 40, 40, 39.59 and 36.18 respectively. *Microbacterium* sp. was observed to cause the least mortality of 33.18 per cent in epilachna grubs and *L. lecanii* did not cause any mortality even after 5 days of treatment.

It took 7 days to cause a mortality of 50 per cent or more in epilachna grubs in case of *M. anisopliae* (66.58), *B. bassiana* (63.25), *P. fluorescens* (phylloplane

isolate) (63.25), *C. flaccumfaciens* (50) and *P. fluorescens* (Deepa pseudo®) (50). No significant difference were observed in the effect of *P. aeruginosa*, phylloplane isolated *P. fluorescens* and the commercial fungal entomopathogens *M. anisopliae* and *B. bassiana*, while the effect of *C. flaccumfaciens* was found to be on par with *P. fluorescens* (Deepa pseudo®) and *B. thuringiensis* at 7 DAT. The mortality caused by *Microbacterium* sp and *B. megaterium* were too little (33.18 and 36.51 per cent respectively) even after 7 days of treatment. No mortality was observed in untreated check and *L. lecanii* treatments. All microbial treatments were statistically superior to these treatments at 1, 3, 5 and 7 DAT. None of the treatments when infected in the larval stage caused any developmental irregularity except *M. anisopliae* causing wing malformation in newly emerged adults (Plate 6).

Over all, *S. marcescens* succeeded in causing a higher mortality of epilachna beetle and was on par with chemical control under laboratory evaluation.

4.5.2 Pathogenicity to Adults Epilachna Beetle

The results of efficacy of different treatments against the adults of epilachna beetle are given in Table 13.

Results reveal that none of the treatments were effective in causing death of adult beetles. A very negligible mortality of 0.07 per cent was observed in beetles treated with phylloplane isolated *P. fluorescens* at 24 h after treatment which slightly increased to 0.13 per cent at 5 DAT. The treatment was followed by *B. thuringiensis* and *C. flaccumfaciens* each causing a mortality of 0.01 per cent at 3 DAT. At 5 DAT the mortality caused by phylloplane isolated *P. fluorescens* was found to be on par with that of *M. anisopliae* (0.13 per cent each). The treatment was closely followed by *B. bassiana* and *C. flaccumfaciens* each causing 0.01 per cent mortality of adult beetle at 5 DAT.

Table 13. Mortality of Epilachna, *H. vigintioctopunctata* adults treated with different pathogenic and phylloplane microorganisms

Treatments		Per cent mortality *			
		1DAT	3 DAT	5 DAT	7 DAT
T1	*M. anisopliae* (10^8 cfu ml^{-1})	0 (0)	0 (0)	0.13 (21.14)^{b}	0.16 (23.36)^{b}
T2	*B. bassiana* (10^8 cfu ml^{-1})	0 (0)	0 (0)	0.01 (18.43)^{bc}	0.16 (23.85)^{b}
T3	*L. lecanii* (10^8 cfu ml^{-1})	0 (0)	0 (0)	0 (0)	0 (0)
T4	*B. thuringiensis* (0.25 %)	0 (0)	0.01 (6.14)^{bc}	0.045 (12.23)^{cd}	0.045 (12.23)^{c}
T5	*P. fluorescens* (Deepa pseudo®) (2 %)	0 (0)	0 (0)	0 (0)	0 (0)
T6	*Microbacterium* sp. (10^8 cfu ml^{-1})	0 (0)	0 (0)	0 (0)	0 (0)
T7	*S. marcescens* (10^8 cfu ml^{-1})	0 (0)	0 (0)	0 (0)	0 (0)
T8	*P. aeruginosa* (10^8 cfu ml^{-1})	0 (0)	0 (0)	0 (0)	0 (0)
T9	*P. fluorescens* (10^8 cfu ml^{-1})	0 (0)	0.07 (15) ^{b}	0.13 (21.14)^{b}	0.13 (21.14)^{b}
T10	*C. flaccumfaciens* (10^8 cfu ml^{-1})	0 (0)	0.01 (6.14)^{bc}	0.01 (6.14)^{de}	0.01 (6.14)^{cd}
T11	*B. megaterium* (10^8 cfu ml^{-1})	0 (0)	0 (0)	0 (0)	0 (0)
T12	Chemical control (Chlorantraniliprole 18.5 SC, 0.006 %)	0.33 (35.22)^{a}	0.77 (61.22)^{a}	0.77 (61.22)^{a}	0.77 (61.22)^{a}
T13	Untreated control	0 (0)	0 (0)	0 (0)	0 (0)
	CD (0.05)	1.61	9.62	7.86	8.81

*Mean of 3 replications comprising 10 adults each

Plate 6. Developmental irregularity in insect treated with *Metarhizium anisopliae*

At 7 DAT *M. anisopliae*, *B. bassiana* and *P. fluorescens* isolated from the phylloplane recorded percentage mortality of 0.16, 0.16 and 0.13 respectively followed by *B. thuringiensis* (0.05 per cent) and *C. flaccumfaciens* (0.01 per cent). During the entire period of study chemical control remained statistically superior to all the other treatments. The microbial treatments had little effect on adult beetles.

4.5.3 Leaf Area Damage Caused by Epilachna Grubs

The leaf area damage by epilachna grubs on brinjal treated with different microorganisms was studied to document feeding activity in addition to mortality and the results are presented in Table 14 (Plate 7).

The minimum leaf area damage caused by epilachna grubs were recorded in chemical control (chlorantraniliprole 18.5 SC) (0.08 per cent). This treatment was followed by *S. marcescens* causing a leaf area damage of 51.47 per cent at 24 h of treatment. Even though *M. anisopliae* and *B. bassiana* were inferior in causing mortality to epilachna grubs at 24 h after treatment, it was noted that these treatments could considerably reduce the leaf area damage (55.49 and 56.47 per cent respectively) when compared with other treatments. The leaf area damage caused by phylloplane isolated *P. fluorescens* (60 per cent) was on par with *P. aeruginosa* (62.82 per cent) at the same time frame. The treatments with *C. flaccumfaciens* and *B. megaterium* were on par and recorded a leaf area damage of 83.39 and 84.30 per cent respectively, followed by treatments with *Microbacterium* sp., *P. fluorescens* (Deepa pseudo®) and *B. thuringiensis* constituting 85.16, 89.52 and 96.57 per cent damage to leaf respectively. *L. lecanii* failed to produce mortality to grubs as in the case of untreated check and caused 100 per cent damage to leaves.

At 3 days of treatment *P. aeruginosa* succeeded in reducing the leaf area damage to 3.52 per cent making it next best to chemical control which did not cause any damage. *P. fluorescens* (phylloplane isolate) and *P. aeruginosa* followed this

Table 14. Leaf area damage caused by Epilachna, *H. vigintioctopunctata* grubs treated with different pathogenic and phylloplane microorganisms

Treatments		Per cent leaf area damage *			
		1DAT	3 DAT	5 DAT	7 DAT
T1	*M. anisopliae* (10^8 cfu ml^{-1})	55.49 (48.16)^{ef}	64.84 (53.63)^{e}	54.49 (47.56)^{f}	54.49 (47.56)^{e}
T2	*B. bassiana* (10^8 cfu ml^{-1})	56.47 (48.72)^{ef}	45.39 (42.36)^{f}	22.47 (28.23)^{g}	22.47 (28.23)^{f}
T3	*L. lecanii* (10^8 cfu ml^{-1})	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	81.25 (64.34)^{b}
T4	*B. thuringiensis* (0.25 %)	96.57 (79.33)^{b}	94.96 (77.03)^{b}	83.13 (65.75)^{b}	73.29 (58.88)^{c}
T5	*P. fluorescens* (Deepa pseudo®) (2 %)	89.52 (71.11)^{c}	86.03 (68.05)^{c}	73.94 (59.33)^{c}	73.88 (59.28)^{c}
T6	*Microbacterium* sp. (10^8 cfu ml^{-1})	85.16 (67.35)^{cd}	74.78 (59.85)^{d}	69.36 (56.34)^{d}	68.44 (55.82)^{cd}
T7	*S. marcescens* (10^8 cfu ml^{-1})	51.47 (45.84)^{f}	27.99 (31.95)^{g}	0.0 (0)^{j}	0.0 (0)^{h}
T8	*P. aeruginosa* (10^8 cfu ml^{-1})	62.82 (52.43)^{e}	3.52 (10.81)^{I}	1.96 (8.05)^{h}	1.89 (7.92)^{g}
T9	*P. fluorescens* (10^8 cfu ml^{-1})	60.00 (50.77)^{e}	8.59 (17.04)^{h}	1.03 (5.82)^{I}	0.89 (5.44)^{g}
T10	*C. flaccumfaciens* (10^8 cfu ml^{-1})	83.39 (65.95)^{d}	73.03 (58.71)^{d}	63.93 (53.09)^{e}	64.95 (53.69)^{d}
T11	*B. megaterium* (10^8 cfu ml^{-1})	84.30 (66.66)^{d}	70.14(56.88)^{de}	64.05 (53.16)^{e}	65.63 (54.11)^{d}
T12	Chemical control (Chlorantraniliprole 18.5 SC, 0.006 %)	0.08 (1.65)^{g}	0.0 (0)^{j}	0.0 (0)^{j}	0.0 (0)^{h}
T13	Untreated control	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}
	CD (0.05)	4.47	3.75	2.13	3.88

*Mean of 3 replications comprising 10 grubs each

Values in the parentheses are arc transformed

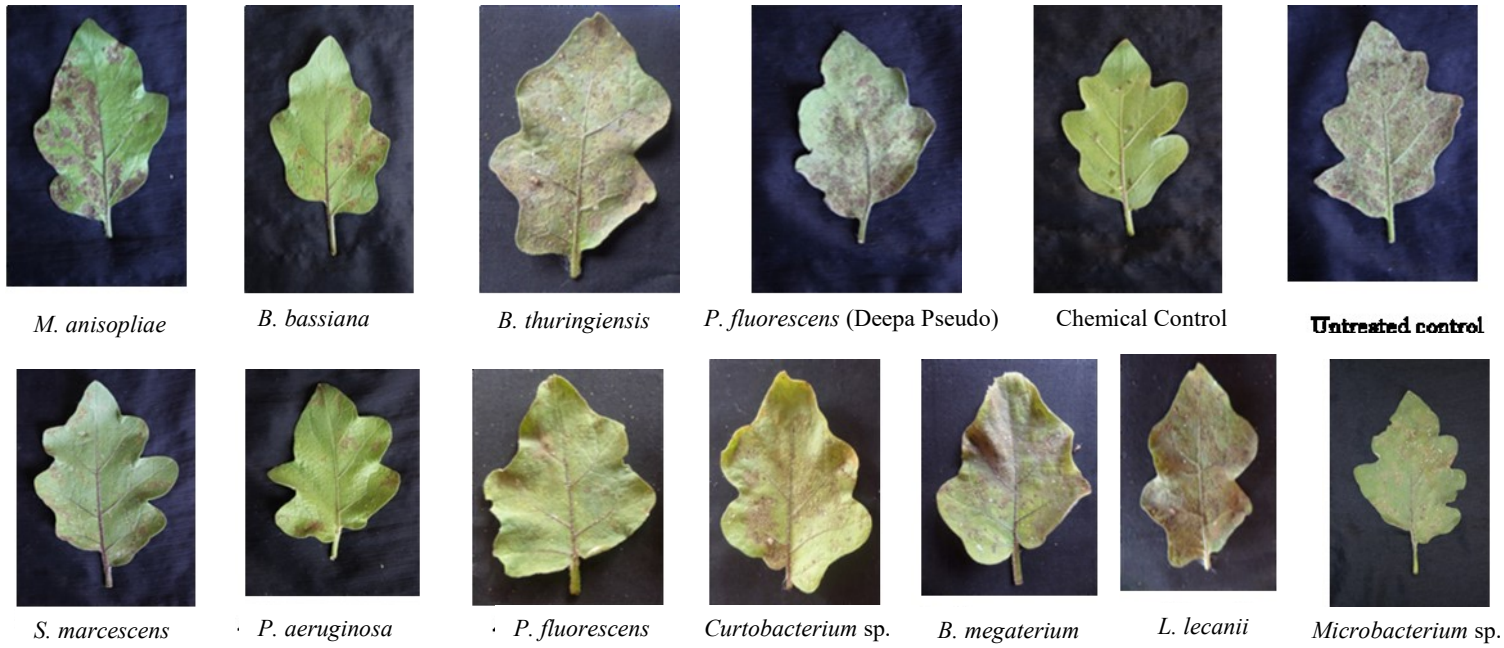


Plate 7. Leaf area damaged by epilachna, *H. vigintioctopunctata* grubs treated with different pathogenic and phylloplane microorganisms

treatment causing 8.59 and 27.99 per cent damage of leaf respectively which were superior to treatments with commercial fungal entomopathogens like *B. bassiana* (45.39 per cent) and *M. anisopliae* (64.84 per cent). Leaves treated with *B. megaterium*, *C. flaccumfaciens* and *Microbacterium* sp showed 70.14, 73.03 and 74.78 per cent damage respectively at 3 days after treatment. The leaf area protection by commercial bacterial entomopathogens *P. fluorescens* (Deepa pseudo®) and *B. thuringiensis* were very poor as the grubs subjected to these treatments caused a damage of 86.03 and 96.96 per cent respectively of leaf area.

The grubs treated with *S. marcescens* did not cause leaf damage making it on par with chemical control at 5 and 7 days after treatment. At 5 DAT a leaf damage of only 1.96 and 1.03 per cent were recorded in case of *P. aeruginosa* and phylloplane isolated *P. fluorescens* respectively, a trend that continued at 7 DAT also (0.89 and 1.89% leaf damage respectively). Moderate leaf damage was caused by grubs treated with *B. bassiana* (22.47 per cent at 5 and 7 DAT) and *M. anisopliae* (54.49 per cent damage at 5 and 7 DAT). The effects of *C. flaccumfaciens and B. megaterium* on leaf damage were on par (63.93 and 64.05 per cent at 5 DAT and 65.95 and 65.63 per cent at 7 DAT respectively). The leaf area protection offered by *Microbacterium* sp. (69.36 and 68.44 per cent damage at 5 and 7 DAT) was low but superior over *P. fluorescens* (Deepa pseudo®) (73.94 and 73.88 per cent damage at 5 and 7 DAT) and *B. thuringiensis* treatments (83.13 and 81.25 per cent damage respectively at 5 and 7 days of treatment). 100% leaf area damage was noted in untreated check at all intervals. *L. lecanii* treated grubs caused 100% leaf damage in all treatments except at 7 DAT.

The leaf area damage caused by *S. marcescens* treated grubs was low denoting that it's a better biocontrol agent than other treatments. This effect is directly caused by fast death of the test insects rather than feeding deterrence. Treatment with phylloplane isolated *P. fluorescens* in spite of having a comparable

mortality with *M. anisopliae* and *B. bassiana* caused less leaf area damage showing feeding deterrence effects.

4.6 POT CULTURE EVALUATION OF SELECTED ENTOMOPATHOGENIC AND PHYLLOPLANE MICROORGANISMS

Pot culture experiment was carried out at the department of Agricultural Entomology, College of Agriculture, Vellayani. The results of the study are presented below.

4.6.1 Efficacy of Entomopathogenic and Phylloplane Microorganism against Epilachna Beetle

Table 15 indicates the per cent mortality of epilachna beetle grubs treated with different entomopathogenic and phylloplane microorganisms.

On the first day of treatment no mortality was recorded from plants treated with *M. anisopliae*, *B. bassiana*, *L. lecanii*, *B. thuringiensis*, *P. fluorescens* (Deepa pseudo®) and *B. megaterium*. Chemical control (Chlorantraniliprole 18.5 SC) recorded the highest mortality of 76.59 per cent followed by *S. marcescens* recording 20 per cent. The mortality percentage in treatments with *S. marcescens* was found to be on par with that of *P. aeruginosa* (16.31 percent) and phylloplane isolated *P. fluorescens* (12.89 per cent). *Microbacterium* sp. and *C. flaccumfaciens* merely caused a mortality of 5.47 per cent each after 24 h of treatment.

Three days after treatment a mortality of 100 per cent was observed in chemical (Chlorantraniliprole 18.5 SC) treated plants. 46.55 per cent mortality of epilachna grubs was noted in *S. marcescens* followed *P. aeruginosa* (35.51 per cent), phylloplane isolated *P. fluorescens* (26.45 per cent), *P. fluorescens* (Deepa pseudo®) (16.31 per cent), *B. megaterium* (15.54 per cent), *B. thuringiensis* (12.98 per cent), *Microbacterium* sp. (12.98 per cent) and *C. flaccumfaciens* (12.97 per cent).

Table 15. Mortality of Epilachna, *H. vigintioctopunctata* grubs on plants treated with different pathogenic and phylloplane microorganisms

Treatments		Per cent mortality *			
		1 DAT	3 DAT	5 DAT	7 DAT
T1	*M. anisopliae* (10^8cfu ml^{-1})	0 (1)^{d}	5.47 (2.54)^{fg}	32.59 (5.79)^{de}	59.72 (7.79)^{bc}
T2	*B. bassiana* (10^8 cfu ml^{-1})	0 (1)^{d}	2.14 (1.77)^{gh}	26.45 (5.24)^{ef}	46.55(6.89) ^{de}
T3	*L. lecanii* (10^8 cfu ml^{-1})	0 (1)^{d}	0 (1)^{h}	0 (1)^{h}	0 (1)^{i}
T4	*B. thuringiensis* (0.25 %)	0 (1)^{d}	12.98 (3.74)^{ef}	20 (4.58)^{f}	23.11(4.91)^{g}
T5	*P. fluorescens* (Deepa pseudo®) (2 %)	0 (1)^{d}	16.31 (4.16)^{de}	39.59 (6.37) ^{cd}	46.23 (6.87) ^{de}
T6	*Microbacterium* sp. (10^8 cfu ml^{-1})	5.47 (2.54)^{c}	12.98 (3.74)^{ef}	26.45 (5.24)^{ef}	30 (5.57)^{fg}
T7	*S. marcescens* (10^8 cfu ml^{-1})	20 (4.58)^{b}	46.55 (6.89)^{b}	93.27 (9.71)^{a}	93.27 (9.71)^{a}
T8	*P. aeruginosa* (10^8 cfu ml^{-1})	16.31 (4.16)^{b}	35.51 (6.04)^{bc}	69.76 (8.41)^{b}	73.02 (8.60)^{b}
T9	*P. fluorescens* (10^8 cfu ml^{-1})	12.98 (3.74)^{b}	26.45 (5.24)^{cd}	46.54 (6.89)^{c}	53.23 (7.36) ^{cd}
T10	*C. flaccumfaciens* (10^8 cfu ml^{-1})	5.47 (2.54)^{c}	12.97 (3.79)^{ef}	36.51 (6.12)^{cd}	36.51 (6.12)^{ef}
T11	*B. megaterium* (10^8 cfu ml^{-1})	0 (1)^{d}	15.54 (4.07)^{de}	26.45 (5.24)^{ef}	33.18 (5.85)^{f}
T12	Chemical control (Chlorantraniliprole 18.5 SC, 0.006 %)	76.59 (8.81)^{a}	100 (10.05) ^{a}	100 (10.05) ^{a}	100 (10.05) ^{a}
T13	Untreated control	0 (1)^{d}	0 (1)^{h}	0 (1)^{h}	0 (1)^{i}
	CD (0.05)	1.38	1.44	0.87	0.84

*Mean of 3 replications

Values in the parenthesis are √x+1 transformed; figures followed by same letter in a column do not differ significantly

B. megaterium was found to be on par with *P. fluorescens* (Deepa pseudo®) while *Microbacterium* sp. and *C. flaccumfaciens* were on par with *B. thuringiensis. M. anisopliae* as well as *B. bassiana* treatments caused less mortality in epilachna grubs (5.47 and 2.14 per cent respectively) at 3 DAT.

At 5 DAT, *S. marcescens* recorded 93.27 per cent mortality in epilachna grubs and was found to be on par with chemical control (Chlorantraniliprole 18.5 SC). *P. aeruginosa* followed this treatment with a mortality of 69.76 per cent. *P. fluorescens* isolated from the phylloplane was the next best treatment recording 46.54 per cent mortality at 5 DAT. All other treatments caused a mortality of less than 40 per cent. *C. flaccumfaciens* (36.51 per cent mortality) was found to be on par with *P. fluorescens* (Deepa pseudo®) and *B. megaterium* was on par with *B. bassiana* each recording a mortality of 26.45 per cent. A mortality of 32.59 and 20 per cent were noted in case of *M. anisopliae* and *B. thuringiensis* respectively at 5 DAT.

There was no change in the mortality percentage caused by *S. marcescens* (93.27) at 7 days of treatment while remained on par with chemical control (Chlorantraniliprole 18.5 SC). The mortality resulted by isolated 9 increased to 73.02 per cent making it the third best treatment. These treatments were followed by *M. anisopliae* causing a mortality of 59.72 per cent and phylloplane isolated *P. fluorescens* recording 53.23 per cent. The treatments *B. bassiana* and *P. fluorescens* (Deepa pseudo®) were on par causing a mortality of 46.55 and 46.23 per cent respectively. *C. flaccumfaciens*, *B. megaterium*, *Microbacterium* sp. and *B. thuringiensis* were able to cause a mortality of 36.51, 33.18, 30 and 23.11 per cent respectively after 7 days of treatment. All treatments except *L. lecanii* (0% mortality) were found superior to untreated check (0% mortality) at 1, 3, 5 and 7 DAT.

As in laboratory trial, in pot culture also *S. marcescens* was found to be superior with *P. aeruginosa* being the next best alternative in causing mortality to

epilachna grubs. In both cases *L. lecanii* was found to be inferior in causing mortality of epilachna grubs.

4.6.2 Mean population of epilachna grubs after treatment

The mean population of epilachna grubs on plants after treatment with different phylloplane and pathogenic microorganisms at different time intervals is presented in Table 16. Post-treatment counts of epilachna grubs were statistically adjusted with pre-treatment so as to make it non - significant. 24 h after treatment chemical control (Chlorantraniliprole 18.5 SC) recorded the lowest mean population of epilachna beetle of 2.31 per plant. The next best treatment was that of *S. marcescens* which recorded 8.00 grubs per plant. This treatment was found to be on par with *P. aeruginosa* treated ones constituting 8.33 grubs per plant. A mean population of 9.33 grubs per plant was observed in plants treated with isolates 1 and 16 and the treatment was found to be on par. All the other treatments proved inferior having 10 grubs per plant at 24 h of treatment.

Three days after treatment the population of epilachna grubs were reduced to zero in chemical (Chlorantraniliprole 18.5 SC) treated plants. Plants sprayed with *S. marcescens* was proved to be the second best treatment recording 5.32 grubs per plant and *P. aeruginosa* treated one was the third best with a mean population of 6.93 grubs. *B. megaterium* treated plants having a mean population of 8.31 grubs per plant was on par with *P. fluorescens* (Deepa pseudo®) with a population of 8.33. *C. flaccumfaciens.* was on par with *M. anisopliae*, *B. bassiana* and *B. thuringiensis* treatments with a mean population of 8.66, 9.33, 9.66 and 8.66 grubs per plant. The population levels in plants treated with *L. lecanii* and untreated control were significantly higher than all other treatments at 5 DAT.

At 5 days of treatment chemical control (Chlorantraniliprole 18.5 SC) was superior followed by *S. marcescens* with a mean population of 0 and 0.63 grubs per

Table 16. Mean population of Epilachna, *H. vigintioctopunctata* grubs on brinjal at different time intervals after treatment with different pathogenic and phylloplane microorganism

Treatments		Mean population *				
		1DAT	3 DAT	5 DAT	7 DAT	14 DAT
T1	*M. anisopliae* (10^8 cfu ml^{-1})	10 (3.32)^{a}	9.33 (3.21)^{ab}	6.64 (2.76)^{cde}	12.13 (3.62)^{abc}	17.73 (4.33)^{abc}
T2	*B. bassiana* (10^8cfu ml^{-1})	10 (3.32)^{a}	9.66 (3.27)^{ab}	7.33 (2.89)^{cd}	16.18 (4.15)^{ab}	22.32 (4.83)^{abc}
T3	*L. lecanii* (10^8 cfu ml^{-1})	10 (3.32)^{a}	10 (3.32)^{a}	9 (3.16)^{ab}	13.48 (3.81)^{abc}	23.72 (4.97)^{ab}
T4	*B. Thuringiensis* (0.25 %)	10 (3.32)^{a}	8.66 (3.11)^{ab}	8 (3)^{bc}	13.58 (3.82)^{abc}	24.18 (5.02)^{ab}
T5	*P. fluorescens* (Deepa pseudo®) (2 %)	10 (3.32)^{a}	8.33 (3.05)^{bc}	5.98 (2.64)^{ef}	11.11 (3.48)^{abc}	18.96 (4.47) ^{abc}
T6	*Microbacterium* sp. (10^8 cfu ml^{-1})	9.33 (3.21)^{ab}	8.66 (3.11)^{ab}	7.33 (2.89)^{cd}	18.19 (4.36)^{a}	21.33 (4.73) ^{abc}
T7	*S. marcescens* (10^8 cfu ml^{-1})	8 (3)^{c}	5.32 (2.51)^{e}	0.63 (1.28)^{h}	7.54 (2.92)^{c}	12.92 (3.73)^{c}
T8	*P. aeruginosa* (10^8 cfu ml^{-1})	8.33 (3.05)^{c}	6.93 (2.82)^{d}	2.96 (1.99)^{g}	9.86 (3.29)^{bc}	15.64 (4.08) ^{bc}
T9	*P. fluorescens* (10^8 cfu ml^{-1})	8.66 (3.10)^{bc}	7.33 (2.89)^{cd}	5.32 (2.51)^{f}	10.80 (3.44)^{abc}	18.43 (4.41)^{abc}
T10	*C. flaccumfaciens* (10^8 cfu ml^{-1})	9.33 (3.21)^{ab}	8.66 (3.11)^{ab}	6.33 (2.70)^{def}	18.26 (4.44)^{a}	28.49 (5.43)^{a}
T11	*B. megaterium*(10^8 cfu ml^{-1})	10 (3.32)^{a}	8.31 (3.05)^{bc}	7.33 (2.89)^{cd}	18.19 (4.36)^{a}	22.78 (4.88)^{abc}
T12	Chemical control(Chlorantraniliprole 18.5 SC, 0.006 %)	2.31 (1.82)^{d}	0 (1)^{f}	0 (1)^{i}	2.05 (1.75)^{d}	5.14 (2.48)^{d}
T13	Untreated control	10 (3.32)^{a}	10 (3.32)^{a}	10 (3.32)^{a}	18.26 (4.44)^{a}	24.46 (5.05)^{ab}
	CD (0.05)	0.11	0.21	0.23	0.99	1.22

*Mean of 3 replications (Adjusted with pre-treatment population)

Values in the parentheses are √x+1 transformed

plant. *P. aeruginosa* with 2.96 grubs per plant followed the treatment. A mean population of 5.32, 5.98, 6.33, 6.64, 8.00 and 9.00 were recorded from phylloplane isolated *P. fluorescens*, *P. fluorescens* (Deepa pseudo®), *C. flaccumfaciens*, *M. anisopliae*, *B. thuringiensis* and *L. lecanii* respectively. *Microbacterium* sp. and *B. megaterium* were found to be on par with *B. bassiana* with a mean population of 7.33 grubs each at 5 DAT.

As stated earlier, chemical control was superior over other treatments with 2.05 grubs per plant followed by *S. marcescens* with 7.54 grubs and *P. aeruginosa* having 9.86 grubs per plant. Phylloplane isolated *P. fluorescens* (10.80 grubs per plant) was found to be on par with *P. fluorescens* (Deepa pseudo®), *M. anisopliae*, *L. lecanii* and *B. thuringiensis* with a population of 11.11, 12.13, 13.48 and 13.58 grubs per plant respectively. The mean population on the control plants increased to 18.26 after 7 days of treatment.

By the end of 14 days of treatment the control plants attained a mean population of 24.46 grubs per plant which was almost five times greater than that in the chemical (Chlorantraniliprole 18.5 SC) treated plants (5.14 grubs per plant). The population count in *L. lecanii* (23.72) and *B. thuringiensis* (24.18) treated plants were found to be on par with that of control treatment. The mean population in plants treated with *S. marcescens* reached 12.92 per plant making it second best to chemical control followed by *P. aeruginosa* with 15.64 grubs per plant. The population level in *M. anisopliae*, *P. fluorescens* (phylloplane isolate), *P. fluorescens* (Deepa pseudo®), *Microbacterium* sp., *B. bassiana* and *B. megaterium* were 17.73, 18.43, 18.96, 21.33, 22.32 and 22.78 respectively.

4.6.3 Leaf area damage caused by epilachna grubs

The leaf area damage caused by epilachna grubs on brinjal plants treated with different phylloplane and pathogenic microorganisms are indicated in Table 17. The

Table 17. Leaf area damaged by Epilachna, *H. vigintioctopunctata* grubs on plants treated with different pathogenic and phylloplane microorganisms

Treatments		Per cent leaf area damage*			
		1DAT	3 DAT	5 DAT	7 DAT
T1	*M. anisopliae* (10^8 cfu ml^{-1})	59.73 (50.61)^{c}	74.73 (48.35)^{d}	45.62 (42.48)^{d}	35.13(36.35)^{d}
T2	*B. bassiana* (10^8 cfu ml^{-1})	51.08 (45.62)^{c}	70.55 (44.87)^{d}	42.75 (40.83)^{d}	32.42 (34.71)^{d}
T3	*L. lecanii* (10^8 cfu ml^{-1})	81.79 (64.74)^{b}	96.26 (74.28)^{a}	83.98 (66.41)^{b}	73.75 (59.18)^{b}
T4	*B. thuringiensis* (0.25 %)	73.97 (59.32)^{b}	86.98 (60.43)^{bc}	65.40 (53.97)^{c}	55.93 (48.41)^{c}
T5	*P. fluorescens* (Deepa pseudo®) (2 %)	76.70 (61.14)^{b}	88.14 (61.81)^{bc}	67.38 (55.17)^{c}	57.26 (49.18)^{c}
T6	*Microbacterium* sp. (10^8 cfu ml^{-1})	80.07 (63.49)^{b}	90.15 (64.35)^{b}	71.40 (57.67)^{c}	60.89 (51.46)^{c}
T7	*S. marcescens*(10^8 cfu ml^{-1})	38.57 (38.39)^{d}	47.28 (28.22)^{e}	18.71 (25.63)^{e}	12.13 (20.38)^{f}
T8	*P. aeruginosa* (10^8 cfu ml^{-1})	47.99 (43.85)^{cd}	50.10 (30.06)^{e}	19.58 (26.26)^{e}	15.25 (22.99)^{ef}
T9	*P. fluorescens* (10^8 cfu ml^{-1})	56.50 (48.73)^{c}	52.15 (31.43)^{e}	25.43 (30.28)^{e}	18.05 (25.14)^{e}
T10	*C. flaccumfaciens* (10^8 cfu ml^{-1})	80.27 (63.63)^{b}	89.42 (63.40)^{b}	71.14 (57.67)^{c}	61.18 (51.46)^{c}
T11	*B. megaterium* (10^8 cfu ml^{-1})	75.55 (60.37)^{b}	83.75 (56.88)^{c}	66.21 (54.46)^{c}	56.32 (48.63)^{c}
T12	Chemical control(Chlorantraniliprole 18.5 SC, 0.006 %)	11.34 (19.68)^{e}	0.0 (0)^{f}	0.0 (0)^{f}	0.0 (0)^{g}
T13	Untreated control	91.45 (72.99)^{a}	96.68 (75.19)^{a}	97.54 (80.97)^{a}	98.39 (82.71)^{a}
	CD (0.05)	7.07	5.54	5.17	4.17

*Mean of 3 replications

Values in the parentheses are arc transformed

per cent damage was the mean of 3 replications each containing 6 leaves (2 from upper, middle and lower strata of each plant).

Chemical control (Chlorantraniliprole 18.5 SC) was significantly superior over all other treatments at time intervals of 1, 3, 5 and 7 days after treatment. *S. marcescens* followed this recording per cent damage of 38.57, 47.28, 18.71 and 12.13 at 1, 3, 5 and 7 days after treatment. The leaf area damage of 47.99 per cent was seen in *P. aeruginosa* treated plants at 24 h after treatment. Treatment with phylloplane isolated *P. fluorescens* (56.50 per cent) was observed to be on par with that of *B. bassiana* and *M. anisopliae.* All the other treatments except *B. thuringiensis*, *B. megaterium*, *P. fluorescens*, *Microbacterium* sp., *C. flaccumfaciens* and *L. lecanii* were statistically on par causing per cent leaf area damage of 73.97, 75.55, 76.70, 80.07, 80.27 and 81.79 respectively after 24 h of treatment.

At 3 DAT the leaf area damage in *S. marcescens* treated plants (47.28) was found to be statistically on par with that of *P. aeruginosa* (50.10) and phylloplane isolated *P. fluorescens* (52.15) treated ones and were next best treatment to chemical control. Leaf area damage by *B. bassiana* and *M. anisopliae* treatments were on par (70.55 and 74.73 per cent respectively). *B. megaterium* recorded a damage of 83.75 per cent. This was followed by *B. thuringiensis* (86.98 per cent) and *P. fluorescens* (Deepa pseudo®) (88.14 per cent) treatments which were on par. There was no significant difference in the damage caused by treatments with *C. flaccumfaciens* (89.42 per cent) and *Microbacterium* sp. (90.15 per cent) at 3 DAT. The damage caused on *L. lecanii* treated plants was on par with that of untreated control (96.26 and 96.68 per cent respectively).

After 5 days, the treatments *S. marcescens*, *P. aeruginosa*, and phylloplane isolated *P. fluorescens* were on par with per cent leaf area damage of 18.71, 19.58 and 25.43. 42.75 per cent leaf damage observed in *M. anisopliae* treatment was on

par with 45.62 per cent caused in *B. bassiana.* The leaf area damage in *B. thuringiensis*, *B. megaterium*, *P. fluorescens* (Deepa pseudo®), *C. flaccumfaciens* and *Microbacterium* sp. treatments were on par with each other recording 65.40, 66.21, 67.38, 71.14 and 71.40 per cent respectively. *L lecanii* with 83.98 per leaf area damage was the last but second treatment 5 days after treatment while damage in untreated control was 97.54 per cent at the same time frame.

It could be noted that leaf area damage caused by epilachna grubs reduced significantly at 7 DAT in isolates 3 (12.13 per cent), *P. aeruginosa* (15.25 per cent) and *P. fluorescens* (18.05 per cent) treated plants. The percentage leaf area damage produced by *M. anisopliae* (35.13) was on par with *B. bassiana* (32.42). The treatments *B. thuringiensis* (55.93 per cent), *B. megaterium* (56.32 per cent), *P. fluorescens* (Deepa pseudo®) (57.26 per cent), *Microbacterium* sp. (60.89 per cent) and *C. flaccumfaciens* (61.18 per cent) were statistically on par and superior over *L. lecanii* (73.75 per cent) and untreated plants (98.39 per cent) after 7 days of treatment. All treatments except *L. lecanii* at 3 DAT were statistically superior over untreated check at 1, 3, 5 and 7 DAT.

4.6.4 Mean population of other pests

The mean population of other pests of brinjal treated with different phylloplane and pathogenic microorganisms in pot culture experiment during the time of study were assessed and recorded in Table 18.

The various treatments did not show any significant difference in mean population of *Leucinodes orbonalis* and *Bemisia tabaci* at 5 DAT. In case of *Antoba olevaceae* the mean population was found to be 0.29 insects per plant in both *S. marcescens* and *B. bassiana* treatments which was on par with *P. aeruginosa*, phylloplane isolated *P. fluorescens* and chemical control (Chlorantraniliprole) each having a population of 0.63 insects per plant at 5 DAT. These treatments were

Table 18. Mean population of other pests in brinjal with different pathogenic and phylloplane microorganism

	Treatments	*Leucinodes orbonalis*	*Antoba olevaceae*	*Aphis gossypii*	*Bemisia tabaci*	*Coccidohystrix insolitus*
		5 DAT *	5 DAT *	5 DAT *	5 DAT *	5 DAT *
T1	*M. anisoplia* (10^8 cfu ml^{-1})	1 (1.41)	1.64 (1.63)^{bcd}	19.93 (4.57)^{cde}	12.84 (3.72)	2.73 (1.93)^{bcd}
T2	*B. bassiana*(10^8 cfu ml^{-1})	0.63 (1.28)	0.29 (1.14)^{d}	17.41 (4.29)^{def}	12.49 (3.67)	1.59 (1.61)^{cd}
T3	*L. lecanii* (10^8 cfu ml^{-1})	0.63 (1.28)	1.64 (1.63)^{bcd}	10.48 (3.39)^{ef}	4.73 (2.39)	0.29 (1.14)^{d}
T4	*B. thuringiensis* (0.25 %)	0.29 (1.14)	1 (1.41)^{cd}	26.68 (5.26)^{bcde}	5.43 (2.53)	33.55 (5.88)^{a}
T5	*P. fluorescens* (Deepa pseudo®) (2 %)	0.91 (1.38)	4.18 (2.26)^{a}	46.82 (6.91)^{ab}	13.14 (3.76)	12.14 (3.62)^{abcd}
T6	*Microbacterium* sp. (10^8 cfu ml^{-1})	0.63 (1.28)	3.62 (2.15)^{ab}	52.61 (7.32)^{ab}	6.74 (2.78)	11.54 (3.54)^{abcd}
T7	*S. marcescens* (10^8 cfu ml^{-1})	0.55 (1.24)	0.29 (1.14)^{d}	42.86 (6.62)^{abc}	12.41 (3.66)	14.52 (3.94)^{abcd}
T8	*P. aeruginosa* (10^8 cfu ml-1)	0.77 1.33)	0.63 (1.28)^{d}	56.27 (7.57)^{a}	10.56 (3.40)	21.13 (4.70)^{ab}
T9	*P. fluorescens* (10^8 cfu ml^{-1})	0.63 (1.28)	0.63 (1.28)^{d}	59.83 (7.79)^{a}	12.27 (3.64)	19.2 (4.49)^{abc}
T10	*C. flaccumfaciens* (10^8 cfu ml-^{1})	0.91 (1.38)	4.26 (2.29)^{a}	35.99 (6.08)^{abcd}	14.52 (3.94)	24.41 (5.04)^{a}
T11	*B. megaterium* (10^8 cfu ml^{-1})	0.63 (1.28)	2.48 (1.87)^{abc}	34.99 (5.99)^{abcd}	7.41 (2.90)	21.11 (4.70)^{ab}
T12	Chemical control(Chlorantraniliprole 18.5 SC, 0.006 %)	1.16 (1.47)	0.63 (1.28)^{d}	5.44 (2.54)^{f}	2.88 (1.97)	0.29 (1.14)^{d}
T13	Untreated control	1.31 (1.52)	2.48 (1.87)^{abc}	54.24 (7.43)^{a}	18.11 (4.37)	22.21 (4.82)^{ab}
	CD (0.05)	NS	0.54	2.09	NS	3.01

*Adjusted with pre-treatment population

Values in the parentheses are √x+1 transformed

followed by *B. thuringiensis* (1), *L. lecanii* (1.64) and *M. anisopliae* (1.64). Approximately, the mean per plant population of *Antoba* sp. in plants treated with *B. megaterium* (2.48) was on par with untreated check (2.48). Plants treated with *Microbacterium* sp. retained a per plant population of 3.62 followed by *P. fluorescens* (Deepa pseudo®) and isolate 6 having 4.18 and 4.26 insects per plant 5 days after treatment.

The population of *Aphis gossypii* was kept under check by chemical control having 5.44 insects per plant and was observed to be superior over other treatments. This was followed by *L. lecanii* with a population of 10.48 per plant. *B. bassiana* and *M. anisopliae* recorded a mean population of 17.41 and 19.93 aphids per plant. These treatments were followed by *B. thuringiensis*, *B. megaterium*, *C. flaccumfaciens, S. marcescens*, *P. fluorescens* (Deepa pseudo®) and *Microbacterium* sp. having mean population of 26.68, 34.99, 35.99, 42.86, 46.82 and 52.61 aphids per plant. The control treatment had a population count of 54.24 aphids per plant.

The mean population of *Coccidohystrix insollitus* (mealy bug) was observed to be minimum in plants treated with chemical control (Chlorantraniliprole 18.5 SC) and was found to be on par with *L. lecanii* each having a population of 0.29 mealy bugs per plant after 5 days of treatment. The treatment was followed by *B. bassiana* (1.59) and *M. anisopliae* (2.73). The treatments *Microbacterium* sp., *P. fluorescens* (Deepa pseudo®) and *S. marcescens* were on par recording a mean population of 11.54, 12.14 and 14.52 insects per plant at 5 DAT. Phylloplane isolated *P. fluorescens* had a population of 19.2. The mean population was found to be on par in plants treated with *B. megaterium* (21.11), *P. aeruginosa* (21.13) and untreated check (22.21). *C. flaccumfaciens* and *B. thuringiensis* retained a population of 24.41 and 33.55 insects in the treated plants.

4.6.5 Yield

The per plant yield of brinjal treated with various phylloplane and pathogenic microorganisms are presented in Table 19.

A superior yield of 0.58 kg per plant each was obtained from plants treated with *M. anisopliae* and *L. lecanii.* This was followed by *B. thuringiensis,* B. *bassiana*, and chemical treated plants recording an yield of 0.48, 0.47 and 0.48 kg plant^{-1}. All the other treatments were found to be on par recoding 0.44, 0.41, 0.41, 0.41, 0.39, 0.39, 0.38 and 0.35 kg plant^{-1} in treatments *P. fluorescens* (Deepa pseudo®)*, P. aeruginosa*, untreated check, phylloplane isolated *P. fluorescens*, *B. megaterium*, *S. marcescens* and *Microbacterium* sp. respectively. Though *S. marcescens* had a batter pest control potential, its quality was not reflected in the yield. Because of unexpected attack of Giant African snail during fruiting phase which destroyed a large proportion of fruits.

Table 19. Yield of brinjal treated with different pathogenic and phylloplane microorganisms

	Treatments	Yield (kg/plant)*
T1	*M. anisopliae* (10^8 cfu ml-1)	0.58^{a}
T2	*B. bassiana* (10^8 cfu ml-1)	0.47^{ab}
T3	*L. lecanii* (10^8 cfu ml-1)	0.58^{a}
T4	*B. thuringiensis* (0.25 %)	0.48^{ab}
T5	*P. fluorescens*(Deepa pseudo®) (2 %)	0.44^{b}
T6	*Microbacterium* sp. (10^8 cfu ml-1)	0.35^{b}
T7	*S. marcescens* (10^8 cfu ml-1)	0.38^{b}
T8	*P. aeruginosa* (10^8 cfu ml-1)	0.41^{b}
T9	*P. fluorescens* (10^8 cfu ml-1)	0.39^{b}
T10	*C. flaccumfaciens* (10^8 cfu ml-1)	0.41^{b}
T11	*B. megaterium* (10^8 cfu ml-1)	0.39^{b}
T12	Chemical control (Chlorantraniliprole 18.5 SC, 0.006 %)	0.48^{ab}
T13	Untreated control	0.41^{b}
	CD (0.05)	0.13

*Mean of 3 replications

Values in the parentheses are $\sqrt{x+1}$ transformed

Discussion

5. DISCUSSION

Epilachna beetle, *Henosepilachna vigintioctopunctata* (Fab.) is a vicious pest that infests plants belonging to solanaceous group (Sreedevi *et al.,* 1993, Omprakash and Raju, 2014a). Both adults and grubs feed on foliage of brinjal causing substantial yield reduction (Bhagat and munshi, 2004). The incidence of epilachna beetle was monitored in the present study from three isolated brinjal growing plots within the instructional farm of College of Agriculture, Vellayani during July 2014 – June 2015, as part of isolating disease causing microorganisms.

Population of different stages of epilachna beetle reached its peak during the summer months of March to May (Figure 1) during which the mean maximum temperature remained high and relative humidity and rainfall remained low. This suggests that hot summer months with low RH was congenial for growth and multiplication of this insect. The results of the study was in agreement with reports of several researchers who observed that epilachna beetle population attained its peak during the months of March – April and declined thereafter with the onset of rains in June (Raghuraman and Veeravel, 1999; Muthukumar and Kalyansundaram, 2003; Shaw and Dutta, 2004; Hasseb *et al.*, 2009). It was noted that the mean population of the insect had a positive correlation with maximum and minimum temperature and negative correlation with RH and rainfall. This was in accordance with the results obtained earlier (Raghuraman and Veeravel, 1999; Muthukumar and Kalyansundaram, 2003; Omprakash and Raju, 2014b).

The population of pest remained low from June to January and increased thereafter. An increase in population of pest was noticed from January onwards. Parasitization of eggs and pupae of epilachna beetle which remained low also increased from January. Maximum egg and pupal parasitization was noticed during January – March (Figure 2). A decline in parasitic activity was noticed from second

Figure 1. Seasonal incidence of Epilachna beetle, *H. vigintioctopunctata* in brinjal

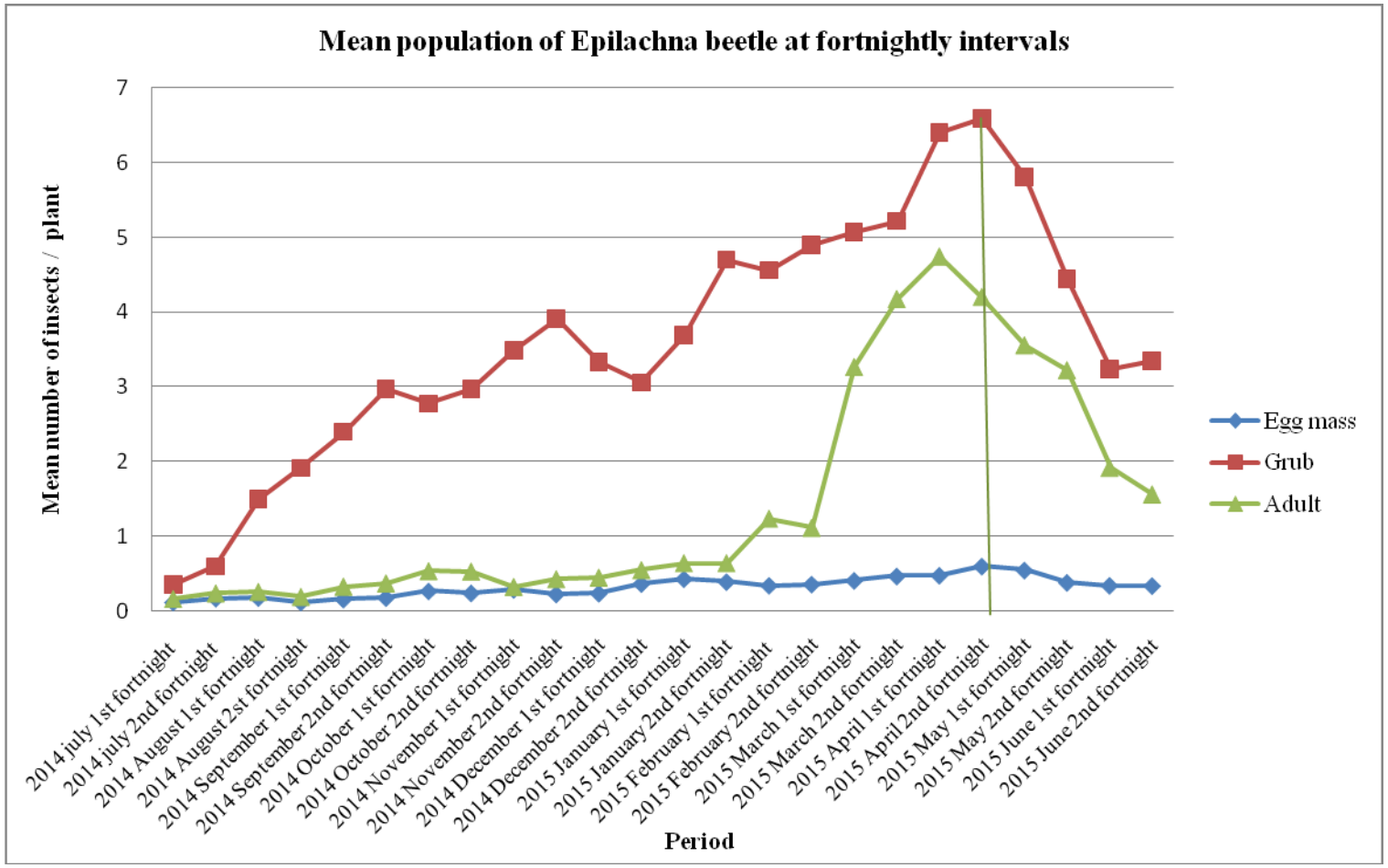
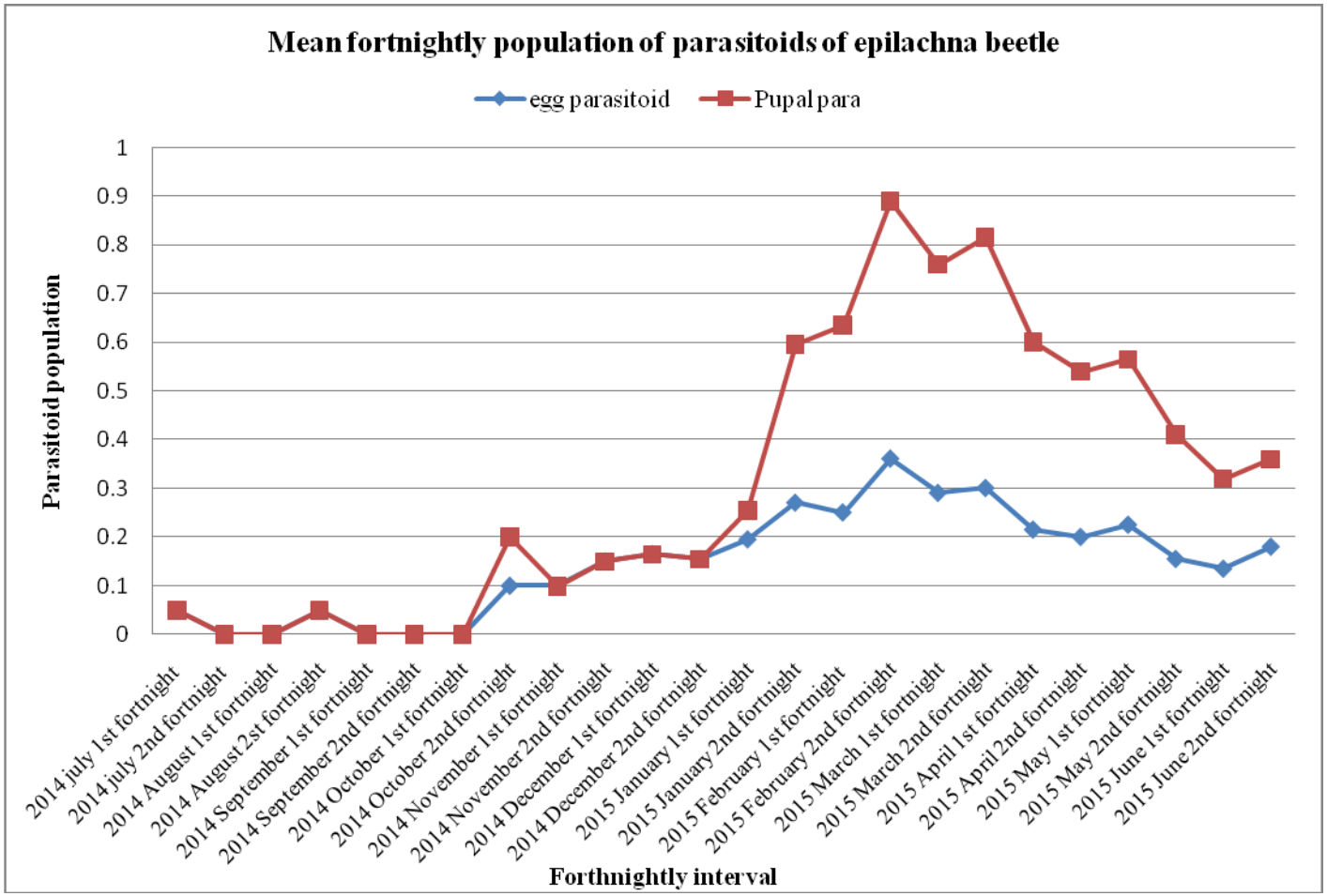


Figure 2. Extend of parasitization of Epilachna beetle, *H. vigintioctopunctata*



fortnight of March with a corresponding increase in population of the pest whichpeeked during April, suggesting the population regulation role of parasitoids. Both pest and parasitic activity decreased with the onset of monsoon. Parasitization, particularly by egg and larval-pupal parasitiods were identified as a major population regulation factor of epilachna beetle under natural conditions (Halder and Srinivasan, 2011). Varma and Anandhi (2008), reported highest activities of different parasitoids (*Tetrastichus* sp., *Pediobius foveolatus* and *Brachymeria* sp.) during February. This information will be helpful in devising timely management strategies for the pest during March – April when population reaches maximum with decline in parasitic activity.

Quick action and easiness in application make chemical pesticides the most attractive option to manage epilachna beetle. Several workers suggested different insecticides for the control of *H. vigintioctopunctata* (Peter and Govindarajalu, 1989; Rao *et al.*, 1989; Thomas and Jacob, 1991; Umapathy and Baskaran, 1991; Mala *et al.*, 1992; Rao *et al.*, 1992; Prasad and Logiswaran, 1993; Gupta and Dogra, 1994; Reddy, 1997; Rai *et al.*, 2014). But the emerging issues of resistance of pests to insecticides, resurgence and unseen health hazards calls for a change. Epilachna beetle is reported have developed resistance against many commercially available pesticides (Jeyasanker *et al.*, 2014), warranting investigations on alternate control strategies. Better efficacy and safety makes microbial control an attractive alternative to chemical pesticides. A search for naturally occurring microorganisms capable of causing diseases in epilachna beetle was done in the present study. Monitoring the naturally occurring diseases in epilachna beetle during July 2014 – June 2015 revealed low incidence of diseases. However, twelve entomopathogenic microorganisms (eleven bacteria and one fungus) were obtained from field collected dead epilachna grubs.

Epilachna beetle being an exposed leaf surface feeder constantly ingest phylloplane microorganisms that stably colonize the surface of plant leaves. Some of the phylloplane microorganisms, capable of producing chitinase are reported to suppress insect defoliators (Andrews, 1992). So, isolation and characterization of phylloplane microorganisms were also attempted in the present study in addition to isolation of entomopathogens. Phylloplane microorganisms were isolated from brinjal leaf surface by stamping the lower side of leaves on minimal media containing chitin as the sole carbon source. The microorganisms which subsequently grew on minimal media were isolated. Six phylloplane bacteria were obtained.

All eighteen microorganisms isolated were serially numbered as Isolate 1 to Isolate 18. Out of the eleven bacterial isolates obtained from diseased grubs only three were found to be pathogenic to epilachna beetle in the preliminary screening. The lone fungal agent obtained was found to be non infective to the test insect and hence discarded. Out of the six phylloplane bacteria isolated three were infective and caused mortality in epilachna grubs. These six infective isolates (isolates 1, 3 and 9 from diseased epilachna grubs and isolates 13, 16 and 18 from phylloplane) were brought to pure culture and Koch's postulates were proved.

The insects infected with all the isolates exhibited liquefaction of body content and colour change from light to dark. The treated grubs shrinked in size following oozing out of body content with foul odour. These disease symptoms shown by the infected insects were typical to bacterial infections. Insects killed by bacteria rapidly darken in colour and often become very soft. Internal tissues and organs are rapidly broken down to viscid consistency accompanied by a putrid odour (Tanada and Kaya, 1993). Grubs treated with isolate 1 swelled prior to oozing out of body content. Isolate 3 treated grubs turned reddish - orange and expelled reddish - orange fluid from the body. Amber dicolouration is typical to infection by *Serratia* sp. (Tanada and Kaya, 1993).

The pathogenic isolates of bacteria obtained from the grubs (1, 3 and 9) and phylloplane (13, 16 and 18) were characterized morphologically and biochemically with an intension of utilizing them for management of epilachna beetle.

The isolates differed in their growth pattern and colony morphology. Isolates 1 and 16 produced bright yellow colonies with dawdling growth while, Isolates 9 and 13 developed pale yellow colonies. Isolate 9 was spreading and turned green after two days. Isolate 9 and 13 also exhibited fluorescens under UV Transilluminator GeNei®. Isolate 3 produced bright red colonies which rapidly grew on NA plates. Isolate 18 had cream to white rough colonies with wavy margins.

Upon Gram's staining, isolates 1, 16 and 18 were positive forming purple coloured cells when observed under oil immersion objective (100X), while isolates 3, 9 and 13 were negative producing pink colour.

Isolate 1, 3, 9, 13, 16 and 18 were identified as *Microbacterium* sp., *Serratia marcescens*, *Pseudomonas aeruginosa, P. fluorescens*, *C. flaccumfaciens* and *Bacillus megaterium* respectively based 16s rRNA sequence homology. *Serratia* is reported to infest over 70 insect species in Orthoptera, Isoptera, Coleoptera, Lepidoptera, Diptera and Hymenoptera (Tanada and Kaya, 1993). *Pseudomonas* sp. is a ubiquitous bacterium that orally infects larvae of insects in different orders determining extensive gut cell damages (Ruiu, 2015). *Bacillus megaterium* is reported to be pathogenic to eggs of European corn borer *Ostrinia nubilalis* (Lynch *et al.*, 1976).

Bacterial invasion to healthy insects is possible mainly through the midgut region since all the exterior regions as well as foregut and hindgut of alimentary are protected with chitinous exoskeleton. The cylindrical chitinous peritrophic membrane present in the midgut region of chewing insects act as a mechanical barrier to protect the midgut epithelial cells from pathogenic microorganisms ingested along

with food (Brandt *et al*., 1978; Pimenta *et al.,* 1997). Since breaching of the chitinous peritrophic membrane is essential to make a bacterial pathogen invasive (Wu *et al*., 2010), detection of chitinolytic enzyme production by the isolated bacteria was attempted by growing them in culture media containing colloidal chitin as the sole carbon source.

S. marcescens, *P. aeruginosa*, *P. fluorescens* and *B. megaterium* produced chitinase as evidenced by formation of clear transparent zones of varying diameter around the colony which is an indication of degradation of chitin in the media. Sowmya *et al.* (2012) observed clear zone formation around colonies of bacteria capable of producing chitinase upon culturing them in media containing chitin. Bacterial chitinase grouped under PR8, have exochitinolytic activity that enable them to degrade the chitinous exoskeleton of insect pests (Roberts and Selitrennikoff, 1988). Maximum clear halo having diameter of 1.8 cm was produced by *B. megaterium* followed by *P. aeruginosa*, *P. fluorescens* and *S. marcescens* producing halos of diameter of 1.3 cm, 0.7 cm and 0.4 cm respectively. Chitinase producing microbes have been used in the control of insect pests (Melchers and Stuiver 2000). These enzymes are produced by a number of entomopathogenic microorganisms including fungi such as *Metarhizium anisopliae*, *Beauveria bassiana* and bacteria namely *Bacillus cereus, Bacillus pumilus* and *Serratia marcescens* (Hamid *et al.*, 2013). Several bacterial species capable of invading insects including *Pseudomonas, Serratia* and *Bacillus* are known to produce chitinase in culture (Ordentlich *et al.*, 1988; Viswanathan and Samiyappan, 2001).

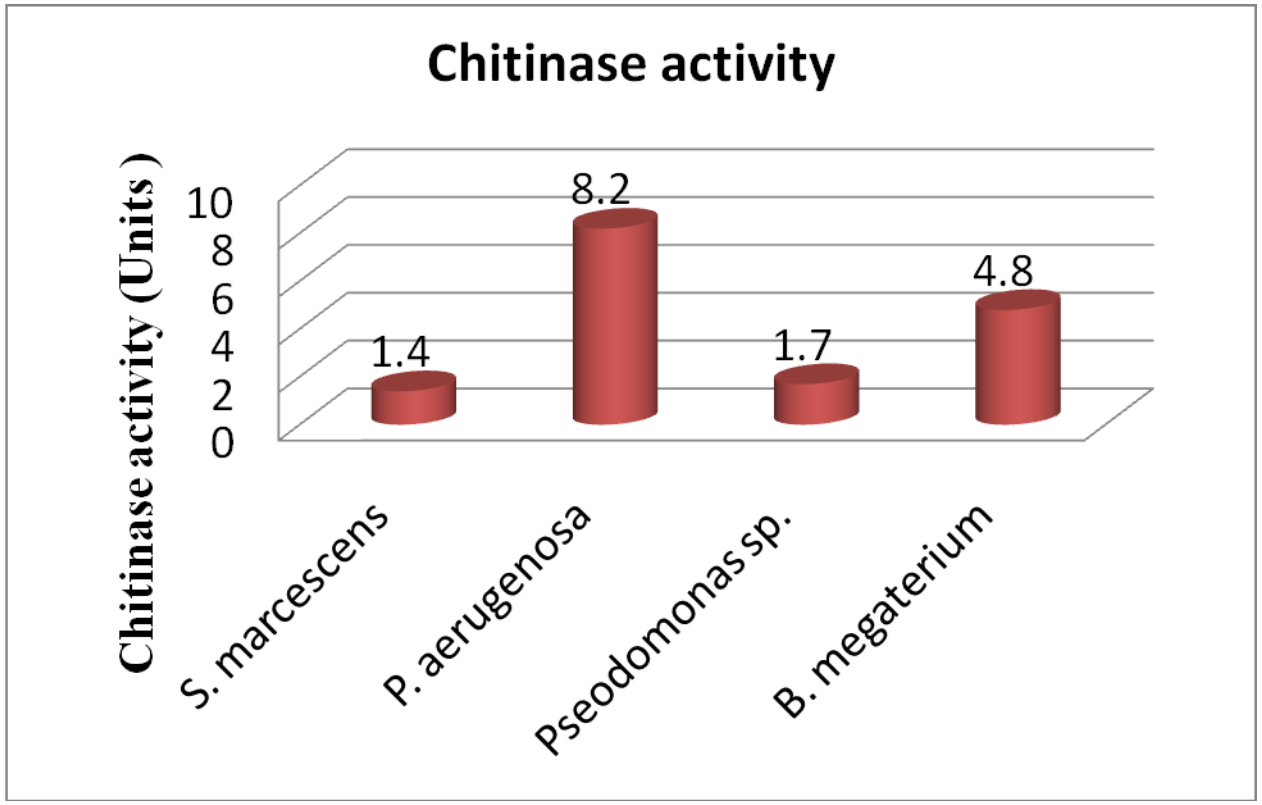
Since biocontrol efficiency have been correlated with chitinase production, the chitinase activity of bacteria obtained in the present study was quantified adopting the method of Ramírez *et al.* (2004) by mixing 48 h old culture broth containing bacterial chitinase with chitin-azure and 0.2 M sodium phosphate buffer (pH 7). The extracellular chitinase produced by the bacteria caused hydrolysis of chitin-azure,

releasing proportionately a violet coloured soluble dye whose absorbance was maximum at 560 nm which was evaluated spectrometrically. This one-step process of chitinase estimation showed that *P. aeruginosa* produced maximum chitinase of 8.2 units ml^{-1}. *B. megaterium* which generated the largest clear halo produced only 4.8 units ml^{-1} of chitinase indicating that diameter of halo produced is not in proportion to the quantity of chitinase. Ramírez *et al.* (2004), also observed differences in preliminary data generated by clear halo production and secondary information obtained through quantitative evaluation of chitinase. Both *P. fluorescens* and *S. marcescens* produced chitinase of 1.7 and 1.4 units ml^{-1} respectively (Figure 3).

Wang and Chang (1997), reported that *P. aeruginosa* K-187 is capable of secreting chitinase into the culture medium. *Serratia marcescens* MO-1, isolated from *Poecilimon tauricola* (Orthoptera: Tettigoniidae) exhibited a chitinase activity of 36.6 Units ml^{-1} (Okay *et al.*, 2013). Similar results were obtained by Wang *et al.*, (2014), who reported that *Serratia marcescens* JPP1 produced 23.09 units ml^{-1} of chitinase when grown in minimal medium containing 12. 7 g l^{-1} colloidal chitin. According to Aggarwal *et al.* (2015) Serratia marcescens strain SEN showed chitinase activity.

In the laboratory evaluation of different microbial control agents, epilachna larvae fed with *S. marcescens* produced 93.28 per cent mortality 5 DAT which was statistically on par with the 100 per cent mortality obtained in chemical treatment (Chlorantraniliprole 18.5 SC). *S. marcescens* is considered as potential entomopathogen and was reported to caused mortality in apple maggot fly, *Rhagoletis pomonella* (Lauzon *et al.*, 2003) and *Spodoptera litura* (Aggarwal 2015). In the present study *S. marcescens* could bring about considerable mortality (26.45 per cent) as early as 24 h after treatment. The production of prodigiosin pigment by *Serratia* might be one of the reasons for rapid death of the insect. Patel *et al.* (2011) for the first time reported the larvicidal activity of prodigiosin, the red colouring

Figure 3. Chitinase activity of different entomopathogenic and phylloplane bacteria



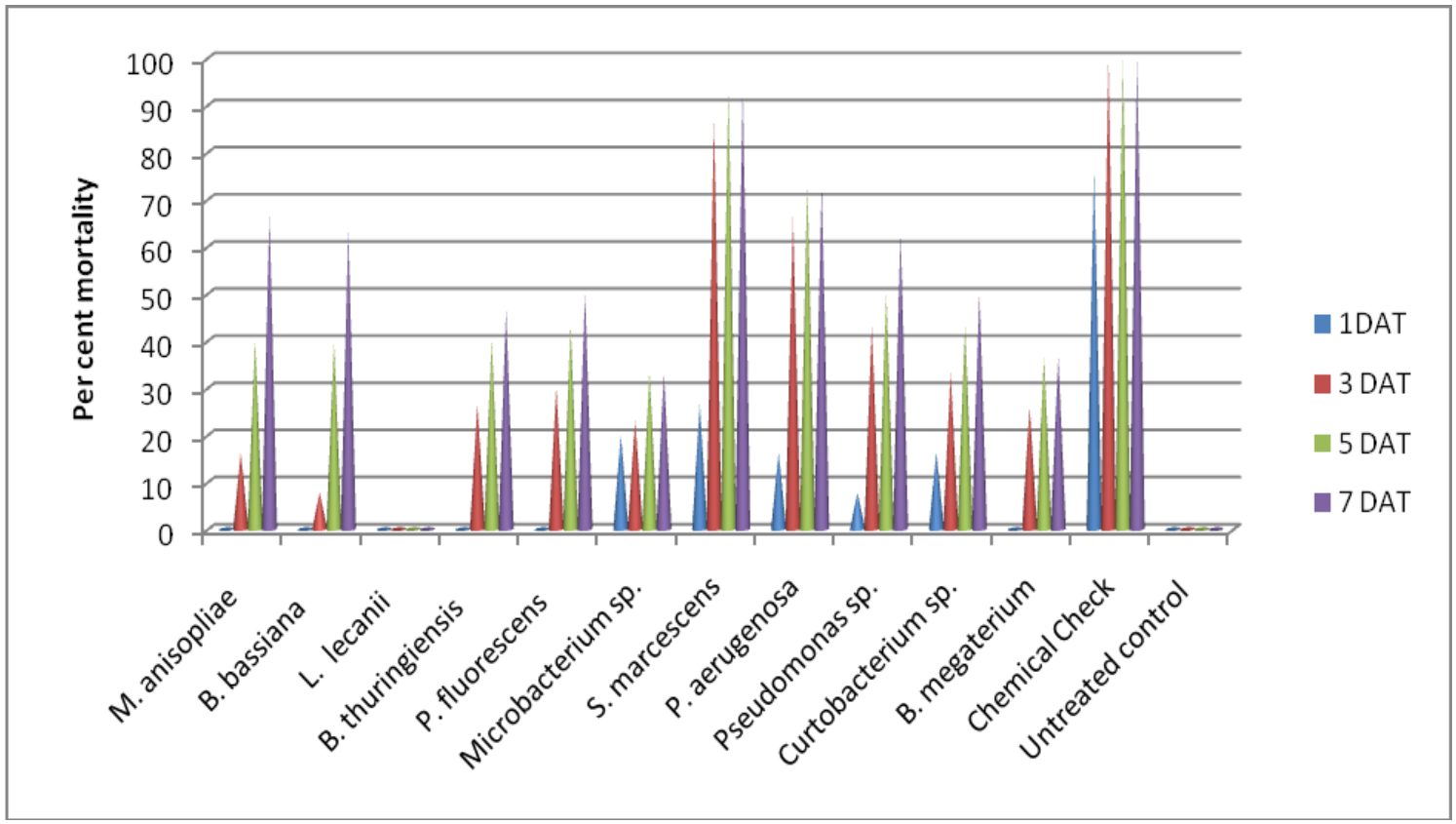
pigment produced by *S. marcescens*. Reports of Ishii *et al.* (2014), suggests that *S. marcescens* are competent in producing adhesion-inhibitory factors against the host immune-surveillance cells thereby curbing the host cellular immunity leading to fast death of the host.

Treatment with *P. aeruginosa* caused a mortality of 73.01 per cent at 5 DAT which was statistically superior to *P. fluorescens* (Deepa pseudo®), *M. anisopliae*, *B. thuringiensis*, *B. bassiana*, and *B. megaterium* which were on par with each other recording a mortality per cent of 43.21, 40, 40, 39.59 and 36.18 respectively (Figure 4). Within the family pseudomonadaceae, *P. aeruginosa* is the most often isolated one from insects and is highly pathogenic once within the haemocoel (Tanada and Kaya, 1993).

P. fluorescens obtained from the phylloplane was able to produce significantly superior mortality to epilachna beetle than other commercially used microbial control agents tested at 5 DAT. At 7 DAT it could produce a mortality of 63.25 per cent making it statistically on par with *P. aeruginosa*. It was reported by several researchers that *P. fluorescens* are successful in causing mortality in coleopteran insects (Sezen *et al.*, 2004; Sezen *et al.*, 2007). Otsu *et al* (2003), isolated a chitinolytic bacterium, *Alcaligenes paradoxus* KPM-012A from the phylloplane of tomato and used it to suppress leaf feeding and oviposition of ladybird beetle, *Epilachna vigintioctopunctata.*

None of the microbial treatments in the present study were effective against adult of epilachna except *M. anisopliae*, *B. bassiana* and phylloplane isolated *P. fluorescens* which produced slight mortality. Rajendran (2002) reported that *M. anisopliae* was not as potent against adults of epilachna beetle and caused only 17.4 per cent mortality.

Figure 4. Mortality of Epilachna, *H. vigintioctopunctata* grubs treated with different pathogenic and phylloplane microorganisms



Leaf area damage by epilachna beetle exposed to microbial treatments was also documented since suppression of leaf feeding by *E. vigintioctopunctata* by phylloplane isolated *Alcaligenes paradoxus* KPM-012A has been reported by Otsu *et al.* (2003). In the present study treatment with *S. marcescens* recorded least leaf area damage at different intervals proving that it is a better biocontrol agent (Figure 5). This leaf area protection is brought about by fast death of the test insects rather than feeding deterrence as evidenced by the high mortality of grubs. In case of *P. fluorescens*, in spite of having a comparable mortality with *M. anisopliae* and *B. bassiana* the leaf area damage was very less (Figure 6) indicating the possibility of feeding deterrence activity. Report show that feeding by epilachna grubs considerably decreased when fed on tomato leaves treated with *P. fluorescens* (Otsu *et al*., 2004)

The fundamental function of any biocontrol agent is to check the population build up of the pest in the natural ecosystem. As a measure to evaluate the efficacy of isolated bacteria, a pot culture experiment was carried out. Brinjal variety, Surya was used for the trial with an assumption that purple varieties of brinjal are more susceptible to pests and diseases than green varieties. Treatments of microbial control agents were applied as foliar spray.

Comparable results were obtained in feeding treatments in the laboratory trial and foliar treatment in pot culture experiments. Mortality of pre adjusted population of insects was recorded in order to assess the efficacy of the treatments. It was found that *S. marcescens* caused a mortality of 93.27 per cent in epilachna grubs at 5 DAT and was on par with chemical control (Chlorantraniliprole 18.5 SC). As in laboratory experiment, *P. aeruginosa* followed this treatment causing a mortality of 69.76 per cent 5 days after treatment. More than 65 per cent mortality of grubs (Figure 7) and more than 70 per cent reduction in population of grubs over control (Figure 8) were observed in *S. marcescens* and *P. aeruginosa* treated plants at 5 DAT indicating their

Figure 5. Per cent reduction in leaf area damage over control caused by Epilachna, *H. vigintioctopunctata* grubs treated with different pathogenic and phylloplane microorganisms

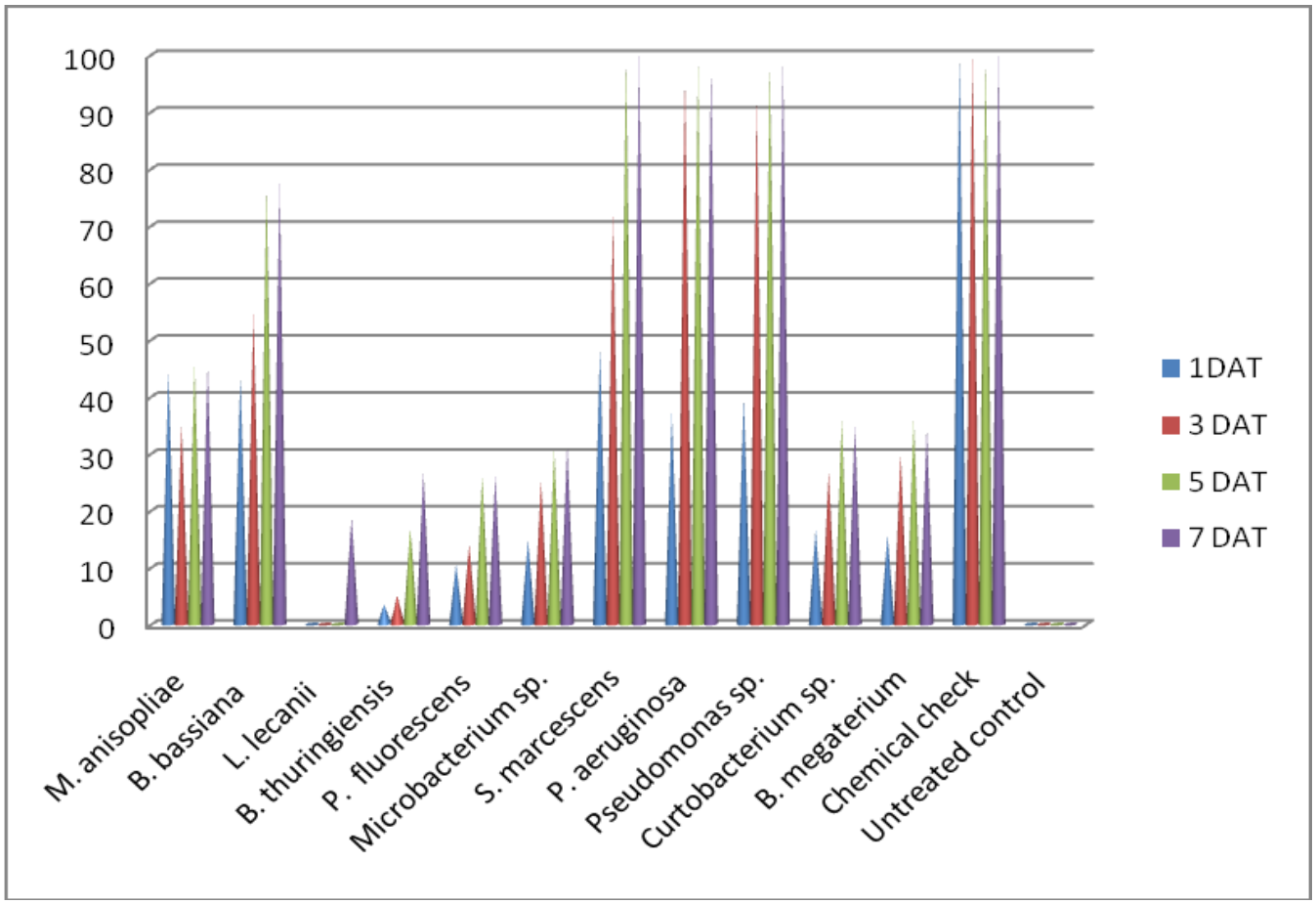


Figure 6. Relation between per cent mortality and Leaf area damaged

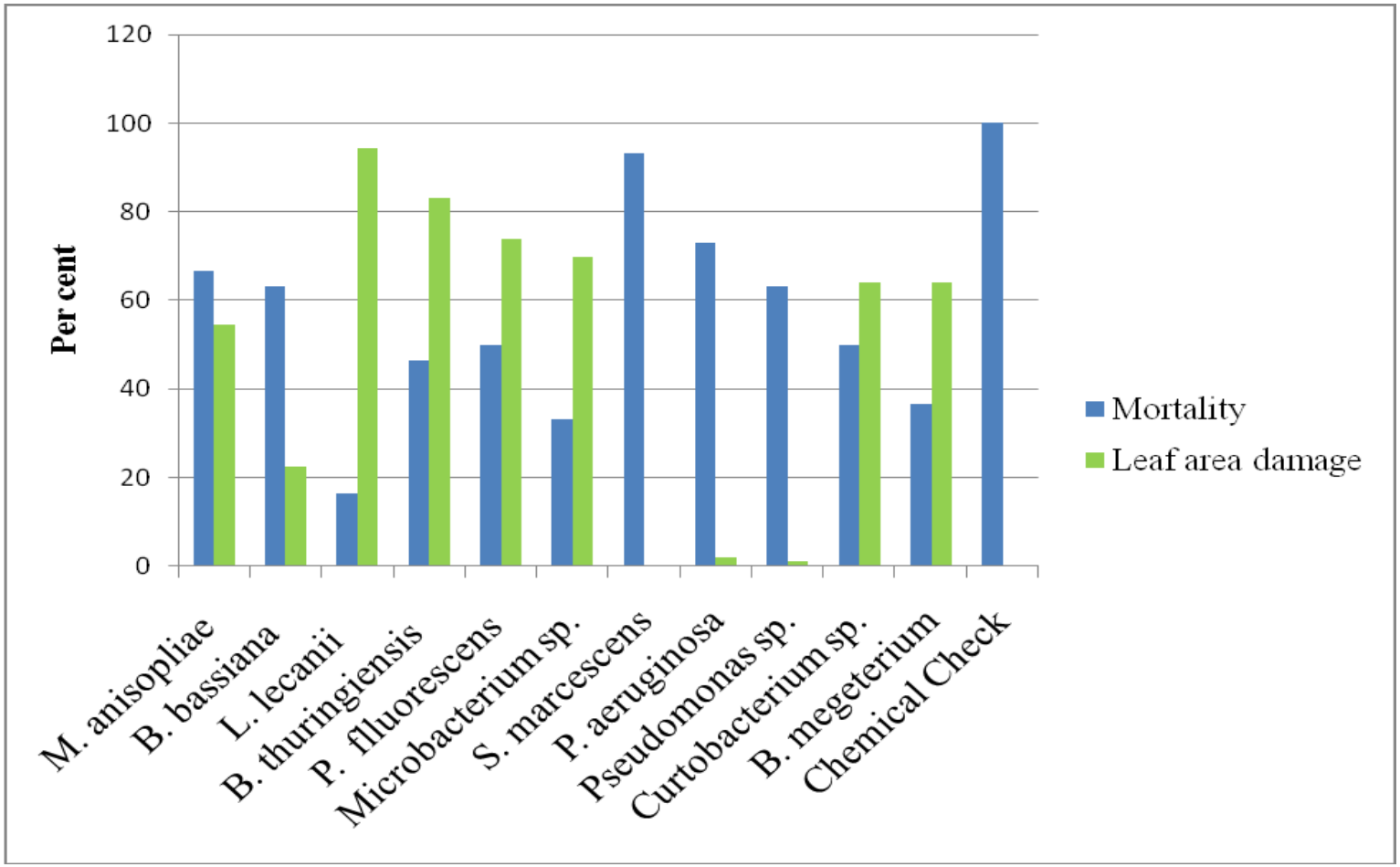


Figure 7. Mortality of Epilachna, *H. vigintioctopunctata* grubs on plants treated with different pathogenic and phylloplane

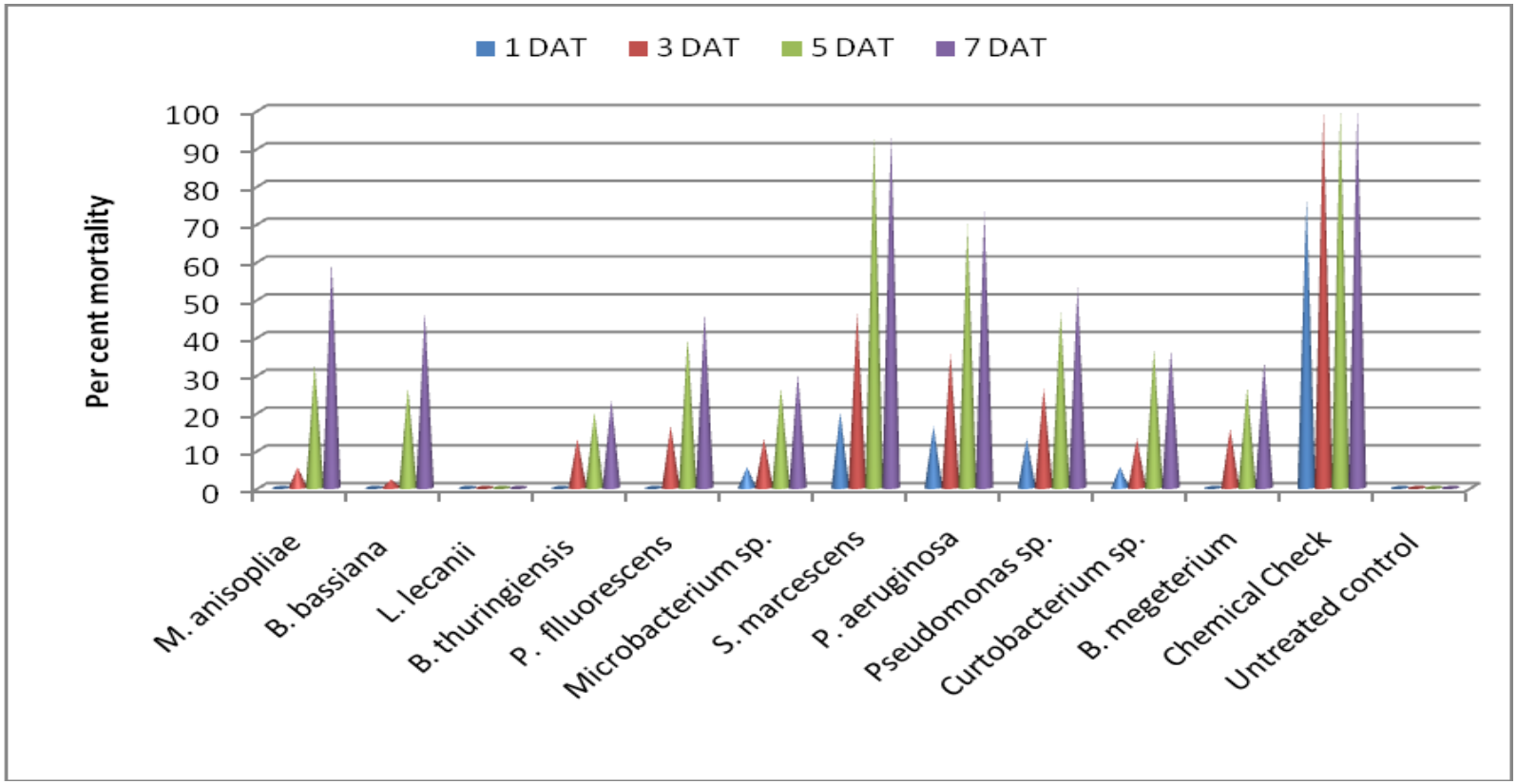
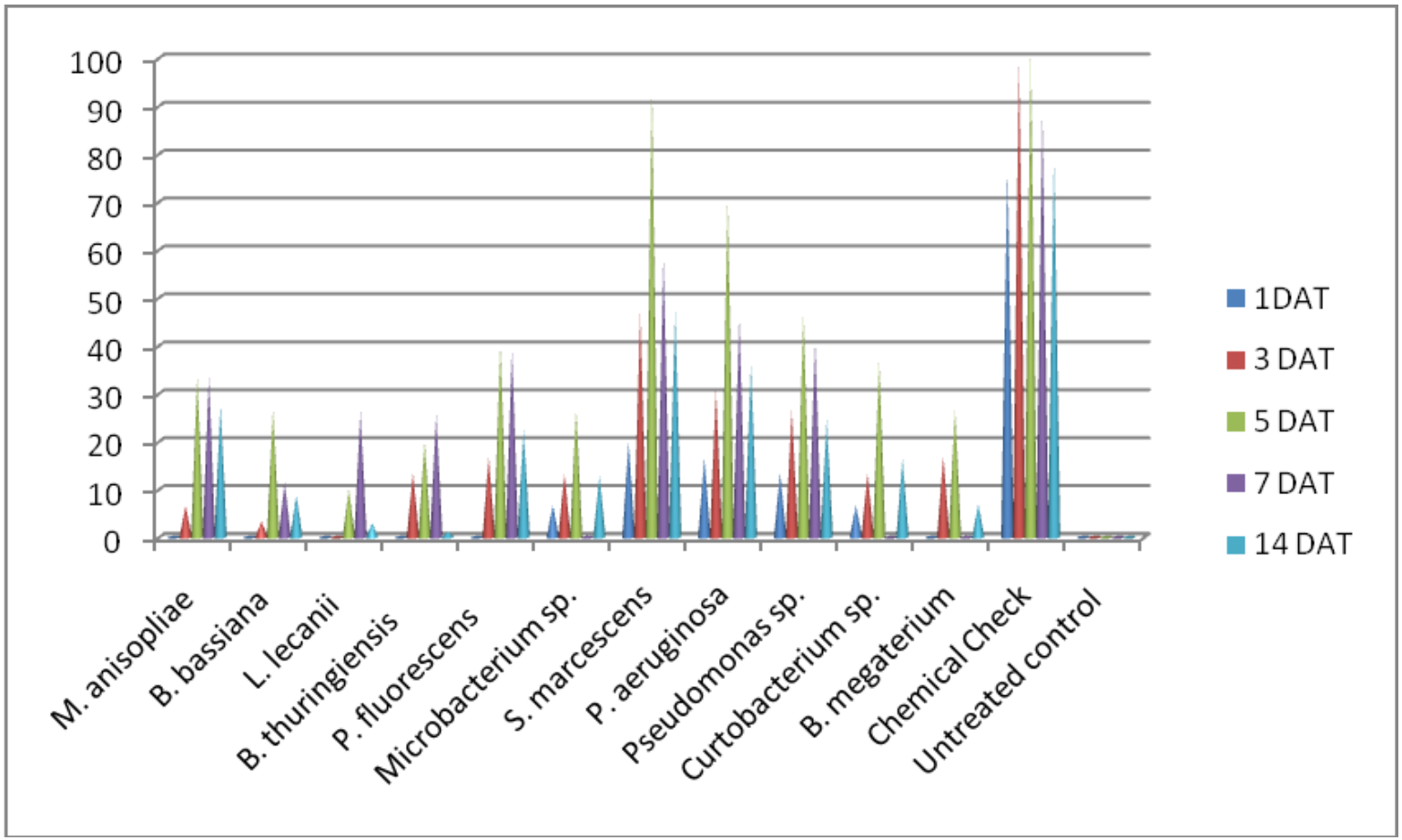


Figure 8. Per cent reduction over control of Epilachna, *H. vigintioctopunctata* grub population on plants treated with different pathogenic and phylloplane microorganisms



population regulation capacity. The population was under check in these two treatments even after 14 days of treatment. Leaf area damage was reduced by about 80 per cent over untreated check in plants subjected to these treatments (Figure 9). With respect to all three important parameters, *viz*. mortality, population reduction and leaf area damage reduction, the treatments with *S. marcescens* and *P. aeruginosa* were found to be superior to commercially used fungal entomopathogens like *M. anisopliae*, *B. bassiana*, *L. lecanii* and bacterial entomopathogens like *B. thuringiensis* and *P. fluorescens* (Deepa pseudo®) (Figure 10). Thus these two microorganisms are potential alternatives to chemical control and superior over the currently employed microbial control agents in the management of epilachna beetle. A *Serratia* sp. which offers promise for insect control of New Zealand Grass grub *Costelytra zealandica* was reported by Trought *et al.* (1982).

The phylloplane isolated *P. fluorescens* was able to produce mortality of 50 per cent and above at 5 and 7 DAT and could also bring about around 50 per cent reduction in population and more than 70 per cent reduction in leaf area damage over untreated check. These results are comparable to that of commercially employed microbial control agents *M. anisopliae, B. bassiana* and superior over *B. thuringiensis* formulations in managing epilachna beetle. Being a phylloplane microorganism, it has the potential to stably colonize the leaf surface and hence can be exploited as a permanent population regulation factor of epilachna in brinjal. Phylloplane isolated microorganisms are increasingly being utilized for pest management (Takikawa *et al.*, 2002; Otsu, *et al*, 2003; Otsu *et al.*, 2004).

Epilachna beetle, *H. vigintioctopunctata,* the well known pest of brinjal has been recently reported to have expanded its host range (Rai *et al.*, 2014) besides developing resistance to various pesticides necessitating a re-orientation in its management strategy. The information generated in the present study about virulent entomopathogenic and phylloplane microorganisms isolated from brinjal ecosystem

Figure 9. Per cent reduction over control in leaf area damaged by Epilachna, *H. vigintioctopunctata* grubs on plants treated with different pathogenic and phylloplane microorganisms

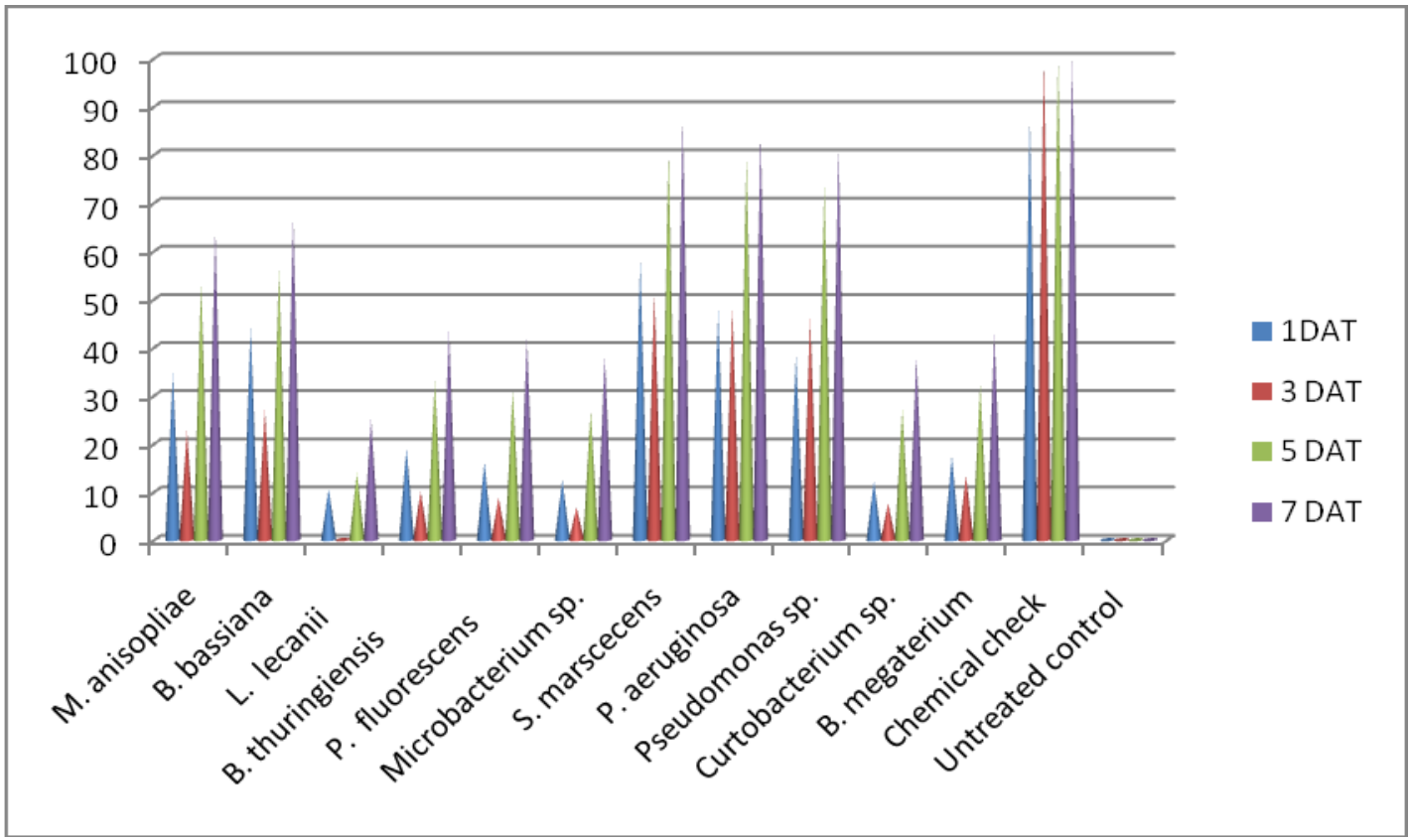
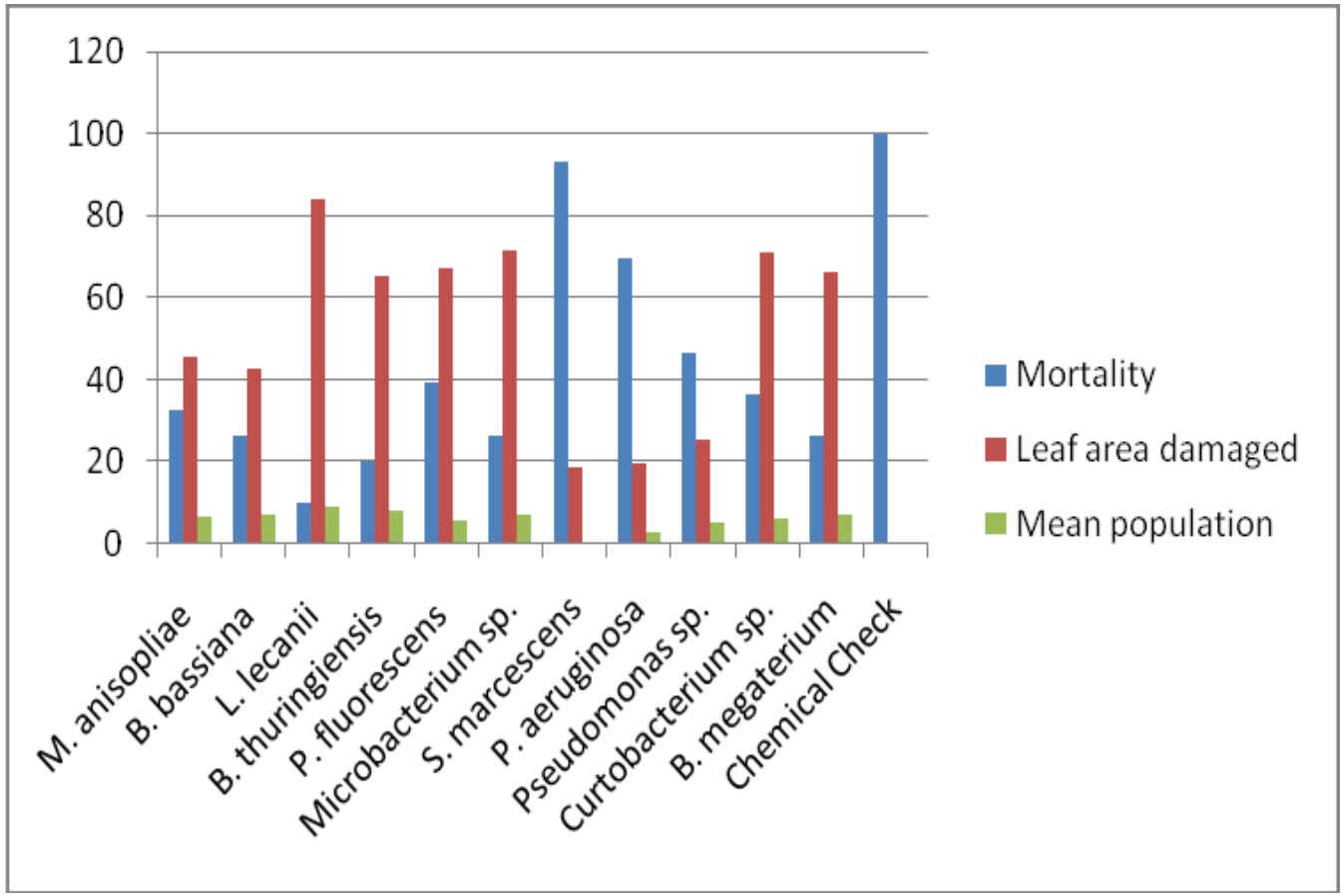


Figure 10. Relation between mortality, mean population and leaf area damage



will be helpful in evolving sustainable and ecologically feasible integrated pest management (IPM) strategies against this pest.

Summary

6. SUMMARY

Yield of brinjal, one of the most important vegetable crops in India is substantially reduced by the infestation of foliage feeding epilachna beetle, *Henosepilachna vigintioctopunctata.* Large quantities of insecticides are regularly applied by the farmers to tackle this menace. The increased awareness and anxiety in the minds of people regarding the ill effects of pesticides warrant development of newer and safer management measures. To reduce the use of chemical pesticides, several efficient and environment friendly measures have been generated by researchers around the world. One such method is microbial control of insect pests using insect pathogenic microorganisms. A less explored area under microbial control is the utilization of phylloplane microorganism dwelling the leaf surface for insect pest management. Against this backdrop the work entitled "Management of epilachna beetle, *Henosepilachna vigintioctopunctata* (Fab.) with phylloplane and pathogenic microorganisms" was undertaken at Department of Entomology, College of Agriculture, Vellayani during 2013 – 2015.

The main objective of the present study was to isolate, characterize and evaluate virulent entomopathogenic and phylloplane microorganisms from brinjal ecosystem for the management of epilachna beetle.

The salient findings of the present study are as follows.

Survey conducted in brinjal fields during 2014 – 2015 revealed that population of epilachna beetle reached its peak during the hot summer months of March – May during which time the mean maximum temperature was high and relative humidity and rainfall was low. The mean number of epilachna beetle population during this period was 4.74 adults per plant while that of grubs and egg masses were 6.59 grubs and 0.60 egg mass per plant respectively. The population started to decline with the onset of monsoon and remained low till January.

A significant positive correlation with maximum temperature (+0.84 and +0.83) and significant negative correlation with relative humidity (-0.41 and -0.42) were observed in relation to number of egg masses and population of grubs of epilachna beetle respectively. The adult beetle had significant positive correlation with maximum temperature (+0.91) and non-significant negative correlation with relative humidity (-0.19) and rainfall (-0.02).

High parasitization of epilachna beetle was observed in January – March. Peak egg parasitization (0.36 egg mass per plant) and pupal parasitization (0.53 pupae per plant) were observed during February. Egg parasitization exhibited significant negative correlation with minimum temperature, relative humidity and rainfall (-0.6, -0.51 and -0.44 respectively) whereas, pupal parasitization showed significant positive correlation with maximum temperature (+0.83) and significant negative correlation with relative humidity (-0.59).

Twelve entomopathogenic microorganisms from diseased epilachna grubs collected from the field (11 bacteria and one fungus) and six bacteria from brinjal phylloplane were isolated during the survey. Upon preliminary screening for pathogenicity to epilachna beetle isolates 1, 3 and 9 obtained from diseased epilachna grub and isolates 13, 16 and 18 obtained from the phylloplane were found to be infective.

Symptoms typical to bacterial infections were noticed in grubs treated with different entomopathogenic and phylloplane bacteria. The infected grubs showed discolouration and change in consistency of body content. The body of the dead insect turned dark and shrinked subsequently.

Isolates 1, 3, 9, 13, 16 and 18 were morphologically and biochemically characterized and identified as *Microbacterium* sp., *Serratia marcescens*,

Pseudomonas aeruginosa, Pseudomonas fluorescens, *Curtobacterium flaccumfaciens* and *Bacillus megaterium* respectively based on 16s rRNA sequence homology.

Chitinase production was detected in the form of clear halo formation around the colony in *S. marcescens, P. aeruginosa, P. fluorescens* and *B. megaterium* when incubated in media containing chitin as the sole carbon source. *B. megaterium* produced the largest halo with a diameter of 1.8 cm. The halo produced by *P. aeruginosa* measured 1.3 cm followed by *P. fluorescens* (0.7 cm) and *S. marcescens* (0.4 cm).

P. aeruginosa had highest activity of 8.2 units followed by *B. megaterium* (4.8 units). *P. fluorescens* and *S. marcescens* recorded a chitinase activity of 1.7 and 1.4 units respectively.

In oral and topical exposure studies conducted in the laboratory, *S. marcescens* produced 93.28 per cent mortality to epilachna grubs at 5 DAT which was statistically on par with that of chemical check (Chlorantraniliprole 18.5 SC). This was followed by *P. aeruginosa* with a mortality of 73.01 per cent *P. fluorescens* caused a mortality of 63.25 per cent at 7 DAT which was on par with that of recommended fungal biocontrol agents *Metarhizium anisopliae* and *Beauveria bassiana* and superior to recommended bacterial pathogens *P. fluorescens* (Deepa pseudo®) and *Bacillus thuringiensis.* The phylloplane isolated *P. fluorescens* caused 50 per cent mortality. *C. flaccumfaciens* isolated from phylloplane caused 43.21 per cent and 50 per cent mortality of grubs at 5 and 7 DAT respectively.

None of the microbial treatments were successful in causing mortality in adult beetles of epilachna. A very negligible mortality of 0.07 per cent was observed in beetles treated with isolate 13 at 24 h of treatment which slightly increased to 0.13 per cent at 5 DAT.

Epilachna grubs exposed to *S. marcescens, P. aeruginosa* and *P. fluorescens* caused leaf area damage of 51.47 per cent, 62.82 per cent and 60 per cent respectively. Even though *M. anisopliae* and *B. bassiana* were inferior in causing mortality of epilachna grubs 24 h after treatment, it was noted that these treatments could reduce the leaf area damage (44.51 and 43.53 per cent respectively).

In pot culture experiment with foliar application of microbial treatments *S. marcescens* caused 93.27 per cent mortality in epilachna grubs and was on par with chemical control (Chlorantraniliprole 18.5 SC) at 5 DAT. *P. aeruginosa* followed this treatment with a mortality of 69.76 per cent. At 7 DAT *S. marcescens* and *P. aeruginosa* reduced the per plant population of epilachna grubs to 7.54 and 9.86 respectively which was significantly superior to that of untreated check (18.26) and was next best to Chlorantraniliprole 18.5 SC (2.05). At 5 DAT in plants treated with *S. marcescens* (18.71 %), *P. aeruginosa* (19.58 %) and phylloplane isolated *P. fluorescens* (25.43 %) recorded significantly low leaf area damage compared to untreated check.

With respect to all three important parameters, *viz*. mortality, population reduction and leaf area damage reduction, the treatment with *S. marcescens* and *P. aeruginosa* was found to be superior to commercially used fungal entomopathogens like *M. anisopliae*, *B. bassiana* and *L. lecanii* and bacterial entomopathogens like *B. thuringiensis* and *P. fluorescens* (Deepa pseudo®).

The phylloplane isolated *P. fluorescens* was able to produce mortality of 50 per cent and above at 5 and 7 DAT and could also bring about around 50 per cent reduction in population and more than 70 per cent reduction in leaf area damage over untreated check.

Thus, it was noticed that *S. marcescens* at a dose of 10^8 cfu ml^{-1} is an effective entomopathogenic bacteria for the management of epilachna beetle in

brinjal and has the potential to replace chemical insecticides. *P. aeruginosa* and phylloplane isolated *P. fluorescens* were also found to be effective. Since some strains of *Serratia marcescens* and *Pseudomonas aeruginosa* are opportunistic mammalian pathogens the pest control advantage of the present strains can be taken advantage of only after suitable bio-safety evaluations or by employing right methods of application.

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Appendix

APPENDIX-I

COMPOSITION OF MEDIA USED

1. Nutrient Agar

Peptone	- 5g
NaCl	- 5g
Beef extract	- 3g
Agar	- 20g
Distilled water	- 1000 ml

Peptone, NaCl and beef extract were dissolved in 500 ml distilled water and volume made up to 1000 ml. 20 g agar-agar was added into this mixture and autoclaved at 15 lsb pressure and 121 ºC for 15 min.

2. King's Medium B

Peptone	- 20g
K_2HPO_4	- 1.5g
$MgSO_4$	- 1.5g
Glycerol	- 10 ml
Agar	- 20g
Distilled water	- 1000 ml

Peptone, K_2HPO_4 and $MgSO_4$ were dissolved in distil water containing glycerol. Agar-agar was added into this mixture and autoclaved at 15 lbs pressure and 121 ºC for 15 min.

3. Potato Dextrose Agar

Peeled and sliced potatoes	- 200g
Dextrose ($C_6H_{12}O_6$)	- 20g
Agar-agar	- 20g

Distilled water - 1000 ml

Potatoes were boiled in 500 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was dissolved separately in 500 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was dissolved in to the mixture. The volume was made up to 1000 ml with distilled water and medium was sterilized at 15 lsb pressure and 121 ºC for 15 min.

3. Martin's Rose Bengal agar

Dextrose - 10g

Peptone - 5g

KH_2PO_4 - 1g

$MgSO_4$. $7H_2O$ - 0.5g

Rose Bengal - 33mg/l

Agar - 20g

Distilled water - 1000ml

APPENDIX - II

COMPOSITION OF STAIN USED

1. Crystal violet

One volume saturated alcohol solution of crystal violet in four volumes of one per cent aqueous ammonium oxalate.

2. Gram's iodine

Iodine crystals	- 1.0g
Potassium iodide	- 2.0g
Distilled water	- 300ml

3. Safranin

Ten ml saturated solution of safranin in 100 ml distilled water.

4. Malachite green

Malachite green	- 5.0g
Distilled water	- 100ml

APPENDIX-III

Sequence producing significant alignment

a. Isolate 1

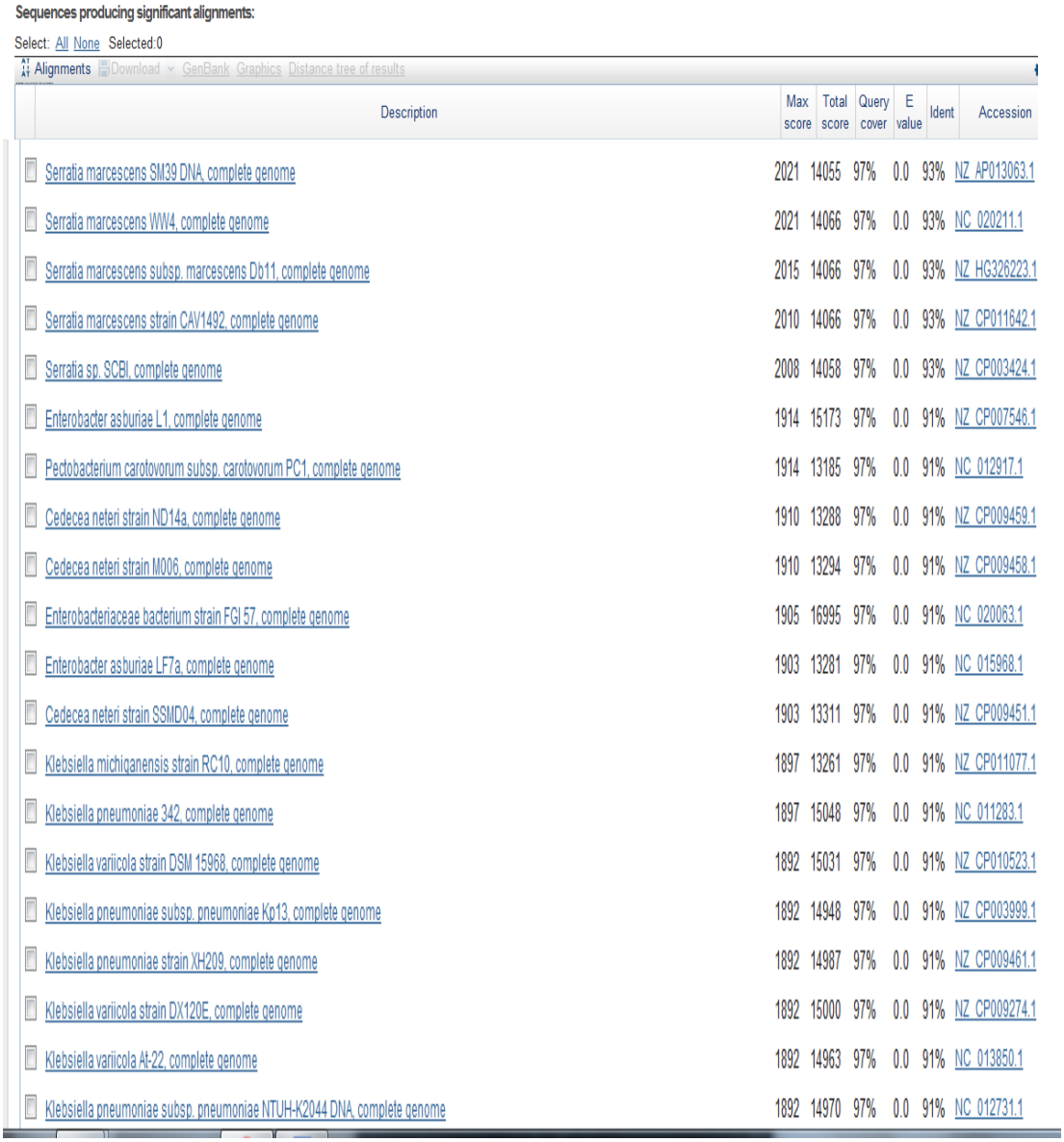
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Microbacterium sp. CGR1, complete genome	1934	3869	97%	0.0	92%	NZ_CP012299.1
Leifsonia xyli subsp. cynodontis DSM 46306, complete genome	1735	1735	97%	0.0	89%	NC_022438.1
Leifsonia xyli subsp. xyli str. CTCB07, complete genome	1718	1718	97%	0.0	89%	NC_006087.1
Clavibacter michiganensis subsp. sepedonicus complete genome	1670	3335	97%	0.0	89%	NC_010407.1
Clavibacter michiganensis subsp. insidiosus strain R1-1, complete genome	1664	3329	97%	0.0	88%	NZ_CP011043.1
Clavibacter michiganensis subsp. michiganensis NCPPB 382 complete genome	1664	3329	97%	0.0	88%	NC_009480.1
Rathayibacter toxicus strain 70137, complete genome	1661	3322	97%	0.0	88%	NZ_CP010848.1
Clavibacter michiganensis subsp. nebraskensis NCPPB 2581 complete genome	1659	3318	97%	0.0	88%	NC_020891.1
Kocuria palustris strain MU14/1, complete genome	1620	4861	96%	0.0	88%	NZ_CP012507.1
Sanguibacter keddieii DSM 10542, complete genome	1613	6436	97%	0.0	88%	NC_013521.1
Micrococcus luteus NCTC 2665, complete genome	1613	3236	96%	0.0	88%	NC_012803.1
[Cellvibrio] gilvus ATCC 13127, complete genome	1596	3191	96%	0.0	88%	NC_015671.1
Isoptericola variabilis 225, complete genome	1594	4784	96%	0.0	88%	NC_015588.1
Intrasporangium calvum DSM 43043, complete genome	1583	3161	93%	0.0	88%	NC_014830.1
Beutenbergia cavernae DSM 12333, complete genome	1583	3167	95%	0.0	88%	NC_012669.1
Cellulomonas fimi ATCC 484, complete genome	1561	3123	96%	0.0	88%	NC_015514.1
Arthrobacter arilaitensis RE117 chromosome, complete sequence	1559	9358	96%	0.0	87%	NC_014550.1
Kocuria rhizophila DC2201 DNA, complete genome	1557	4673	93%	0.0	88%	NC_010617.1
Arthrobacter sp. FB24, complete genome	1557	7789	96%	0.0	87%	NC_008541.1

b. Isolate 3



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Serratia marcescens SM39 DNA, complete genome	2021	14055	97%	0.0	93%	NZ_AP013063.1
Serratia marcescens WW4, complete genome	2021	14066	97%	0.0	93%	NC_020211.1
Serratia marcescens subsp. marcescens Db11, complete genome	2015	14066	97%	0.0	93%	NZ_HG326223.1
Serratia marcescens strain CAV1492, complete genome	2010	14066	97%	0.0	93%	NZ_CP011642.1
Serratia sp. SCBI, complete genome	2008	14058	97%	0.0	93%	NZ_CP003424.1
Enterobacter asburiae L1, complete genome	1914	15173	97%	0.0	91%	NZ_CP007546.1
Pectobacterium carotovorum subsp. carotovorum PC1, complete genome	1914	13185	97%	0.0	91%	NC_012917.1
Cedecea neteri strain ND14a, complete genome	1910	13288	97%	0.0	91%	NZ_CP009459.1
Cedecea neteri strain M006, complete genome	1910	13294	97%	0.0	91%	NZ_CP009458.1
Enterobacteriaceae bacterium strain FGI 57, complete genome	1905	16995	97%	0.0	91%	NC_020063.1
Enterobacter asburiae LF7a, complete genome	1903	13281	97%	0.0	91%	NC_015968.1
Cedecea neteri strain SSMD04, complete genome	1903	13311	97%	0.0	91%	NZ_CP009451.1
Klebsiella michiganensis strain RC10, complete genome	1897	13261	97%	0.0	91%	NZ_CP011077.1
Klebsiella pneumoniae 342, complete genome	1897	15048	97%	0.0	91%	NC_011283.1
Klebsiella variicola strain DSM 15968, complete genome	1892	15031	97%	0.0	91%	NZ_CP010523.1
Klebsiella pneumoniae subsp. pneumoniae Kp13, complete genome	1892	14948	97%	0.0	91%	NZ_CP003999.1
Klebsiella pneumoniae strain XH209, complete genome	1892	14987	97%	0.0	91%	NZ_CP009461.1
Klebsiella variicola strain DX120E, complete genome	1892	15000	97%	0.0	91%	NZ_CP009274.1
Klebsiella variicola At-22, complete genome	1892	14963	97%	0.0	91%	NC_013850.1
Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044 DNA, complete genome	1892	14970	97%	0.0	91%	NC_012731.1

c. Isolate 9

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Pseudomonas aeruginosa VRFPA04, complete genome	1306	15928	97%	0.0	98%	NZ_CP008739.1
Pseudomonas aeruginosa strain F9676, complete genome	1306	7964	97%	0.0	98%	NZ_CP012066.1
Pseudomonas aeruginosa DSM 50071, complete genome	1306	7964	97%	0.0	98%	NZ_CP012001.1
Pseudomonas aeruginosa B136-33, complete genome	1306	7936	97%	0.0	98%	NC_020912.1
Pseudomonas aeruginosa SCV20265, complete genome	1306	7964	97%	0.0	98%	NC_023149.1
Pseudomonas aeruginosa LES431, complete genome	1306	7916	97%	0.0	98%	NC_023066.1
Pseudomonas aeruginosa NCGM2.S1 DNA, complete genome	1306	7964	97%	0.0	98%	NC_017549.1
Pseudomonas aeruginosa M18, complete genome	1306	7964	97%	0.0	98%	NC_017548.1
Pseudomonas aeruginosa DNA, complete genome, strain: NCGM 1984	1306	7964	97%	0.0	98%	NZ_AP014646.1
Pseudomonas aeruginosa DNA, complete genome, strain: NCGM 1900	1306	7964	97%	0.0	98%	NZ_AP014622.1
Pseudomonas aeruginosa PA1, complete genome	1306	7956	97%	0.0	98%	NC_022808.1
Pseudomonas aeruginosa PA1R, complete genome	1306	7956	97%	0.0	98%	NC_022806.1
Pseudomonas aeruginosa strain F22031, complete genome	1306	7964	97%	0.0	98%	NZ_CP007399.1
Pseudomonas aeruginosa RP73, complete genome	1306	7945	97%	0.0	98%	NC_021577.1
Pseudomonas aeruginosa LESB58 complete genome sequence	1306	7958	97%	0.0	98%	NC_011770.1
Pseudomonas aeruginosa strain Carb01 63, complete genome	1306	7964	97%	0.0	98%	NZ_CP011317.1
Pseudomonas aeruginosa DK2, complete genome	1306	7964	97%	0.0	98%	NC_018080.1
Pseudomonas aeruginosa MTB-1, complete genome	1306	7964	97%	0.0	98%	NC_023019.1
Pseudomonas aeruginosa PA7, complete genome	1306	7958	97%	0.0	98%	NC_009656.1
Pseudomonas aeruginosa UCBPP-PA14, complete genome	1306	7964	97%	0.0	98%	NC_008463.1
Pseudomonas aeruginosa PAO1 chromosome, complete genome	1306	7963	97%	0.0	98%	NC_002516.2

d. Isolate 13

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Pseudomonas fluorescens NCIMB 11764, complete genome	2313	13950	98%	0.0	96%	NZ_CP010945.1
Pseudomonas fluorescens F113, complete genome	2302	11510	98%	0.0	96%	NC_016830.1
Pseudomonas chlororaphis strain PA23, complete genome	2296	11476	98%	0.0	96%	NZ_CP008696.1
Pseudomonas sp. MRSN12121, complete genome	2290	11454	98%	0.0	96%	NZ_CP010892.1
Pseudomonas chlororaphis strain PCL1606, complete genome	2290	11454	98%	0.0	96%	NZ_CP011110.1
Pseudomonas chlororaphis subsp. aurantiaca strain JD37, complete genome	2285	11399	98%	0.0	96%	NZ_CP009290.1
Pseudomonas brassicacearum strain DF41, complete genome	2285	11427	98%	0.0	96%	NZ_CP007410.1
Pseudomonas sp. TKP, complete genome	2285	11421	98%	0.0	96%	NC_023064.1
Pseudomonas fluorescens SBW25 complete genome	2285	11427	98%	0.0	96%	NC_012660.1
Pseudomonas mandelii JR-1, complete genome	2278	13640	97%	0.0	96%	NZ_CP005960.1
Pseudomonas syringae pv. syringae HS191, complete genome	2272	11358	97%	0.0	96%	NZ_CP006256.1
Pseudomonas syringae pv. phaseolicola 1448A, complete genome	2272	11362	97%	0.0	96%	NC_005773.3
Pseudomonas syringae pv. syringae B728a chromosome, complete genome	2266	11307	97%	0.0	96%	NC_007005.1
Pseudomonas trivialis strain IHBB745, complete genome	2263	11310	98%	0.0	96%	NZ_CP011507.1
Pseudomonas fluorescens strain PCL1751, complete genome	2263	13557	98%	0.0	96%	NZ_CP010896.1
Pseudomonas sp. UW4, complete genome	2259	15702	97%	0.0	96%	NC_019670.1
Pseudomonas fluorescens PICF7, complete genome	2257	13546	98%	0.0	96%	NZ_CP005975.1
Pseudomonas poae RE*1-1-14, complete genome	2257	11288	98%	0.0	96%	NC_020209.1
Pseudomonas syringae pv. syringae B301D, complete genome	2255	11279	97%	0.0	96%	NZ_CP005969.1

e. Isolate 16

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Curtobacterium flaccumfaciens UCD-AKU contig_83, whole genome shotgun sequence	1746	3287	98%	0.0	98%	NZ_APJN01000083.1
[strain=DSM 22003], whole genome shotgun sequence	1633	2973	98%	0.0	96%	NZ_AULK01000004.1
Rathayibacter toxicus DSM 7488 H594DRAFT_scaffold00012.12_C, whole genome shotgun sequence	1616	2993	98%	0.0	95%	NZ_AUDF01000015.1
Rathayibacter toxicus DSM 7488 H594DRAFT_scaffold00008.8_C, whole genome shotgun sequence	1616	2993	98%	0.0	95%	NZ_AUDF01000011.1
Zimmermannella faecalis ATCC 13722 strain B187 genomic scaffold G328DRAFT_scaffold00017.17, whole genome shotgun sequence	1581	2919	98%	0.0	95%	NZ_KB907090.1
Humibacter albus DSM 18994 H623DRAFT_scaffold00007.7_C, whole genome shotgun sequence	1580	2903	97%	0.0	95%	NZ_ATXT01000018.1
Humibacter albus DSM 18994 H623DRAFT_scaffold00015.15_C, whole genome shotgun sequence	1580	2903	97%	0.0	95%	NZ_ATXT01000006.1
Humibacter albus DSM 18994 H623DRAFT_scaffold00013.13_C, whole genome shotgun sequence	1580	2903	97%	0.0	95%	NZ_ATXT01000004.1
Clavibacter michiganensis subsp. sepedonicus complete genome	1578	5838	98%	0.0	95%	NC_010407.1
Leifsonia aquatica H1aii Contig6, whole genome shotgun sequence	1574	2868	98%	0.0	95%	NZ_AYMR01000006.1
Leifsonia xyli subsp. xyli str. CTCB07, complete genome	1574	2873	98%	0.0	95%	NC_006087.1
Cryobacterium roopkundense strain RuG17 contig00180, whole genome shotgun sequence	1563	2761	98%	0.0	94%	NZ_JPXF01000180.1
Mycetocola saprophilus strain NRRL B-24119 contig10.1, whole genome shotgun sequence	1552	2916	98%	0.0	94%	NZ_JOEC01000010.1
Microbacterium luticocti DSM 19459 H577DRAFT_scaffold00007.7_C, whole genome shotgun sequence	1544	2770	96%	0.0	94%	NZ_AULS01000007.1
Agrococcus lahaulensis DSM 17612 genomic scaffold H508DRAFT_scaffold00003.3, whole genome shotgun sequence	1544	2822	98%	0.0	94%	NZ_KE384303.1
Agromyces italicus DSM 16388 H516DRAFT_scaffold00009.9_C, whole genome shotgun sequence	1543	2862	98%	0.0	94%	NZ_ATXF01000012.1
Agromyces subbeticus DSM 16689 H521DRAFT_scaffold00026.26_C, whole genome shotgun sequence	1537	2846	98%	0.0	94%	NZ_ATXG01000026.1
Glaciibacter superstes DSM 21135 K323DRAFT_scaffold00032.32_C, whole genome shotgun sequence	1515	2846	98%	0.0	94%	NZ_ATWH01000032.1
Glaciibacter superstes DSM 21135 K323DRAFT_scaffold00015.15_C, whole genome shotgun sequence	1515	2846	98%	0.0	94%	NZ_ATWH01000015.1
Glaciibacter superstes DSM 21135 K323DRAFT_scaffold00002.2_C, whole genome shotgun sequence	1515	2846	98%	0.0	94%	NZ_ATWH01000002.1
Microbacterium testaceum StLB037 DNA, complete genome	1507	5694	98%	0.0	93%	NC_015125.1

f. Isolate 18

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Bacillus megaterium strain Q3, complete genome	2202	28547	97%	0.0	95%	NZ_CP010586.1
Bacillus megaterium WSH-002, complete genome	2202	21888	97%	0.0	95%	NC_017138.1
Bacillus megaterium DSM319, complete genome	2202	24181	97%	0.0	95%	NC_014103.1
Bacillus megaterium QM B1551, complete genome	2202	24140	97%	0.0	95%	NC_014019.1
Bacillus megaterium NBRC 15308 = ATCC 14581, complete genome	2202	28547	97%	0.0	95%	NZ_CP009920.1
Bacillus megaterium strain Q3 plasmid p1, complete sequence	2202	2202	97%	0.0	95%	NZ_CP010587.1
Bacillus megaterium QM B1551 plasmid pBM400, complete sequence	2191	2191	97%	0.0	95%	NC_004604.2
Bacillus megaterium WSH-002 plasmid WSH-002_p1, complete sequence	2187	2187	97%	0.0	94%	NC_017139.1
Bacillus sp. 1NLA3E, complete genome	1912	22802	97%	0.0	91%	NC_021171.1
Bacillus infantis NRRL B-14911, complete genome	1890	16967	97%	0.0	91%	NC_022524.1
Bacillus sp. X1(2014), complete genome	1847	22073	97%	0.0	90%	NZ_CP008855.1
Bacillus methanolicus MGA3, complete genome	1829	16436	97%	0.0	90%	NZ_CP007739.1
Bacillus cytotoxicus NVH 391-98, complete genome	1796	23238	97%	0.0	90%	NC_009674.1
Bacillus mycoides strain 219298, complete genome	1792	24926	97%	0.0	90%	NZ_CP007626.1
Bacillus thuringiensis strain Al Hakam, complete genome	1792	25030	97%	0.0	90%	NZ_CP009651.1
Bacillus cereus NC7401 genomic DNA, complete genome	1790	24880	97%	0.0	90%	NC_016771.1
Bacillus cereus AH187, complete genome	1790	24880	97%	0.0	90%	NC_011658.1
Bacillus endophyticus strain Hbe603, complete genome	1786	19605	97%	0.0	90%	NZ_CP011974.1
Bacillus thuringiensis serovar indiana strain HD521, complete genome	1786	22898	97%	0.0	90%	NZ_CP010106.1

Abstract

Management of Epilachna Beetle,

Henosepilachna vigintioctopunctata (Fab.) with Phylloplane and Pathogenic Microorganisms

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ABSTRACT

The study entitled "Management of epilachna beetle, *Henosepilachna vigintioctopunctata* (Fab.) with phylloplane and pathogenic microorganisms" was undertaken in the Department of Agricultural Entomology at College of Agriculture, Vellayani during the period 2013 – 2015 with an objective to manage epilachna beetle in brinjal utilizing phylloplane and pathogenic microorganisms.

Twelve entomopathogenic microorganisms from diseased epilachna grubs and six phylloplane bacteria were isolated. Upon preliminary screening for pathogenicity to epilachna beetle, six bacterial isolates (three from diseased epilachna grubs and three from phylloplane) were found to be infective. The bacteria were identified as *Microbacterium* sp., *Serratia marcescens*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *C. flaccumfaciens* and *Bacillus megaterium* based on 16s rRNA sequence homology.

The highest chitinase activity was recorded in *P. aeruginosa* (8.2 units ml^{-1}) followed by *B. megaterium* (4.8 units ml^{-1}). Chitinase activity was also seen in *S. marcescens* and *P. fluorescens* (1.4 and 1.7 units ml^{-1} each).

In oral and topical exposure studies conducted in the laboratory, *S. marcescens* produced 93.28 per cent mortality to epilachna grubs at 5 DAT which was statistically on par with that of chemical check (Chlorantraniliprole 18.5 SC). This was followed by *P. aeruginosa* with a mortality of 73.01 per cent. The phylloplane isolated *P. fluorescens* and *C. flaccumfaciens* caused 63.25 and 50 per cent mortality at 7 DAT respectively. Epilachna grubs exposed to *S. marcescens* caused a leaf area damage of 51.47 per cent as against 100 per cent in untreated check.

In pot culture experiment with foliar application of microbial treatments *S. marcescens, P. aeruginosa* caused 93.27 per cent and 69.76 per cent mortality in

epilachna grubs at 5 DAT and were on par with chemical control (Chlorantraniliprole 18.5 SC). *S. marcescens* and *P. aeruginosa* reduced the per plant population of epilachna grubs to 7.54 and 9.86 respectively which was significantly superior to that of untreated check (18.26) and was next best to Chlorantraniliprole 18.5 SC (2.05) at 7 DAT. With respect to all three important parameters, *viz*. mortality, population reduction and leaf area damage reduction, the treatment with *S. marcescens* and *P. aeruginosa* was found to be superior to commercially used fungal entomopathogens like *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium lecanii* and bacterial entomopathogens like *B. thuringiensis* and *P. fluorescens.* The phylloplane isolated *P. fluorescens* was able to produce mortality of 50 per cent and above at 5 and 7 DAT and could also bring about around 50 per cent reduction in population and more than 70 per cent reduction in leaf area damage over untreated check.

Thus, *S. marcescens* at a dose of 10^8 cfu ml^{-1} is an effective entomopathogenic bacterium in controlling epilachna beetle in brinjal and has a potential to replace chemical insecticides. Since some strains of *Serratia marcescens* are known to be opportunistic mammalian pathogens suitable bio-safety evaluations have to be conducted before employing it for field level pest management.