EFFECT OF ENTOMOPATHOGENIC FUNGI TO CONTROL THE SUCKING PESTS ON Bt COTTON CV BIO 20-15 (*Gossypium hirsutum*)

A Project Report

Submitted by

KOWSALYA DEVI.A (71203204012)

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COIMBATORE-641 006

ANNA UNIVERSITY::CHENNAI 600 025

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Certified that this project report **EFFECT OF ENTOMOPATHOGENIC FUNGI TO CONTROL THE SUCKING PESTS ON Bt COTTON CV BIO 20-15 (*Gossypium hirsutum)*** is the bonafide work of **KOWSALYA DEVI.A** who carried out project work under my supervision.

SIGNATURE

Dr. P. Rajasekaran
PROFESSOR AND HEAD
Department of Biotechnology,
Kumaraguru College of Technology,
Coimbatore-641006.

SIGNATURE

Mr.S.Senthil Kumar
SUPERVISOR
Lecturer
Department of Biotechnology,
Kumaraguru College of Technology,
Coimbatore-641006.

Telegram : 'STANES'
Fax : 091 - 422 - 2220432
Email : tstanes@vsnl.com

Telephone : 2221514
: 2223515 to 2223518

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12.4.2007

Dr P Rajasekaran
Professor & Head
Kumaraguru College of Technology
Coimbatore 641 006

Dear Sir

Sub: B. Tech dissertation work for a period of 3 months for your Final year B. Tech. Biotechnology student - Period from 13.01.2007 to 05.04.2007

This is to certify that your student Miss A Kowsalya Devi has carried out the work on "Effect of Entomopathogenic fungi on control of the sucking pest on Bt. cotton" in our Research and Development Centre, recognized by the Department of Scientific & Industrial Research (D.S.I.R.) and also by Bharathiyar University and Annamalai University. She has done various experiments related to the above topic, under my guidance and supervision. She is regular in her attendance and did the entire laboratory and field research works with total involvement.

Thanking you

Yours faithfully
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(S Ramarethinam)
Director/Projects

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COLLEGE : Kumaraguru College of Technology

BRANCH : Industrial Biotechnology

SEMESTER : Eighth Semester

NAME OF THE STUDENT	TITLE OF THE PROJECT	NAME OF THE SUPERVISOR
KOWSALYA DEVI.A (71203204012)	EFFECT OF ENTOMOPATHOGENIC FUNGI TO CONTROL THE SUCKING PESTS ON Bt COTTON CV BIO 20-15 (*Gossypium hirsutum*)	Mr.S.SENTHILKUMAR (Lecturer)

The report of the project work submitted by the above student in partial fulfillment for the award of Bachelor of Technology degree in Industrial Biotechnology of Anna University were evaluated and confirmed to be the report of work done by the above student. It was submitted for evaluation and viva-voce held on 2/5/07

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A. Bharathi
(A. Kousalya devi)

ABSTRACT

Cotton is one of the most important commercial crop in India. The quality of cotton fiber is severely affected by the damage due to bollworms this damage was first controlled by chemical methods such as insecticides and pesticides. But this caused environmental pollution. So an alternate way of using Bt cotton came into exsistance. Though Bt cotton controlled bollworms they are ineffective against sucking pests such as Mealy bugs, Aphids, Jassids, Thrips. Here entomopathogenic fungi used to control the sucking pests. Of the four (*Verticillium lecanii, Beauveria bassiana, Metarhizium anisopilae* and *Paecilomyces fumosoroseus*) different fungi used. *Verticillium lecanii* and *Beauveria bassiana* were found to be effective. This showed a better control effect when *Verticillium lecanii* and *Beauveria bassiana* combined with Nimbicidin.

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1. INTRODUCTION

INTRODUCTION

Agriculture (Cultivation of the soil for any aspect of farming) is the process of producing food, fiber, fuel and other goods by systematic rising of plants and animals. It is the backbone of our Indian economy. Nearly 70% of the people depend upon our agriculture. Thus the word agriculture covers the activities essential to food /feed /fiber production in which cotton is healthiest of all fibers.

1.1. Cotton

Cotton is the most important commercial crop playing a significant role in Indian economy. It is cultivated in 8-8.5mha with a production of 150 to160 lak bales. Approximately 20 million farmers are engaged in the cultivation of this crop in 65 countries of the world. (Ramesh chander. 2006)

Cotton is a soft fiber. The fiber is most often spun into thread and used to make a soft, breathable textile, which is the most widely used natural fiber cloth in clothing today.

Cotton fiber originates from the cotton plant, an important crop in tropical and warm temperature climates. It is a thirsty crop and as water resources get tighter around the world.

Cotton being a cash crop that harbors a wide array of insect pests during its growth cycle has thrown a difficult and often challenging task to farmers and researchers from the time it has been under cultivation to save the crop from ravages of various noxious insects. Generally, insect pest problem in cotton is depended upon genotype, agro-ecology including irrigation, plant population and economic condition of growers.(Rajendran, *et al.*,2006).

Quality of cotton fiber is severely affected by the damage due to pink bollworms. The fiber strength and micronaire are reported to be affected by the concurrent damage of bolls by the three bollworms (Pink bollworm, spotted bollworm, American bollworm).

Modern agriculture uses a wide variety of insecticides to control insect damage most of which are effective in chemically synthesized and through are effective in controlling pest population have the disadvantage of causing environmental pollution, which includes broad toxicity range, potential toxicity to non target organisms, development of resistance and resurgence of insects, residual toxicity, secondary out break of minor insect pests and biological magnification in the ecosystem.

One of the earliest solutions offered to the above problems of chemical control was the use of biological agents in pest control. Biological control of insects has become more popular and provides alternative options to reduce insect damage with clear advantages over chemical pesticides , such as being highly economical selective with no side effects, self propagating and self perpetuating, no development of pest resistance, no harmful effects on humans livestock and other organisms etc...

Among various biological options available for insect pest control, the soil bacterium, Bt has immense potential. The molecular potency of these bacteria is reported to be 300 times higher than synthetic pyrethroids and 80,000 times higher than organophosphates (Krishna raj ,*et al.*, 2001).

1.2. *Bacillus thuringensis*

Bt is short for *Bacillus thuringensis*, gram positive,sporulating bacterium, first discovered in 1902, one of the most successful agent of biological control, synthesizes crystalline proteins coded by the cry genes during sporulation.The Bt gene (Cry 1Ac) encodes a special type of toxin commonly known as "Delta endotoxin".Delta toxin kills bollworm only without any deleterious effects on living creatures .The effort was made towards transferring this gene in cultivars of cotton to produce transgenic cotton plants showing resistance against bollworm.

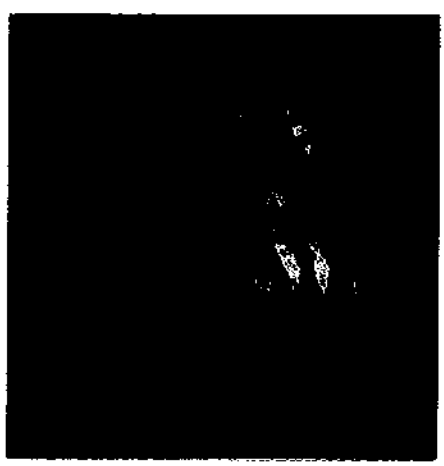

Figure 1.2.1.Structure of *Bacillus thuringensis*

The mechanism of action of Bt cry proteins involves solublisation of crystal in the insect midgut protease binding of the cry toxin to midget receptors and insertion of the cry toxin into the apical membrane to create ion channels or pores, disturbing cellular osmotic balance and causing cell to swell and lyse through a process that has been termed as "colloid –osmotic lysis" which results in death of insects (Jadhav pravin ,2006).

1.3. Bt Cotton

Bt cotton shows specific toxicity against Lepidoteran pests. These Lepidoteran larvae eat the various parts of transgenic plant and die. However, Bt cotton is not effective against non target pests such as sucking pests, beneficial insects and predators. Therefore additional pesticides may have to be applied for effective pest control when pest pressure is very high or if such insect pests are present which are not controlled by the Cry1Ac protein of Bt cotton (Ghosh, 2001).

Bt cotton is also seen to have some control against tobacco caterpillers but it does not kill sucking pest such as Jassids (*Amarasca biguttala biguttala)* mealy bugs (*Pseudococcidae)*,white fly (*Bemisia tabaei Gonnadices*) (Ameta, *et al.*, 2006).In this, Mealy bugs and Aphids cause more ravages in Bt cotton.

1.4. Mealy bugs

Mealy bugs are small in size and occur in small colonies at the bases of leaf. Incidence of this pest is particularly more severe in drought years. (Rain water, 1952)

The insects are oval in shape and measure about 2-3 mm nymphs and adults are soft bodied with a waxy, white powdery covering. Life cycle is completed in 45 days (Gour *et al.*, 1999) the female mealy bugs will lay her legs within a cottony mass that surrounds her body. In about a week, the eggs hatch into nymphs begin crawling to a suitable location on the plant nearby, After four weeks of sucking plants, those nymphs that will become males from a mass of cotton like filaments where they change into winged adults about two weeks later. The females change into their scale-like bodies about two weeks after the males, they mate and the process renews.

An interesting feature of mealy bugs is their association with ants. These ants help in dispersal of the pest from one plant to another in return for a sweet secretion given out by the pest (Rainwater, 1952).

Figure 1.4.1 Mealy bugs on leaves

1.5. Aphids

Aphids active from May-Nov. Adults live for 2-3 weeks and produce 8-22 nymphs/day. Both winged and wingless form breeds parthew genetically. The nymphal period lasts for about 7-9 days. It has 12-14 generations/year. (Regupathy and Palaniswamy)

These insects present in colonies on the underside of leaves and stems, as a result of which the viability of the plant is considerably reduced. The insect produces honeydew on which shooty mould grows in due course. This shooty mould not only impacts of blackish appearance to the plant, but also interferes with its photosynthetic activities. (Sawhney ,1995).

micro organism used biocontrol of plant diseases are termed as 'antagonists'. An association with it. Antagonism is the balance wheel of nature. The antagonists act by (a) reducing the population of pathogens, (b) preventing the pathogen infecting the plant and (c) limiting disease development after infection.

A number of bio-pesticides of fungal and bacterial origin have been introduced in various parts of the world. Fungi are by far the most extensively researched group of biocontrol agents (Mukhopadhyay, 2001).

1.7. Entomopathogenic fungi

Entomopathogenic fungus are most important in the control pests in agricultural. Entomopathogenic fungus is a fungus that can act as a parasite of insects and kills or seriously disable them. These fungi such as *Beauveria bassiana, Verticillium lecanii, Metarhizium anisopilae, Paeciliomyces fumosoroseus* are usually attach to the external body surface of insects in the form of microscopic spores. Under permissive conditions of temperature and moisture, these spores germinate, grow as hyphae and colonize the insect's cuticle, eventually they bore through it and reach the insect's body cavity. Then the fungal cells proliferate in the host body cavity, usually as walled hyphae or in the form of wall-less protoplasts. After some time, the insect is usually killed and new propagules are formed in /on the insect if environmental conditions are again permissive, usually high, humidity is required for sporulation.

1.8. *Beauveria bassiana*

Beauveria bassiana was the first micro organism as to be recognized as contagious agent of animal diseases. It is common soil

borne fungus. It attacks both immature and adults insects. Once inside of the insect's, it produces a toxin called Beauvercin that weakens the host's immune system. After the insect dies, an antibiotic (oospores) is produced that enables the fungus to out compete intestinal bacteria, Eventually, the entire body cavity is filled with fungal mass. When conditions are favorable the fungus will grow through the softer parts of the insect's body. Producing the characteristic "white bloom" appearance'. So this fungus is called as white muscardine fungus (infected larve eventually turn white or gray). (Tanada and Kaya, 1993).

1.9.Verticillium lecanii

Verticillium lecanii (formerly known as *Cephalosporum lecanii*) was first described in 1861 and is a cosmopolitan fungus found on insects. It is a common pathogen of scale insects in tropical and subtropical climates. *Verticillium lecanii* is known as a "white-halo" fungus because of the white mycelial growth on the edges of infected scale insects. The conidia (spores) of *Verticillium lecanii* are slimy and attach to the cuticle of insects. The fungus infects insects by producing hyphae from germinating spores that penetrate the insect's integument; the fungus then destroys the internal contents and the insect dies. The fungus eventually grows out through the cuticle and sporulates on the outside of the body. Infected insects appear as white to yellowish cottony particles. Diseased insects usually appear in 7 days.

1.10. *Paecilomyces fumosoroseus*

The soil borne filamentous fungus *Paecilomyces fumosoroseus* is a common insect pathogen that has been isolated throughout the world. Because of its potential to cause epizootics naturally, *Paecilomyces fumosoroseus* is used for microbial control of insect pests .Conidia are often the means for dispersal and transmission of entomopathogenic fungi, they must come into contact with, germinate on, and then penetrate the insect cuticle.

1.11. Objective

- To find the effect of entomopathogenic fungi to control the sucking pests on Bt cotton
- To check which fungus will effectively control the sucking pests in Bt cotton, meanwhile inducing the plant growth

2. LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. Bt Cotton

2.1.1. Toxicological evaluation of genetically modified cotton

Insecticides derived from the bacterium *Bacillus thuringiensis* (Bt) and plants genetically modified (GM) to express *B. thuringiensis* toxins are important alternatives for insect pest control worldwide. This study investigated the effects of the ingestion of Bt cotton Bollgard and of the *B. thuringiensis* commercial product Dipel WP on the pantropical species *Scheloribates praeincisus* (Scheloribatidae). Ingestion of Bollgard and Dipel did not affect adult and immature survivorship and food consumption (estimated by number of fecal pellets produced daily) or developmental time of immature stages of *S. praeincisus*. These results indicate the safety of Bollgard and Dipel to *S. praeincisus* under field conditions where exposition is lower and other food sources besides leaves of Bt plants are available. The method for toxicological tests described here can be adapted to other species of Oribatida, consisting on a new option to risk assessment studies.(Olivera *et al.*,2007).

2.1.2. Highlevel resistance to *Bacillus thuringiensis* toxin crylac and cadherin genotype

Resistance to transgenic cotton, *Gossypium hirsutum L.*, producing *Bacillus thuringiensis* (Bt) toxin Cry1Ac is linked with three recessive alleles of a cadherin gene in laboratory-selected strains of pink bollworm, *Pectinophora gossypiella* (Saunders), a major cotton pest was analyzed. The strain (MOV97-R) with a high

frequency of cadherin resistance alleles, a high frequency of resistance to 10 micro gram of Cry1Ac per milliliter of diet, and an intermediate frequency of resistance to 1000 micro gram of Cry1Ac per ml of diet. Two strains were selected for increased resistance by exposing larvae from MOV97-R to diet with 1000 micro gram of Cry1Ac per ml of diet. In both selected strains, two to three rounds of selection increased survival at 1000 micro gram of CrylAc per ml of diet to at least 76%, indicating genetic variation in survival at this high concentration and yielding >4300-fold resistance relative to a susceptible strain. This conclusion was confirmed with results showing that when exposure to Cry1Ac stopped, survival at 1000 microgram of Cry1Ac per ml of diet dropped substantially, but survival at 10 microgram Cry1Ac per ml of diet remained close to 100% and all survivors had two cadherin resistance alleles. Although survival at 1000 microgram of Cry1Ac per ml of diet is not required for resistance to Bt cotton, understanding how genes other than cadherin confer increased survival at this high concentration may reveal novel mechanisms of resistance. (Tabashnik *et al.*,2006).

2.1.3. DNA Screening reveals pink bollworm resistance to Bt cotton

Transgenic crops producing toxins from the bacterium *Bacillus thuringiensis* (Bt) kill insect pests and can reduce reliance on insecticide sprays. Resistance to Bt cotton with DNA-based screening, were analyzed which detects single resistance alleles in heterozygotes. Polymerase chain reaction primers are used to amplify three mutant alleles of a cadherin gene linked with resistance to Bt cotton in pink bollworm, *Pectinophora gossypiella* (Saunders), a major pest In

conjunction with data from bioassays and field efficacy tests, the results reported here contradict predictions of rapid pest resistance to Bt crops.(Morin *et al.*,2006)

2.1.4. Effect of resistance to *Bacillus thuringensis* cotton on pink bollworm

Fitness costs associated with resistance to transgenic crops producing toxins from *Bacillus thuringiensis* (Bt) could reduce male response to pheromone traps. Such costs would cause underestimation of resistance frequency if monitoring was based on analysis of males caught in pheromone traps. To develop a DNA-based resistance monitoring program for pink bollworm, *Pectinophora gossypiella* (Saunders). Studies were conducted to compare the response to pheromone traps of males with and without cadherin alleles associated with resistance to Bt cotton (*Gossypium hirsutum*). When irradiated males from two hybrid laboratory strains with an intermediate frequency of resistance alleles were released in large field cages, the probability of capture in pheromone traps was not lower for males with resistance alleles than for males without resistance alleles. These results suggest that analysis of trapped males would not underestimate the frequency of resistance. Thus, the efficiency of a DNA-based resistance monitoring program would be improved by analyzing males remaining in traps for 3 d or less. (Carriere *et al.*,2006).

2.1.5. Bt toxin distribution in transgenic Bt cotton and soil System

Bt toxin distribution in transgenic Bt cotton and soil system study showed that the amounts of Bt toxin expressed in transgenic Bt cotton leaves and stems (103.5 - 134.1 ng x g(-1)) were rather higher than those expressed in transgenic Bt cotton roots (44.7 - 21.2 ng x g(-

1)), indicating that total amount of soil Bt toxin introduced by transgenic Bt cotton could be decreased through treating its above-ground biomass. In comparing with the amount of Bt toxin expressed in transgenic Bt cotton plant, that expressed in its root exudates was rather lower, showing that the effects of plant Bt toxin on soil ecosystem would be limited if other sources of Bt toxin introduced into soil were controlled.(Sun *et al.*,2005).

2.1.6. Measuring gene flow in the cultivation of transgenic cotton

Transgenic Bt cotton NewCott 33B and transgenic tfd A cotton TFD were chosen to evaluate pollen dispersal frequency and distance of transgenic cotton (*Gossypium hirsutum*). The objective was to evaluate the efficacy of biosafety procedures used to reduce pollen movement. The results indicated that the pollen of Bt cotton or tfd A cotton could be dispersed into the environment. Out-crossing was highest within the central test plot where progeny from nontransgenic plants, immediately adjacent to transgenic plants, had resistant plant progeny at frequencies up to 10.48%. Dispersal frequency decreased significantly and exponentially as dispersal distance increased. In this experiment, the farthest distance of pollen dispersal from transgenic cotton was 50 m. These results indicate that a 60-m buffer zone would serve to limit dispersal of transgenic pollen from small-scale field tests.

2.2. Aphids

2.2.1. Parasitism in Aphids

Natural enemies are important ecological and evolutionary forces, and heritable variation in resistance to enemies is a prerequisite for adaptive responses of populations. Such variation in

resistance has been previously documented for pea aphids (*Acyrthosiphon pisum*) attacked by the parasitoid was *Aphidius ervi.* (Hunter *et al.*,2005).

2.2.2. Pathogenicity of hypomycet fungi to Aphids

The aphids *Aphis gossypii* and *Myzus persicae* are cosmopolitan, poliphagous and damage cultivated plants. The effects of the entomopathogenic fungi *Beauveria bassiana* (isolate IBCB 66), *Metarhizium anisopliae* (isolate IBCB 121), *Paecilomyces fumosoroseus* (isolate IBCB 141) and *Lecanicillium (Verticillium) lecanii* (isolate JAB 02) on third instar nymphs of *A. gossypii* and *M. persicae* were evaluated in the laboratory at 25 ^{0} C, 70 +/- 10% RH and 12h photophase. The aphids were transferred to petri dishes with a foliar disk (cotton or pepper) with a layer of 1 cm tick of agar-water. The fungi were applied in a suspension containing 1.0 x 106 to 1.0 x 108 conidia/ml. In the control treatment, 1 ml of sterilized water was added to the foliar disks. The mortality of aphids was evaluated daily. *B. bassiana* and *M. anisopliae* caused 100% mortality at the seventh day after inoculation, for both species. *L. lecanii* was the fungus that provided mortality later in the aphids and *M. persicae* was more susceptible to both fungi than *A. gossypii.* (Lourerio Ede and Oino .,2006).

2.2.3. Transmission of Passion fruit woodiness virus by *Aphis gossypii*

The transmission of Passion fruit woodiness virus (PWV) by *Aphis gossypii* (Glover) was evaluated. In two independent experiments, *A. gossypii* transmitted PWV to passion fruit plants at the rates of 75% and 100%, when eight and twelve viruliferous aphids

were deposited by plant, respectively. At the end of the tests, nymphs of *A. gossypii* were observed in some of the passion fruit plants, suggesting that the aphid species was colonizing the plants. This seems to be the first report of *Passiflora edulis, f. flavicarpa* (Deneger) colonization by a species of aphid. (Di Piero *et al.*, 2006).

2.3. *Paecilomyces fumosoroseus*

2.3.1. Azadirachtin and *Paecilomyces fumosoroseus* to control white fly

Azadirachtin and *Paecilomyces fumosoroseus* (Wize) have been used to control the whitefly *Bemisia argentifolii* Bellows and Perring, but with only moderate effectiveness. Azadirachtin is a botanical insecticide derived from the neem tree, and *P. fumosoroseus* is an entomopathogenic fungus. To test whether these two agents might be more effective for whitefly control if used together, different rates of each were combined in laboratory bioassays in factorial treatment.Both tank mixes and separate sprays were tested. Up to 90% nymphal mortality was obtained when both the fungus and azadirachtin were combined, a significant increase over the 70%, or less, mortality obtained when only one agent was used; however, the combined effects were less than additive.(Brown and Smith.,2003).

2.3.2. Genetic variability of *Paecilomyces fumosoroseus*

Paecilomyces fumosoroseus is a fungus that is potentially useful for the bio-control of economically important agricultural pests, such as whitefly (*Bemisia tabaci*). Arbitrarily primed PCR and PCR with tRNA consensus primers were used to analyze genetic variability among 27 *P. fumosoroseus* isolates, 15 of which came from the same host, *B. tabaci*, one *P.lilacinus* isolate, used as an out group, 9

previously unidentified *Paecilomyces* isolates. Two of the three arbitrary phenetic groups were closely related (76% similarity) with the third group quite different (only 14% similarity) from the first two. The phenetic groups did not correlate with geographical origin or host species. (Tigano-Milani *et al.*,2006).

2.3.3. Impact of Carbon and Nitrogen nutrition on the quality, yield and Composition of blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus*

The impact of growing cultures of *Paecilomyces fumosoroseus* in liquid media containing four combinations of glucose and casamino acids (8 g l^{-1} or 80 g l^{-1} glucose, 1.32 g l^{-1} or 13.2 g l^{-1} casamino acids) was evaluated, based on blastospore production, germination rate, viability after freeze-drying and short-term storage stability. When blastospores were produced using a high casamino acid concentration, blastospore yields and germination rates were significantly higher ($13.2–18.5\times10^7$ blastospores ml^{-1}, 50–60% germination after 4 h), compared to cultures grown in media containing lower casamino acid concentrations ($0.4–2.3\times10^7$ blastospores ml^{-1}, 10–20% germination after 4 h). Chemical analyses of blastospore composition showed that accelerated blastospore germination may be related to increased proteinaceous reserves rather than to glycogen or lipid accumulation. Tolerance to freeze-drying by blastospores suspended in spent medium was enhanced by a high initial casein acid concentration in the culture medium (75% survival) and by the residual glucose concentrations in the spent medium. The storage stability of blastospores of *P. fumosoroseus* was unaffected by

the nutritional condition in which they were produced.(Sophie Cliquet and Mark Jackson ,2002).

2.3.4. Mycoparsitism of *Paecilomyces fumosoroseus*

Paecilomyces fumosoroseus was investigated for its mycoparasitism on the cucumber powdery mildew pathogen. Mycoparasitism was documented by using standard bioassay and SEM. Effects of mycoparasitism were evaluated in three types of experiments. *Paecilomyces fumosoroseus* was applied in the form of graded suspensions into a colony of powdery mildew on a leaf segment. Interaction between both fungi was observed as the percentage of colonized area vs. experimental time. In the second experiment, young cucumber plants were sprayed with a suspension of *Paecilomyces fumosoroseus* 24 h before inoculation of *Sphaerotheca fuliginea*. Pre-treatment with *P. fumosoroseus* reduced development and spreading of powdery mildew infection significantly 15 days post-inoculation in contrast to pre-treatments with sulfur fungicide and distilled water. In the third experiment, mildewed plants were treated with a suspension of *P. fumosoroseus*. The control treatments with sulfur fungicide and distilled water were tested. Effects of *P. fumosoroseus* on the dispersion of powdery mildew during a 21-day period were observed. *P. fumosoroseus* suppressed the development and spread of cucumber powdery mildew significantly during the time of the experiment. (Kavkova and Curn., 2003).

2.4. *Beauveria bassiana*

2.4.1. Screening and screotomic analysis of entomopathogen *Beauveria bassiana*

Entomopathogenic fungi adapt to growth in a culture medium containing an insect-like hydrocarbon as the sole carbon source inducing the beta-oxidation pathway during the alkane degradation. The effect of two carbon sources on the catalase activity was studied in the entomopathogenic fungus *Beauveria bassiana.* Catalase activity was detected both in the peroxisomal and cytosolic fraction. A significant increment in the specific activity of the peroxisomal fraction (12.6-fold) was observed when glucose was replaced by an insect-like hydrocarbon, whereas the specific activity in the cytosol diminished more than 1.2-fold in the same culture condition. After purification to homogeneity by gel filtration and strong anion exchange chromatography, an apparent molecular mass of 54.7 and 84.0 kDa per subunit were determined respectively for the peroxisomal and cytosolic catalase. The enzymes showed different biochemical and kinetic characteristics, but both were inhibited by 3-amino-1, 2, 4 triazole. Measurement of catalase activity is a new approach for evaluating fungal ability to degrade hydrocarbons. (Pedrini *et al.*, 2006).

2.4.2. Reliable alternative for coleopteran pest control

The production of cowpea (*Vigna unguiculata*), is severely affected by damage by the cowpea weevil *Callosobruchus maculatus.* The presence of a single larva in stored seeds can lead to losses of almost 40%.The use of entomopathogenic fungus became a reliable alternative for coleopteran pest control and has been extensively

investigated. Among them, *Beauveria bassiana* was widely evaluated in order to measure their virulence toward many insects. This study could, in the near future, help to establish novel biotechnological tools to use for cowpea weevil control. (Murad *et al* .,2007).

2.4.3. Interaction among the *Metarhizium,Beauveria bassiana* and *Oomyzus sokolowskii*

Research investigated the interactions among the fungi *Metarhizium anisopliae* (Metsch.) Sorok., *Beauveria bassiana* (Bals.) Vuill., and the larval-pupal parasitoid *Oomyzus sokolowskii* (Kurdjumov) before and after application of the fungi on DBM larvae offered to the parasitoid. The experiment was carried out at 26+/-l degreeC, 75+/- 5% RH and 12h photophase using a completely randomized design, with eight treatments with six replications each. The isolates E9 of *M. anisopliae* and ESALQ 447 of B. bassiana, were used at the concentration of 10(7) conidia ml(-1). The results showed that *M. anisopliae* and *B. bassiana* reduced the parasitism of *P. xylostella* by *O. sokolowskii*.(Barros *et al*.,2006).

2.4.4. Isolation of microbial control agents

Selection of *Beauveria bassiana* (Bals.) Vuill. And *Metarhizium anisopliae* (Metsch.) Sorok. Isolates against *Alphitobius diaperinus* was carried out to evaluate entomopathogenic fungi isolates as microbial control agents of the lesser mealworm, *Alphitobius diaperinus* (Panzer). Larvae and adults were inoculated with conidial suspension of 99 isolates of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok. (10(5) to 10(9) conidia/ml). Vegetative growth on culture media and sporulation on culture media, cooked rice and lesser mealworm cadavers were also

evaluated. Isolates of *B. bassiana* were more effective than the *M. anisopliae* isolates and larvae were more susceptible than adults. (Rohde *et al.*,2006).

2.4.5. Effect of nutrition on growth and Virulence of the entomopathogenic fungus *Beauveria bassiana*

Three isolates of the entomopathogen *Beauveria bassiana* along with one strain of *Metarhizium anisopliae* were cultured on seven media with different carbon/nitrogen (C/N) ratios. The effect of nutrition on virulence of the isolates was evaluated via measurement of colony growth, spore yield, germination speed, conidial C/N ratio and Pr1 (a serine protease) activity. 'Osmotic stress' medium produced the lowest colony growth with low numbers of conidia in all isolates. However, these conidia showed a high germination rate and virulence. However, conidial Pr1 activity was low in some isolates. In most but not in all cases,conidia from 1% yeast extract, 2% peptone and low (10 : 1) C/N medium had higher Pr1 activity compared with conidia from other media. C/N ratio of conidia was statistically different among various media and fungal isolates. Conidia with lower C/N ratio generally produced lower LT (50) (lowest median lethal time) values (more virulent). Insect-passaged conidia from different media had lower C/N ratio compared with similar conidia from artificial cultures. Therefore, they should be more virulent than *in vitro* produced conidia. As germination rate, conidial Pr1 activity and C/N ratio are independent of host, it seems that host-related determinants such as insect cuticle and physiology and environmental conditions may influence host susceptibility and therefore fungal isolate virulence towards host insects. (Safavi *et al.*,2007).

2.5. *Metarhizium anisopliae*

2.5.1. Evaluation of cellular response in engorged females of Boophilus *microplus* inoculated with *Metarhizium anisopilae*

The effect of *Beauveria bassiana, Metarhizium anisopliae, Penicillium corylophilum* or *Fusarium oxysporum* on the dynamic of hemocytes presented in the haemolymph of engorged females of *Boophilus microplus* was studied. The inoculation was carried out with conidia suspension of different fungi in the concentration of 10(8) conidia/ml. A negative control group was inoculated with 0.1% Tween 80 water solution and a testimony group was comprised of non inoculated ticks. The haemolymph samples were collected in 24, 48 and 72 hours post-challenge. Prohemocytes, plasmatocytes and spherulocytes were the most cells in the haemolymph. The absence of hemocytes 72h post-challenging was observed prior to the death of the specimens inoculated with *B. bassiana* suggesting a failure in the cellular response. Hyphae and conidia growth was observed in the samples treated with entomopathogenic fungi (*B. bassiana* or *M. anisopliae*). The groups treated with non entomopathogenic fungi (*P. corylophilum* or *F. oxysporum*) did not shown significant differences in relation to the negative control and testimony groups.(Da Silva and Bittercourt ,2006).

2.5.2. Evaluation of *Metarhizium anisopilae* against puparia &adults of *Ceratitis capitata*

Laboratory experiments were done to measure the pathogenicity of 10 autochthonous isolates of *Beauveria bassiana* (Balsamo) Vuill. and of five *Metarhizium anisopliae* (Metsch.) Sorok. toward puparia and adults of the Mediterranean fruit fly, *Ceratitis*

capitata (Wiedemann) (Diptera: Tephritidae). Although all isolates applied via inoculation of the fungal suspensions on the ventral surface of the abdomen were pathogenic to adults, with mortality rates ranging from 30 to 100% and average survival times (ASTs) from 6.5 to 8.6 d, when *C. capitata* puparia were immersed in the conidial suspensions, only *B. bassiana* Bb-1333 and EABb 01/103-Su and M. anisopliae EAMa 01/58-Su isolates caused >50% mortality of puparia.*M. anisopliae* was more effective in reducing fecundity and fertility at 6 d after treatment, with the reduction varying from 58.4 to 72.1% and from 28.6 to 45.9%, respectively. The highest pupal mortalities ranged from 52.5 to 70.0%, as a function of soil moisture and were caused by EAMa 01/58-Su and Bb-1333 isolates. (Quesada-Morga *et al.*,2006).

2.5.3. Pathogenicity of *Metarhizium anisopilae* for control of adult Hematobia irritans

Powder formulations of three species of entomopathogenic fungi were evaluated for their pathogenic effect upon adult horn flies, *Hematobia irritans* (L.) (Diptera: Muscidae). Flies were treated with conidia and blastospores of the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. (strain GHA), *Metarhizium anisopliae* (Metschnikoff) Sorokin (strain ESCI), and *Paecilomyces fimosoroseus* (Wize) .At 4 d postexposure, flies treated with *B. bassiana* had an average of 98.4% mortality versus 43.5% from treatment with M. anisopliae and 13.0% from treatment with *P. fumosoroseus*. At 7 d postexposure, flies treated with *B. bassiana* had an average of 100.0% mortality compared with 73.0% from treatment with *M. anisopliae* and 33.3% from treatment with *P.fumosoroseus*. Entomopathogenic

fungi such as *B. bassiana* and *M. anisopliae* may have the potential for controlling populations of horn flies. These studies indicate that B. bassiana and *M. anisopliae* were not only pathogenic to adult horn flies, but they caused mortality in a short time.(Lohmeyer and Miller .,2006).

2.5.4. Virulence of 11 native strains of entomopathogenic fungi *Metarhizium anisopliae*

Virulence of 11 native strains of entomopathogenic fungi *Metarhizium anisopliae* (three strains), *Beauveria bassiana* (six strains) and *Lecanicillium psalliotae* (two strains) well collected. After the exposure of ticks to the fungal strains in different concentrations (i.e. 10(3), 10(5), 10(7) conidia/ml),various parameters such as mortality rate and reproductive efficiency of engorged females, mortality of unfed tick larvae and eclosion percentage of infected eggs were evaluated to determine the fungal virulence. Based on the obtained results, five strains including *M. anisopliae* (IRAN 437 C and DEMI 001), *B. bassiana* (IRAN 403 C) and *L. psalliotae* (IRAN 468 C and IRAN 518 C) were found to be virulent to various stages of tick developmental cycle. Mortality rate of engorged females was found to be dose-dependent with regard to the conidial concentration used. In general, the entomopathogenic effects of native *M. anisopliae* and *B. bassiana* against various developmental stages of R. (B.) annulatus were confirmed in the present work. Likewise, although *L. psalliotae*, which was introduced for the first time as an entomopathogenic fungus against tick had not more than 13.3% mortality effect against adult females, but its effect on egg

hatchability and reproductive efficiency was remarkable.(Zare *et al.*,2006).

3. MATERIALS AND METHODS

MATERIALS AND METHODS

The present work was carried out at Research and Development Centre of T.Stanes Co Ltd which is recognized by, Department of Scientific and Industrial Research (DSIC). Ministry of Agriculture, Government and India, New Delhi, Annamalai University, Chidambaram and Bharathiar University, Coimbatore.

3.1. Sterlization of Glass wares

The Corning glassware's i.e., Petri dishes, Erlenmeyer flask (conical flasks), test tubes, pipettes and measuring cylinders intended for use in these studies were immersed for 24 hours in the solution containing 5% formalin and5% gramicide per liter of water for cleaning. They were then washed with tap water, rinsed in distilled water, air dried and sterilized in the hot air over at 160^0C for 2 hours pipettes and measuring cylinders were sterilized by autoclaving at 15psi for 20 minutes.

3.2. Preparation of culture media

- Special fungal medium (Appendix 1) consisting the following ingredients were used to culture the Entomopathogenic fungi's *Verticillium lecanii* ,*Metartizium anisopilae* ,*Paecilomyces fumosoroseus.*
- Potato carrt Agar (Appendix 2) used as solid medium for *Verticillium lecanii, Metartizium anisopilae* ,*Paecilomyces fumosoroseus.*
- Standard Dextorage Agar (Appendix 3) used as solid medium for *Beauveria bassiana*

3.3. Maintenance of culture

The fungus (*Verticillium lecanii, Metarhizium anisopilae, Paecilomyces fumosoroseus)* grown in PCA (Potato Carrot Agar) medium were utilized in this experiment. The fungus were multiplied in a complete medium previously autoclaved and poured onto sterilized Petri dishes, previously autoclaved and poured onto sterilized Petri dishes, by maintaining it for 5 days at 27(+ /-)C in BOD incubator. It has been originally isolated by T.Stanes and Co. Ltd and the same has been deposited at Institute of microbial Technology (IMTCH) Chandigarh India (Source, T.Stanes and Co Ltd). These are formulated and sold as the Stanes, Biocatch, Biomagic, Priority pests such as white flies, mealy bugs, aphids and thrips.

The pure stock culture of *Verticillium lecanii, Metarhizium anisopilae, Paecilomyces fumosoroseus* were maintained on potato carrot agar. They were sub cultured at an interval of one month on stanes. To have sufficient amount of inoculum, the fungi's were cultured in PCA slants and re inoculated into PCA media plates and incubated for 5 days in the laboratory at room temperature.

3.4. Inoculum preparation

Potato Carrot Agar and standard Dextrose agar were prepared. Each PCA slants were inoculated with *Verticillium lecanii, Metarhizium anisopilae & Paecilomyces fumosoroseus.* SDA slants was inoculated within *Beauveria bassiana.* They were incubated for 5-6 days at a room temperature of 27^0 (+/-) C.

3.5. Pot culture experiments

3.5.1. Seed Source: Seeds of Bt cotton (Bio 20-15) variety were collected from Kovilpalayam Agro Seed Centre.

3.5.2. Sowing: Seeds were sowed on 17-01-07.

Using Bt cotton bio 20-15 variety of the pest crop, a pot culture experiments were conducted to study the effect of entomopathogenic fungi to control the sucking pests. The Bt cotton seeds were sown in each experimental pots and the pots were placed in green house.

3.5.3. Treatments

1. Measured quantity of distilled water (100 ml) was equally dispensed in 12 conical flasks of 250 ml capacity.

Treatments	Organisms Used
T1	*Beauveria bassiana*
T2	*Verticillium lecanii*
T3	*Paecilomyces fumosoroseus*
T4	*Metarhizium anisopilae*
T5	*Beauveria bassiana* +1ml of Wetting agent
T6	*Beauveria bassiana* + 1ml of Nimbicidine
T7	*Verticillium lecanii* + 1ml of Wetting agent
T8	*Verticillium lecanni* + 1ml of Nimbicidine
T9	*Metarhizium anisopilaE* + 1ml of Wetting agent
T10	*Metarhizium anisopilae*+ 1ml of Nimbicidine
T11	*Paecilomyces fumosoroseus* + 1ml of W.A
T12	*Paecilomyces fumosoroseus*+1ml of Nimbicidine
T13	Control.

2. Approximately 1g of *Beauveria bassiana, Verticillium lecanii, Metarhizium anisopilae and Paecilomyces fumosoroseus* were taken from the slants and it was dispensed into their respective conical flasks (treatments) which contain 100 ml of distilled water.

3. Half of the (50 ml) spore suspension of T1, T2, T3, and T4 (without combination) was separated for serial dilution. The test tubes, tips and Petri dishes were sterilized for serial dilution method.

4. The serial dilution plating method, 1 ml of *Verticillium lecanii* culture (from T2) was suspended in 9 ml sterile distilled water which gave a dilution of 10^{-1}.

5. The serial dilution 10^{-2}, 10-^{3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10} were made by pipetting 1ml into dilution blanks containing 9ml of sterile distilled water.

6. Finally 1ml of various dilutions was added to the sterile Petri dish into which 15ml of sterilized cold molten (45^{0}C) PCA media was added. The dilution10^{-8}, 10^{-9} & 10^{-10} were selected for enumeration.

7. On solidification the plates were incubated 5-6 days at room temperature for fungal growth.

8. The above steps (4, 5, 6, 7, and 8) were repeated to do serial dilution for other fungi's.

9. SDA media was added for the fungi *Beauveria bassiana*

10. The each of the treatments (T1.....T12) were sprayed on Bt cotton plants at vegetative stage.

11. The first spray was done on the 35^{th} day when it reaches the vegetative stage of the growth.

12. The second and third spray's were done on the 45th and 50th day respectively, on the square forming stage. After each spray's the pest count were noted.

3.6. Parameters observed

The following growth parameters were recorded at the 60th day

3.6.1. Morphological Parameters

- Number leaves / 3plants
- Number branches /3 plants

3.6.1.1. Number of leaves

Number leaves were counted for each plant and the mean was taken

3.6.1.2. Number of branches

Number branches were counted for each plant and the mean was taken

4. RESULTS AND DISCUSSIONS

4. RESULTS AND DISCUSSION

Cotton crop often suffers from a series of complex pests. The most dreaded one amongst them is *Helicoverpa armigera.* Bt cotton seems to be an answer to protect the cotton bolls from *Helicoverpa armigera*, but definitely not from other pests, including pink boll worm. Therefore, when Bt cotton is cultivated, it is very much essential for the extension officials, field functionaries and farmers to have a basic knowledge of the pest morphology, biology and their management. Normally cotton plant is affected by sucking pests like cotton aphid, jassids, thrips, whiteflies, mealy bugs, red cotton bug, red spider mite, leaf roller, tobacco caterpillar, pink bollworm and the like. Therefore, it was thought worth, while to undertake the usage of Entomopathogen and botanical pesticide in integrated manner to control the early sucking pest problems in Bt cotton especially and cotton cultivation in general. There are a number of entomopathogens like *Verticillium lecani, Beauveria bassiana,verticillium lecanii,Metarhiziumanisopilae,Paecilomyces fumosoroseus Pseudomonas flourescens, Hirsutella thompsonii* and *Noumeria reili.* In my present study, I have tried entomopathogens like entomopathogens like *Verticillium lecani, Beauveria bassiana,verticillium lecanii,Metarhiziumanisopilae,Paecilomyces fumosoroseus.*There were 12 treatments carried out, different types entomopathogenic fungi were sprayed alone (T1,T2,T3,T4) ,with combination of Nimbicidine (T6,T8,T10,T12) and with combination of Wetting agent(T5,T7,T9,11). Intially, Mealy bugs present on the Bt

cotton plant.Before spray Mealy bugs counts were noted in the below (Table 4.1)

Treatments	Stage of Pest	
	Adults	Nymphs
T1	14	22
T2	7	135
T3	4	57
T4	9	4
T5	11	29
T6	7	29
T7	11	59
T8	8	23
T9	15	67
T10	9	19
T11	6	67
T12	28	47
Control	32	20

Table 4.1 before spray Mealy bugs count

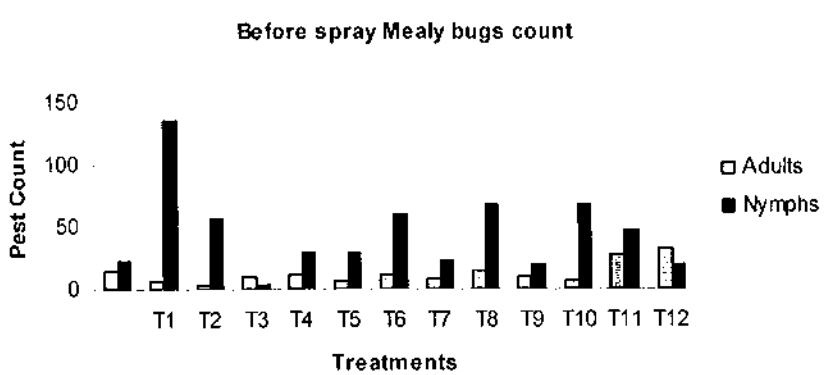

Figure 4.1 before spray Mealy bugs count

Table 4.2 I Spray Mealy bugs Count

Treatments	Stage of Pest									
	1st day		3rd day		5th day		7th day		10th day	
	A	N	A	N	A	N	A	N	A	N
T1	10	6	7	9	7	7	2	3	2	3
T2	7	102	7	102	6	58	6	27	3	23
T3	4	53	6	56	2	26	12	23	14	10
T4	3	3	3	3	7	16	7	15	7	17
T5	10	16	10	16	9	7	6	5	5	3
T6	7	27	6	24	4	8	4	6	2	3
T7	9	56	9	33	8	17	4	12	4	12
T8	8	17	7	13	7	13	5	3	2	0
T9	11	57	15	36	12	22	13	42	13	27
T10	9	17	12	22	17	5	17	9	14	9
T11	6	67	6	32	6	17	6	13	5	11
T12	27	47	27	44	23	47	25	39	11	27
Control	17	25	17	26	22	16	25	26	26	27

A- Adults N - Nymphs

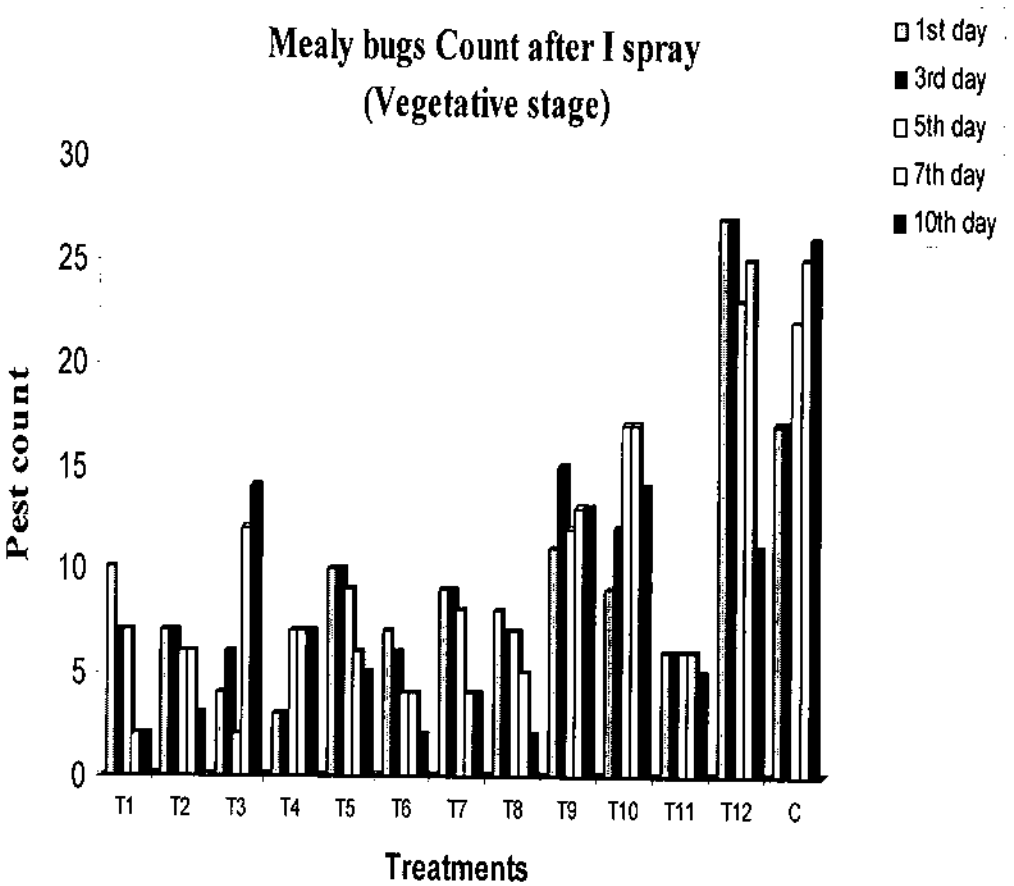

Figure 4.2 I spray Mealy bugs count

The above table 4.2. Shows the pest count was controlled efficiently in the treatment T5, T6, T7 and T8 when compared with other treatments and control.

II spray was carried out, at the end of the 45th day (Square forming stage).The pest counts were noted in alternative days and the results are tabulated below

Treatments	Stage of Pest			
	2nd day		4th day	
	Adults	Nymphs	Adults	Nymphs
T1	7	2	7	0
T2	5	19	4	18
T3	5	12	2	7
T4	5	5	5	9
T5	6	0	2	3
T6	2	12	0	0
T7	4	0	3	7
T8	2	12	0	0
T9	15	0	12	32
T10	14	32	9	7
T11	6	11	6	11
T12	13	27	9	23
Control	26	35	26	39

Table 4.3 II spray Mealy bugs Count

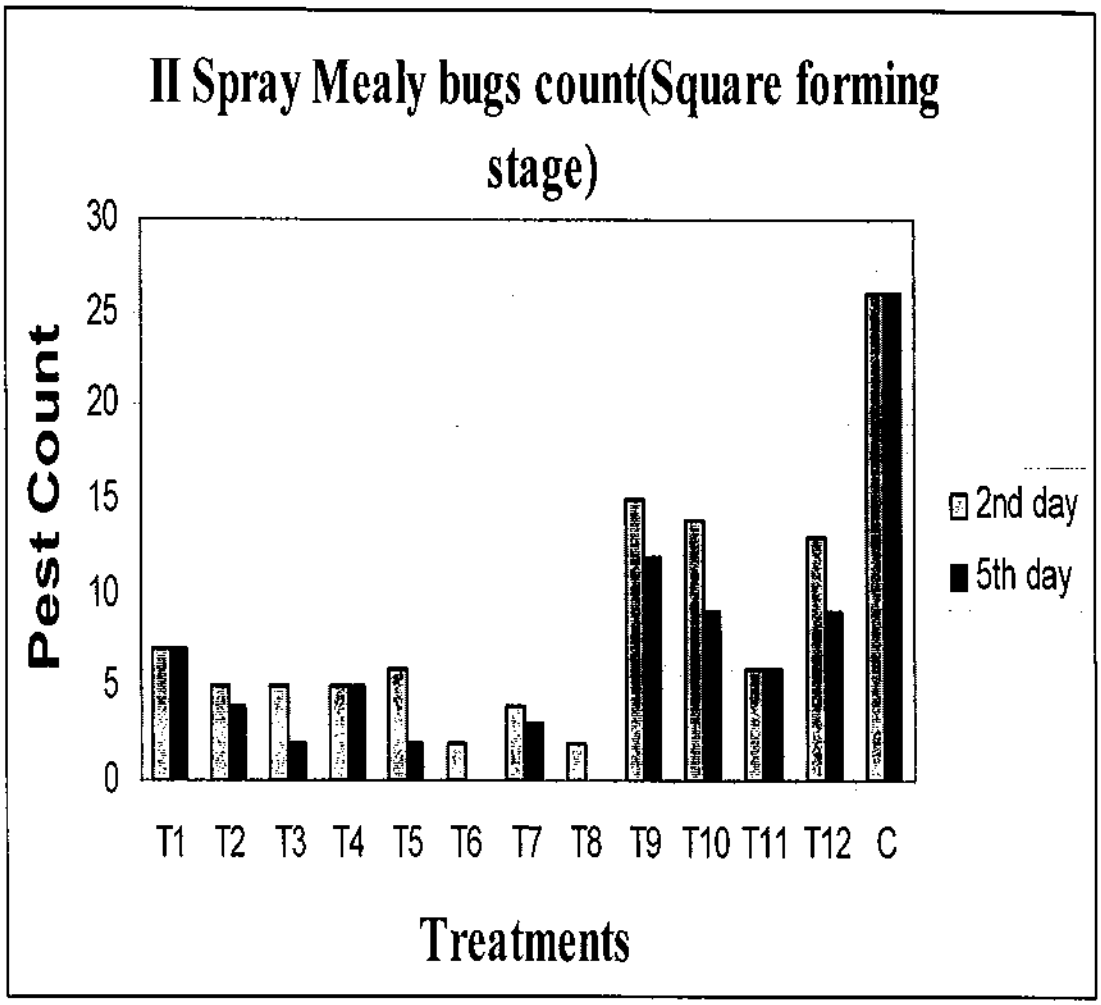

Figure 4.3 II spray Mealy bugs Count

III spray was carried out, at the end of the 50th day (Square forming stage).The pest counts were noted in alternative days and the results are tabulated below.

Treatments	Stage of Pest			
	2nd day		5th day	
	Adults	Nymphs	Adults	Nymphs
T1	3	1	3	0
T2	1	12	1	8
T3	2	9	2	2
T4	3	9	3	6
T5	1	0	0	0
T6	0	0	0	0
T7	2	2	0	0
T8	0	0	0	0
T9	12	29	12	2
T10	9	7	7	7
T11	6	11	5	9
T12	7	20	4	14
Control	28	42	27	53

Table 4.4 III spray Mealy bugs Count

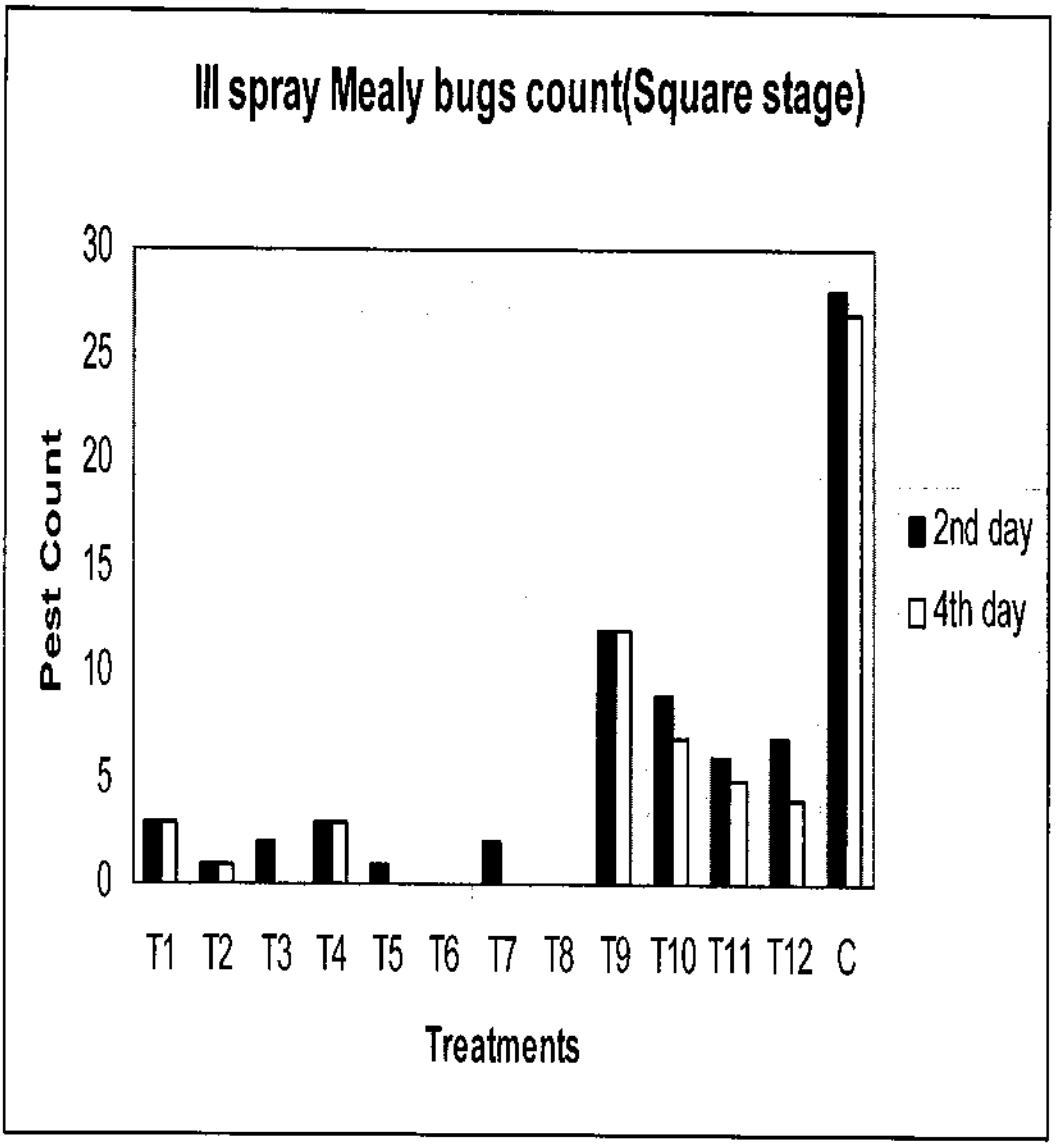

Figure 4.4:III spray Mealy bugs Count

III Spray results show that there were no pests in T6 and T8. And in other treatments also the pest count are very less in number.

4.5 Aphids

At the end of I spray Aphids were found on the Bt cotton. It can be counted and the results are tabulated below.

Table 4.5.1 II Prophylactic Spray Aphids Count

Treatments	Stage of pest						
	Upto 3rd day	5th day		7th day		10th day	
		A	N	A	N	A	N
T1	0	0	0	0	1	0	0
T2	0	0	0	2	33	0	47
T3	0	0	0	28	2	20	4
T4	0	0	0	0	4	0	0
T5	0	0	0	0	17	0	13
T6	0	0	0	0	0	0	0
T7	0	2	0	2	0	3	7
T8	0	0	0	1	5	0	0
T9	0	7	13	7	19	2	13
T10	0	0	0	2	13	1	15
T11	0	1	2	0	7	1	3
T12	0	0	0	14	5	11	7
Control	9	17	212	17	23	35	103

A-Adults N-Nymphs

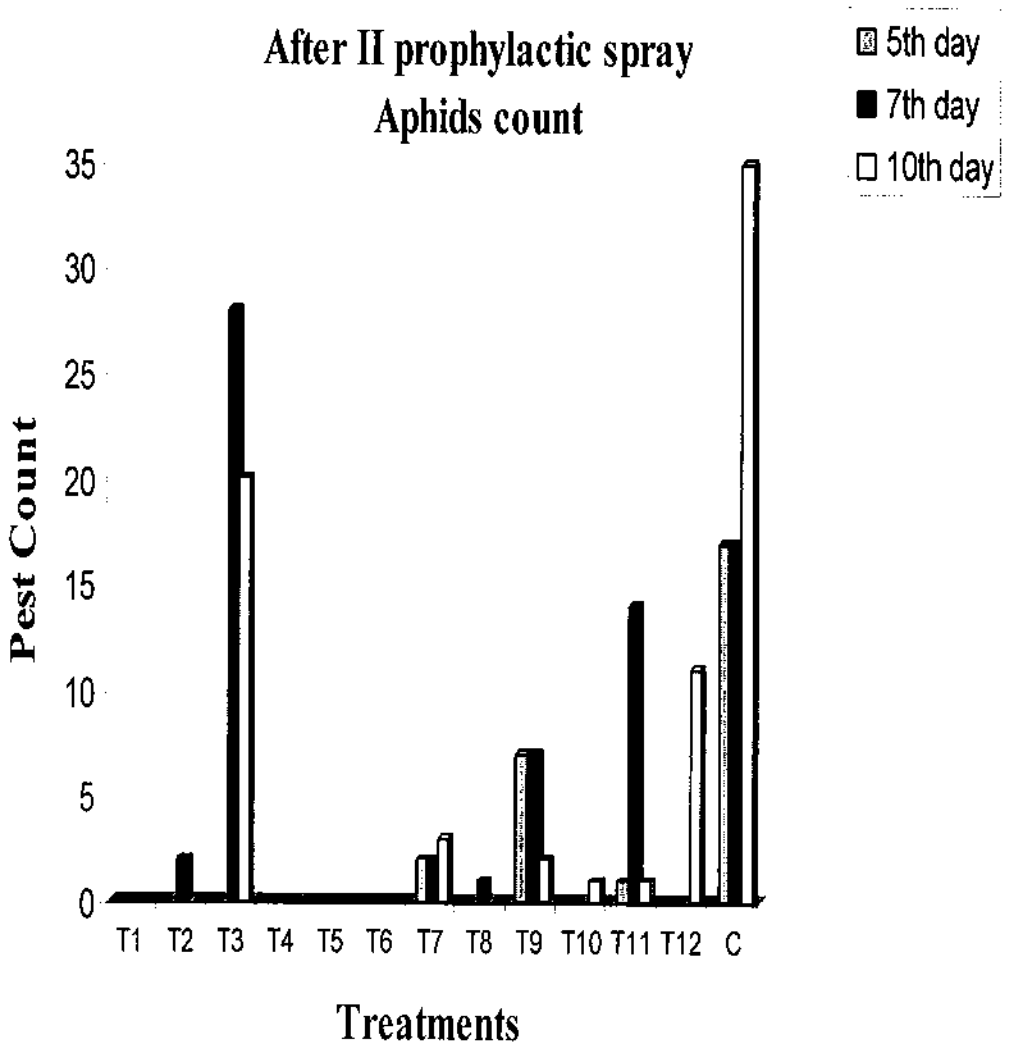

Figure 4.5.1 II Prophylactic Spray Aphids Count

The above table shows that there were no aphids found on T1,T4,T5 and T6.At III spray the aphids count were noted and the results are tabulated below.

Treatments	Stage of pest			
	2nd day		4th day	
	Adults	Nymphs	Adults	Nymphs
T1	1	0	0	0
T2	2	35	0	28
T3	18	11	18	19
T4	0	17	2	17
T5	0	0	0	11
T6	0	0	0	0
T7	2	0	0	0
T8	0	0	0	0
T9	0	17	1	13
T10	1	13	1	3
T11	1	3	1	3
T12	11	9	7	0
Control	17	128	29	227

Table 4.5.2 III Prophylactic spray Aphids count

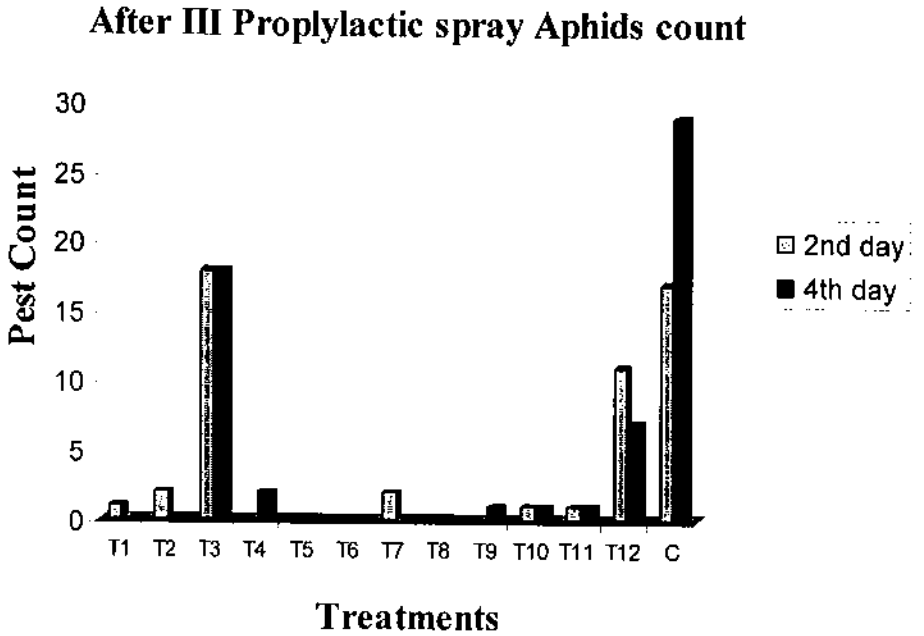

Figure 4.5.2 III Prophylactic spray Aphids count

4.6 Spore Count results

Spore count also done after I spray and the results are tabulated below

Beauveria bassiana

Table4.6.1 Spore count for *Beauveria bassiana*

Dilution	R1	R2	Mean value
10^{-8}	81	82	81.5
10^{-9}	42	41	41.5
10^{-10}	23	19	21

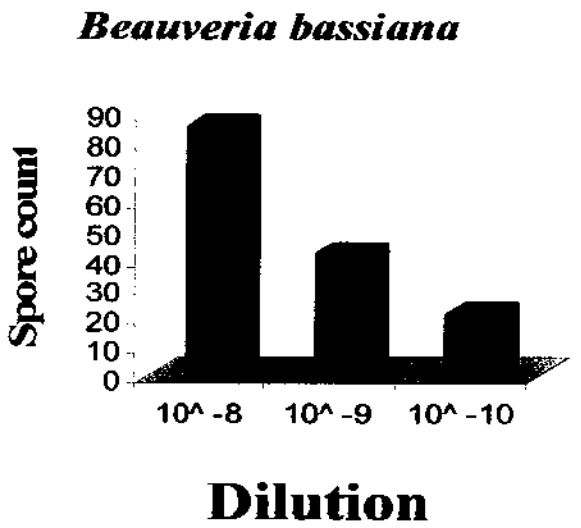

Figure 4.6.1 Spore count for *Beauveria bassiana*

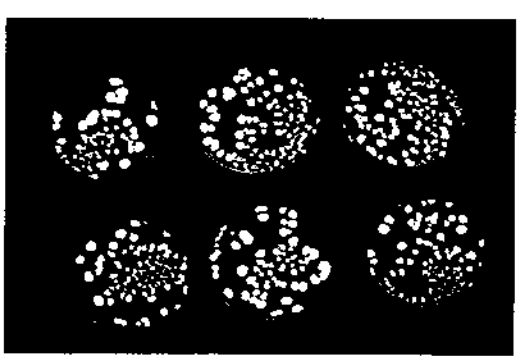

Figure 4.6.2 Spore count for *Beauveria bassiana*

Beauveria bassiana was serially diluted upto dilutions 10^{-10}.The spore count was noted to be more in dilution 10^{8}($81.5x10^{8}$ cfu/ml).Similarly 10^{9}&10^{10}as noted to be ($41.5x10^{9}$ cfu/ml) was noted to be ($21x10^{10}$ cfu/ml).

Verticillium lecanii

Table4.6.3 Spore count for *Verticillium lecanii*

Dilution	R1	R2	Mean value
10^{-8}	127	122	124.5
10^{-9}	104	102	103
10^{-10}	86	79	82.5

Verticillium lecanii was serially diluted upto dilutions 10^{-10}.The spore count was noted to be more in dilution 10^{8}($124.5x10^{8}$ cfu/ml).Similarly 10^{9}&10^{10}as noted to be ($103x10^{9}$ cfu/ml) was noted to be ($82.5x10^{10}$ cfu/ml).

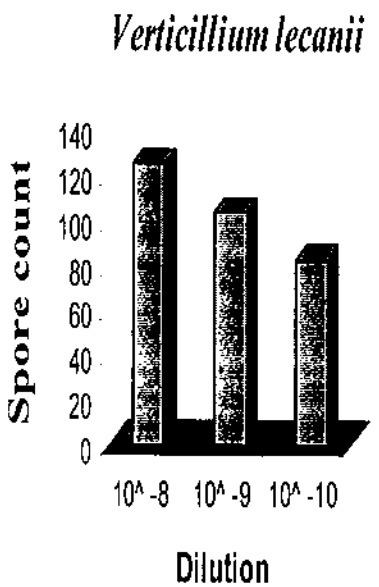

Figure 4.6.3 Spore count for *Verticillium lecanii*

Figure 4.6.3 Spore count for *Verticillium lecanii*

Metarhizium anisopilae

Dilution	R1	R2	Mean value
10^{-8}	191	182	186.5
10^{-9}	102	97	99.5
10^{-10}	84	80	82

Table 4.6.4 Spore count for *Metarhizium anisopilae*

Metarhizium anisopilae was serially diluted upto dilutions 10^{-10}.The spore count was noted to be more in dilution 10^{8}(186.5.5x10^{8} cfu/ml).Similarly 10^{9}&10^{10}as noted to be (99.5x10^{9} cfu/ml) was noted to be (82x10^{10} cfu/ml)

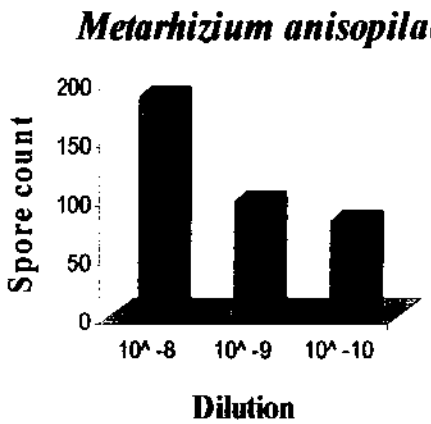

Figure 4.6.4 Spore count for *Metarhizium anisopilae*

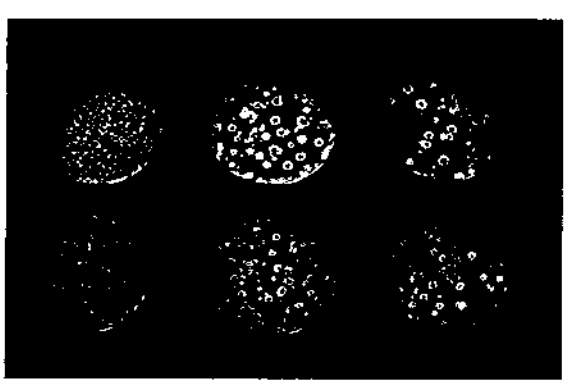

Figure 4.6.5 Spore count for *Metarhizium anispilae*

Paecilomyces fumosoroseus

Dilution	R1	R2	Mean value
10^{-8}	127	122	192.5
10^{-9}	104	102	147
10^{-10}	86	79	70

Table4.6.6 Spore count for *Paecilomyces fumosoroseus*

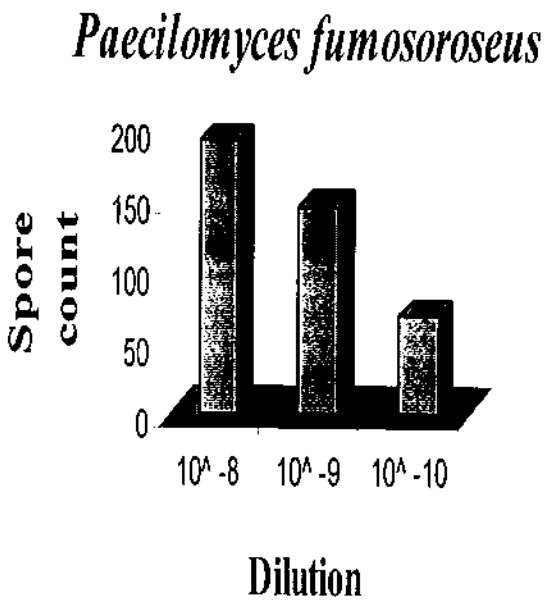

Figure 4.6.6 Spore count for *Paecilomyces fumosoros*

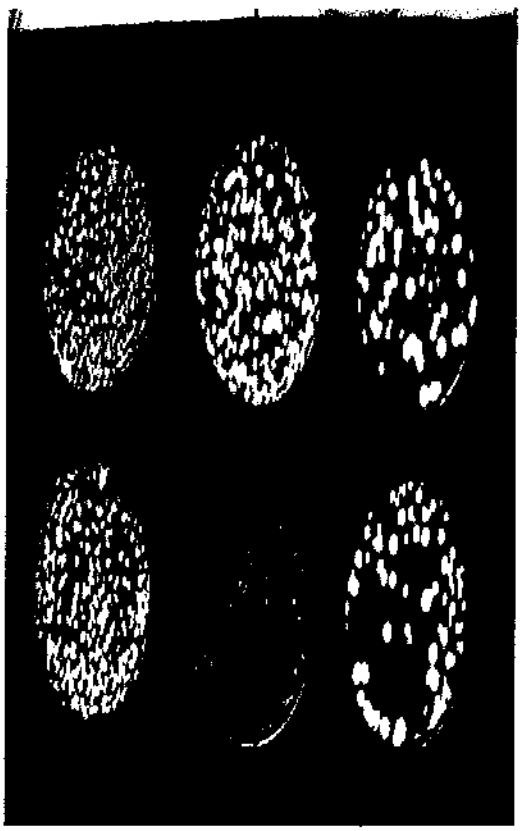

Figure 4.6.7 Spore count for *Paecilomyces fumosoroseus*

Paecilomyces fumosoroseus was serially diluted upto dilutions 10^{-10}.The spore count was noted to be more in dilution 10^{8}(192.5x10^{8} cfu/ml*)*.Similarly 10^{9}&10^{10}as noted to be (147x10^{9} cfu/ml) was noted to be (70x10^{10} cfu/ml).

4.7. Morphological Parameters

Number for branches

Numbers of branches were counted for each plant and the results are tabulated below

Table 4.7.1.Count for branches

Treatments	Numbe r of branches / 3 plants			
	First plant	Second Plant	Third plant	Mean value
T1	6	6	8	6.6
T2	9	11	0	6.7
T3	9	10	15	11.1
T4	8	9	8	8.33
T5	7	9	8	8
T6	14	15	6	11.6
T7	11	9	9	9.6
T8	14	17	8	13
T9	11	5	7	7.6
T10	11	9	10	10
T11	9	9	8	8.66
T12	7	10	10	9
Control	7	6	5	6

The above table show the number of branches was maximum (13) in plant T8 and the minimum (6) number of branches was found in control.little difference was noted between T6 and T8.

Number for leaves

Number of leaves was counted for each plant and the results are tabulated below

Table 4.7.2 Count for Leaves

Treatments	Numbe r of branches / 3 plants			
	First plant	Second Plant	Third plant	Mean value
T1	9	7	9	8.4
T2	8	9	8	8.3
T3	10	10	5	8.3
T4	10	10	6	8.7
T5	13	11	3	9
T6	12	13	7	10.7
T7	8	9	6	7.7
T8	10	13	7	10
T9	12	5	10	9
T10	11	10	8	9.7
T11	8	9	6	7.7
T12	7	9	10	8.7
Control	9	7	5	7

The above table shows the number of leaves was maximum (10.7) in plant T6 and the minimum (7) number of leaves was found in control. little difference was noted between T6 and T8.

5. CONCLUSION

CONLUSION

The table results shows that the Neem based combination with *Verticillium lecanii* and *Beauveria bassiana* shows effective against Mealy bugs. These combination also found to act as prophylactic pressure in controlling on Aphids on Bt cotton plant.Hence,we conclude that different entomopathgens for different pest complexes will be useful in controlling the sucking pest in Bt cotton not only to reduce the pest menace, but also facilitating proliferation of natural predators, in addition to reducing the chemical pesticides application in cotton crop. This will make Bt cotton cultivation more greener and bring the cotton cultivation towards organic farming concept.

6. APPENDICES

Appendix 1

Special Fungal Medium

Chemicals	g/litre
Glucose	10
Peptone	2
$FeSo_4$	0.01
KH_2Po_4	1.5
Kcl	0.5
$MgSo_4$	0.5
Sodium Nitrate	0.001
$CaCl_2$	6
$ZnSo_4$	0.5
Casein	0.5
Yeast	0.5
Chloramphenicol	0.1
Distilled water	1000ml

Appendix 2

Potato carrt Agar used as solid medium for *Verticillium lecanii,Metarhizium anisopilae,Paecilomyces fumosoroseus*

Chemicals	g/litre
Potato	30
Carrot	30
Glucose	10
Agar	20
Distilled water	1000ml
pH	6.5

Appendix 3

Standard Dextorage Agar (SDA) used as solid medium for *Beauveria bassiana*

Chemicals	g/litre
Glucose	20
Peptone	10
Sorbitol	1

Agar Agar.

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