KFRI Research Report 72

EVALUATION OF MICROBIAL PATHOGENS FOR BIOCONTROL AGAINST IMPORTANT INSECT PESTS OF AILANTHUS AND TEAK

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March 1991 Pages: 44

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ABSTRACT

A serious disease of the teak defoliator *Hyblaea puera* caused by a nuclear polyhedral virus was observed in various teak plantations. Bioassay studies revealed that the virus is highly pathogenic to *H. puera.* There was no cross infectivity to three other forest insect pests, viz., *Eutectona machaeralis, Eligma narcissus* and *Atteva fabriciella.* Three species of bacterial pathogens, viz., *Bacillus cereus, B. thuringiensis* var *thuringiensis* and *Enterobacter aerogenes* were also recorded from *H. puera. B. thuringiensis* was recorded as a stray case of infection and was highly pathogenic and caused 100% mortality in laboratory experiments. Low incidence of *B. cereus* infection was seen in *H. puera* while *E. aerogenes* was consistently isolated from field infected larvae. Except a new species of *Hirsutella* no other fungal pathogens could be observed on *H. puera.* No microbial pathogens were recorded from the teak skeletonizer *Eutectona machaeralis.* The fungal pathogen *Beauveria bassiana* was found to cause larval mortality of the teak sapling borer *Sahyadrassus malabaricus.* Two species of fungal pathogens viz. *Paecilomyces farinosus* and *P. fumasoroseus* were consistently recorded on the pupae of the yellow hairy caterpillar *Eligma narcissus,* an insect pest of *Ailanthus triphysa.* Laboratory trials indicated that *P. farinosus* is more effective in bringing about insect mortality than *P. fumasoroseus.* A leaf area bioassay for estimating the conidial activity of *Paecilomyces* spp. was standardised and it can be used for estimating the field spore concentration of *Paecilomyces* spp. Sporadic occurrence of bacterial pathogens, viz., *Bacillus firmus* and *Serratia marsecens,* were observed on larvae of *Eligma narcissus;* the former was effective only at high concentration. From *Atteva fabriciella.* shoot webber of *A. triphysa* only *B. bassiana* was recorded causing larval mortality. *B. thuringiensis* (teak strain) was found infective against both *E. narcissus* and *A. fabriciella.*

1. GENERAL INTRODUCTION

Application of pesticides is one of the earliest methods of pest control in forest plantations and nurseries. However, the use of pesticides in forest ecosystem is not well appreciated due to the suspected development of resistant strains of insect pests necessitating increased dose of pesticide application; damage caused to the non-target biota and the pollution to the environment. With more area likely to come under plantations and with intensive management practices, pest problems may be on the increase. But the lack of safe and effective methods to combat the pest situation in forest plantations force us to go for alternative means.

It is now generally agreed upon that the old system of insecticide.application should be replaced by "pest management" strategies, integrating different methods such as silvicultural, biological, chemical etc, so as to reduce the pest population below economic threshold levels. 'In this context, information on natural biocontrol agents is a prerequisite to develop non-polluting, safe methods of pest control in forest ecosystems. These methods, in general, are termed as "biological control", and one among them is the use of microbial pathogens. The major disease causing organisms are viruses, bacteria and fungi. Every insect pest has a number of natural enemies associated with it, including pathogenic microbes. Though we have some information on the pathogens associated with a few forest pests from India, detailed studies on this aspect are lacking. We require basic information on various pathogens associated with specific pests, host range and their efficacy as control agent under both laboratory and field conditions.

Teak is one of the most economically important forest species raised in plantations. Two important pests of teak are - *Hyblaea puera* Cramer (teak defoliator) and *Eutectona machaeralis* (Walker) (teak skeletoniser), of which the former causes serious economic damage to teak (Nair *et a/,* 1985). *Ailanthus triphysa* is a fast growing tree species and two major pests of this tree are *Eligma narcissus indica* Roth. and *Atreva fabriciella* Swed., of which the latter has been found to be a serious pest (Varma, 1986). Though we find some chemicals to control these pests in the literature (Varma, 1986), safe and effective methods using microbial pathogens are yet to be standardised.

The present study was therefore taken up with a view to generate data on microbial pathogens associated with the major pests of teak and *Ailanthus.* For promising pathogens, their seasonal occurrence in the field, pathogenicity and other pathological characteristics were studied to examine the potential ones to be used as control agents in the field.

2 VIRAL PATHOGENS

2.1. Introduction

Viruses from seven families and a number of as yet unclassified groups are known to occur in insects, in most cases causing lethal infection (Payne and Kelly, 1981). Only one family - Baculoviridae is exclusive to invertebrates, occuring mainly on insects and in some other arthropods. All other families contain members causing diseases in human or other vertebrates and the molecular similarity of some of the insect viruses and vertebrate pathogens is close (Harrap, 1982). For this reason, the development of insect viruses as biopesticides has tended to be concentrated on baculoviruses alone. At present 40 separate baculoviruses are being developed to control a wide variety of pests of field crops, grasslands, forests and stored products (Entwistle. 1976). Fourteen examples of baculoviruses used in Agriculture and Forestry in UK has been tabulated by Hunter *et a/.*,(1984). Several baculoviruses have already been registered as pesticides and available commercially.

Sub groups A (Nuclear Polyhedral Viruses-NPV) and B (Granulosis Virus-GV) are characterised by the presence of protinaceous inclusion bodies within which either a single virus particle (GV) or many virus particles (NPV) are embedded. The matrix protein within which the virus particles are embedded is known as polyhedrin for NPV and granulin for GV. The polyhedral inclusion bodies (PIB) of NPV are upto 5μm in diameter and each may contain several hundred virus particles. Each virus particle may contain one (singly enveloped SNPV) or more than one (multiply enveloped MNPV) nucleocapsid or virions. Initially the host range of individual baculoviruses was thought to be very restricted, but there are a few which can infect atleast two. For example *Autographa californica.* MNPV can infect insects from several genera.

The susceptibility of larvae of Lepidoptera and sawflies to baculovirus infection declines with age, early instars being most susceptible (Evans, 1981). Decrease in susceptibility tends to be related to increase in weight such that the LD_{50} /mg body weight is often independent of host age. Infection occurs most commonly via midgut following infection of PIB, but can also be initiated by infection of a virus into the haemocoel by parasitoid wasps during oviposition. When ingested, under the alkaline gut condition, the PIB break down, releasing virus particles. In Lepidoptera, the virus particles pass through the midgut cells, sometimes with a replicative phase, and enter the haemocoel. The major sites of replication are nuclei of fat body, haemocytes and hypodermis.

Epizootics baculovirus diseases are frequent in both Lepidoptera and sawflies, often with very high larval mortalities resulting in population depression. Epizootic development usually requires a polyvoltine insect and the

tendency of a baculovtrus disease to become epizootic depends on the scale of virus dispersal (spatial component) together with persistence of virus outside and within the host (temporal component). Baculoviruses survive long periods in soil (Thompson *et a/..* 1981). The recycling of virus from soil occurs by movement of animals, rain splash and by wind blown soil particles and PIB onto leaves. Reservoirs of baculovirus in soil probably have a more longterm importance, perhaps initiating fresh epizootics when insect population resurge following a phase of low density (Entwistle, 1976; Evans and Entwistle, 1982).

There have been reports on the occurrence and epizootiology of baculoviruses from forest ecosystems in UK. Canada and USA (Hunter *et a/.,* 1984; Abrahamson and Harper, 1973; Vendol, 1975). Ahmed and Sen Sarma (1983) reported the occurrence of nuclear polyhedrosis on *Pygaera fulgurita* (Notodontidae : Lepidoptera), and no detailed investigations have been carried out in India on the occurrence and epizootiology of baculoviruses in forest insects.

2.2. Materials and Methods

2.2.1 *Search for diseese incidence* : Various teak plantations in Nilambur, Parambikulam, Ranni, Konni, Punalur, Malayattoor, Trichur and Trivandrum Forest Divisions and plantations of *Ailanthus triphysa* in Pothuchady (Trichur Forest Division), Kottappara, Thattekad (Malayatoor Forest Division) and Erumeli (Kottayam Forest Division) were visited from 1986 to 1988 during the peak incidence of various insect pests. Dead and morbid larvae lying on leaves or hanging by their prolegs (Fig. 1A) were collected and brought to the laboratory under asceptic conditions. These specimens were appropriately examined for the presence of causal organism. If any such species indicated viral infection during the postmortem examination the smear was subjected to staining procedures for further observations as described by Poinar and Thomas (1978).

When the virus association with teak defoliator, *H. puera* was confirmed, partially purified virus pellets were subjected to Electron microscopy for ultrastructure.

2.2.2 *Bioassay studies* : Fourth instar larvae of *H. puera* were used for bioassay. The larvae were obtained either from field or from a culture maintained in the laboratory. The polyhedral inclusion bodies were obtained from diseased dead larvae by putrification and were partially purified by filtiation and centrifugation (Ramakrishnan, 1976). The number of PIB in stock suspension was determined through a Neubauer haemocytometer and serial dilutions were made in sterile water for bioassay studies

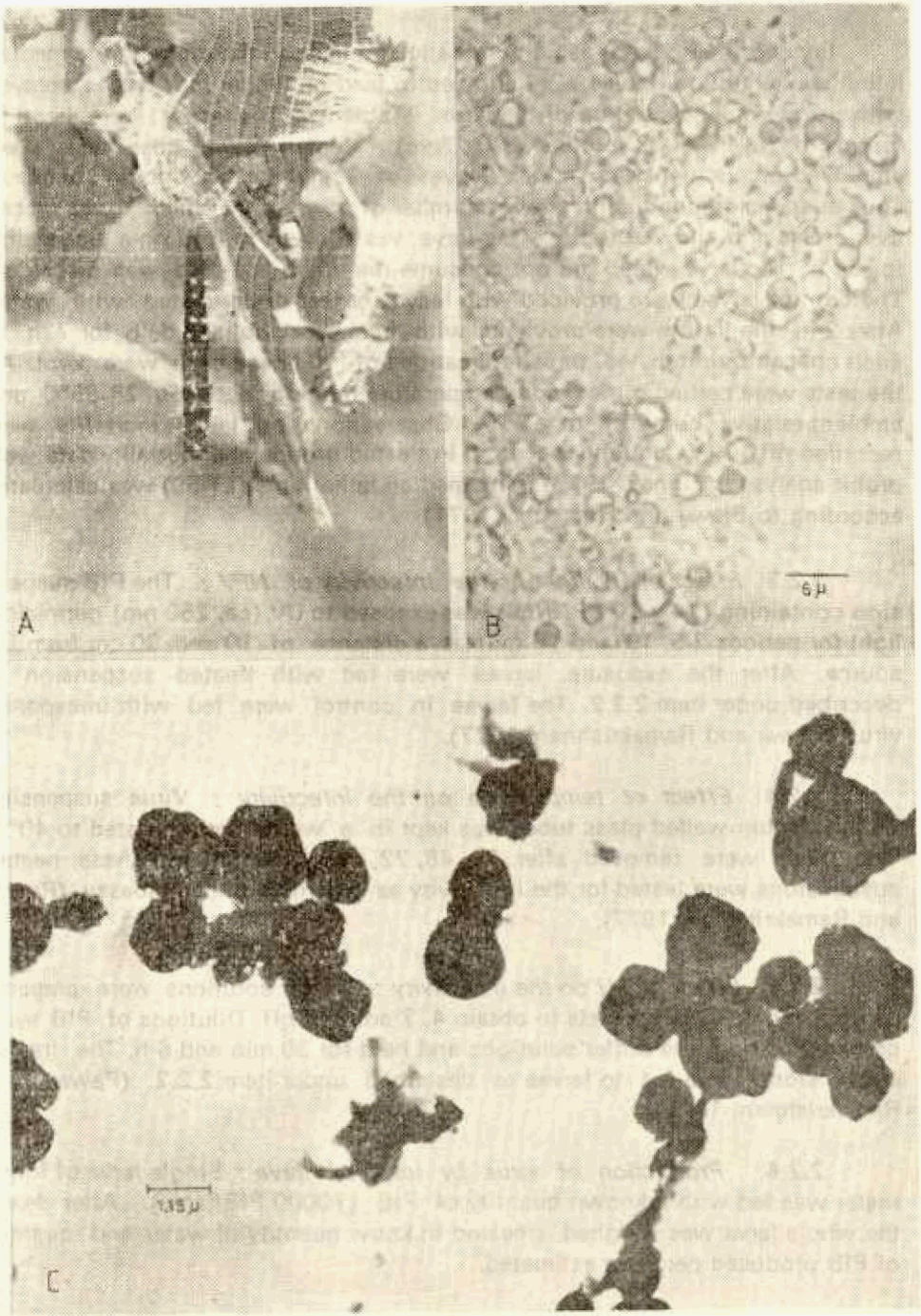


Fig. 1. A. *H. puera* larva infected by NPV; B. Photomicrograph of NPV. of *H. puera*; C. Scanning electron micrograph of NPV of *H. puera*

For confirming pathogenicity initially and later on determining the median lethal concentration, larvae were allowed to feed on tender teak leaves sprayed with a known concentration of the virus. (Table 1). The larvae were kept in groups of 10 in glass bottles (17 x 7cm). Median lethal dose tests were employed on circular discs of teak leaves of 2cm diameter. Known volume of virus suspension containing known number of polyhedra (Table 2) was placed over a disc and allowed to dry. One larva was allowed to feed on a single disc for 24 h. The larva which did not consume the entire leaf disc was discarded. The control larvae were provided with leaves or leaf discs treated with water. After 24h the larvae were provided with fresh teak foliage daily for 72h. In each concentration tested, three replications of 10 larvae each were kept. All the tests were carried out at room temperature ranging between 28-35°C and ambient relative humidity of >75%. Observations on larval mortality were recorded till 100% mortality occurred in treated groups and mortality data were probit analysed (Finney, 1977). The median lethal time (LT 50) was calculated according to Biever and Hostetter (1971).

2.2.3 *Effect of UV light on the infectivity of NPV* : The PIB suspension containing (14 x 10^6 PIB/ml) wasexposed to UV (ca. 250 nm) germicidal light for periods 2.5, 10 and 15 min at a distance of 10 and 30 cm from UV source. After the exposure, larvae were fed with treated suspension as described under item 2.2.2. The larvae in control were fed with unexposed virus (Pawar and Ramakrishnan, 1977).

2.2.4. *Effect of temperature on the infectivity* : Virus suspension (5 ml) in thin-walled glass tubes was kept in a water bathadjusted to 40°C. The tubes were removed after 24, 48, 72, 120 and 168 h. These heated suspensions were tested for the infectivity as described under bioassy (Pawar and Ramakrishnan, 1977).

2.2.5 *Effect of pH on the infectivity* : Buffer solutions were prepared using standard buffer tablets to obtain 4, 7 and 9.2 pH. Dilutions of PIB were prepared using these buffer solutions and held for 30 min and 6 h. The treated suspensions were fed to larvae as described under item 2.2.2. (Pawar and Rarriakrishnan, 1977).

2.2 6. *Production of virus by infected larva* : Single larva of fourth instar was fed with a known quantity of PIB (70000 PIB/larva). After death, the whole larva was weighed, smeared in know quantity of water and quantity of PIB produced per larva estimated.

2.2.7. *Cross infectivity studies* : Since no viral pathogen was isolated from other insect pests included in the study, the baculovirus isolated from

H. puera was subjected to cross infectivity tests on three other insects as per the method included in item 2.2.2. The lepidopteran larvae included in the study were *Eutectona machaeralis* (teak skeletoniser), and *Eligma narcissus* (yellow hairy caterpillar) and *Atteva fabriciella* (shoot webber) the two serious insect pests of *A. triphysa.*

2.3 Results and Discussion

2.3.1 *Search for disease incidence* : Mortality of *H. puera* due to viral infection was prevalent in all the teak plantations visited. Studies undertaken in 3 teak plantations of Nilambur revealed that the infection at moderate levels was prevalent usually from the onset of monsoon (second to third week of June) and continued upto October. Thereafter the insect population became erratic and association of virus continued in stray cases till the next peak of insect build up.

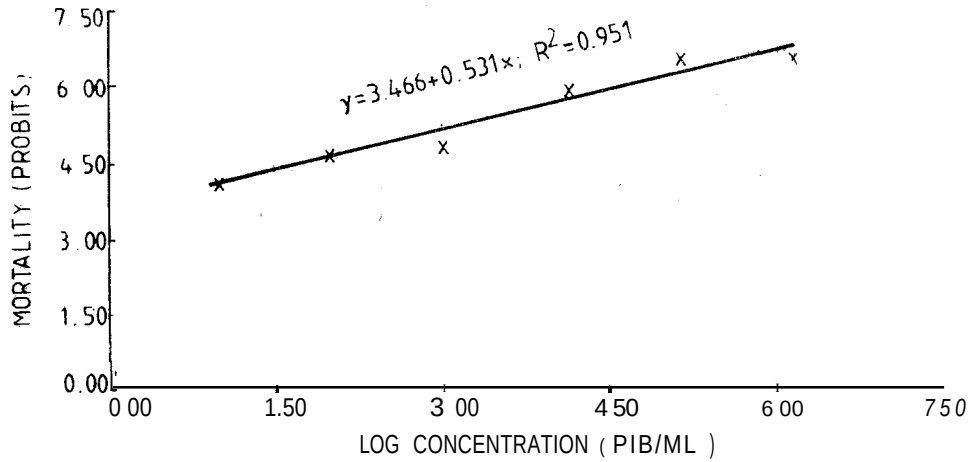
Microscopic examination of the smear of infected *H. puera* larvae revealed the presence of refractile polyhedral inclusion bodies. The inclusion bodies appeared refringent (shining white) under bright field (Fig. 1, B). The inclusion bodies did not get stained with Sudan III (10-15 min) and fat droplets turned red. Inclusion bodies on staining with Giemsa (diluted) became blue and were clearly visible, whereas fat globules stained purple to red. When stained with Buffalo black, the inclusion bodies stained thick blue to black in colour.

Electron microscopy confirmed the presence of PIB, polyhedral in shape which measured 0.9 - 2.4 μm in diameter (Fig. 1C).

2.3.2. *Bioassay studies* :

2.3.2.1 *Pathogenicity studies* : When healthy third and fourth instar larvae of *H. puera* were fed with leaves sprayed with 2×10^6 PIB/ml, the feeding rate of the larvae was found to be slow on the second day and by the third day feeding almost ceased. The larvae became sluggish with flaccid bodies and died in 3-5 days. The body wall ruptured and liquified body contents oozed out. The stained body smear, on examination revealed the presence of refractile polyhedral inclusion bodies thereby confirming the pathogenic nature of the NPV. All stages of larvae or *H. puera* were susceptible to NPV and occurrence of NPV on *H. puera* is the first record of its nature from the country (Sudheendrakumar *et a/..* 1988).

2.3.2.2 *Median lethal concentration (MLC) studies* : It is evident from the Table 1 that NPV is pathogenic to larvae of *H. Puera* even at a low concentration of 10×10^2 PIB/ml wherein 40% of the test insects did



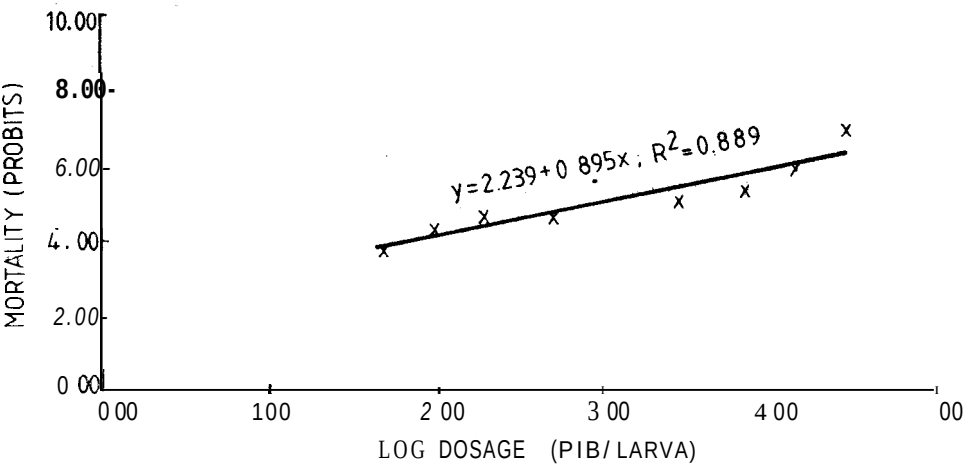


Fig. 2. A. Concentration - mortality curve of fourth instar larvae of *H. puera* exposed to NPV.

B. Dosage - mortality curve of fourth instar larvae of *H. puera* exposed to NPV.

not survive. The LC_{50} value was 796 PIB/ml with fiducial limits of 300-2100 PIB/ml. The LT_{50} value was 61.4 h at a concentration of 14.07 X 10^6 PIB/ml. and 102.0 h at 10 x 102 PIB/ml concentration. The LT_{50} varied with the concentrations of the PIB administered, lower the concentration of PIB, higher the value (Table 1). A minimum period of 3 days was required for the mortality to occur due to virus infection. Concentration - mortality curve is given in Fig. 2 A.

Table 1. Effect of different concentrations of NPV to *H.* puera

Concentration of PIB/ml	%Mortality* at 96 h	LC_{50}	LT_{50} (in h)	Fiducial limit lower	upper	b value
14.07 x 10^6	100.0		61.4			
14.07 x 10^5	93.3		68.0			
14.07 x 10^4	93.3		70.5			
14.07 x 10^3	80.0	796	78.0	300	2110	0.531
10.00 x 10^2	40.0	PIB/ml	102.0	PIB/ml	PIB/ml	
1.00 x 10^2	33.3		—			
1.00 X 10	16.6		—			
0	0		—			

* N = 30 larvae.

2.3.2.3 Median lethal dose *(MLD)* studies

The results of bioassay studies with NPV of *H.* puera are presented in Table 2 and dosage - mortality curve in Fig. 2 B. In various dosages tested, the mortality was directly related to the number of PIB injested orally. The LD_{50} value for a fourth instar larvae was 1427 PIB. The LT_{50} was 60.8 h for the highest concentration and 97.6 h for the dose of 2800 PIB/larva. The LT_{50} varied with the dose of PIB tested, lower the dose higher the value. The regression equation for the doses tested were of the form Y=A+bx where Y= mortality in probits and x=-log10 dose/larva. Ramakrishnan and Chaudhari (1979) recorded the LD_{50} value for 4- and 8-day-old larva of Diacrisia obliqua (Walker) as 741 and 13360 PIB/larva respectively. Bucher and Turnock (1983) when working on dosage-mortality relationship of NPV on bertha army worm *Mamestra configurata* (Wal), determined that susceptibility to infection decreased as larvae aged, the mediam exposure dose (LE_{50}) increased from 18 PIB/larva for the first instar to 21 x 10^6 PIB/larva for half grown larvae. However, quantification of PIB/larva of different instars could not be determined in our study.

Table 2. Effect of different doses of NPV to *H. puera*

Dose of PIB/Larva	% Mortality* at 96 h	LD_{50}	LT_{50} (in h)	Fiducial limit lower	Fiducial limit upper	b value
70000	100.0		60.8			
28000	96.6		68.0			
14000	80.0		70.5			
7000	60.0		92.0			
2800	50.0	1427	97.6	850 PIB/larva	2546 PIB/larva	0.895
500	33.3		—			
200	33.3		—			
100	20.0		—			
50	10.0		—			
0	0		—			

* N = 30 larvae

Based on the observations made in dosage/concentration studies, NPV appeared to be a promising candidate for biocontrol against *H. puera.* However, it would be worth investigating dosage-mortality and the growth of NPV in all the instars of teak defoliator.

2.3.3 *Effect of pH on the infectivity of NPV*

The results obtained on the larval mortality due to NPV held 6 h at different pH levels (Table 3) showed slight change in total mortality (<10.0%) when the virus was held at pH 4, and 9.2 and LT_{50} values changed slightly from 65 to 70h. However, at 30 minutes exposure there was no change in the activity, as compared to pH 7 where there was no loss in activity at both the exposures.

The adverse effect of extreme hydrogen ion concentration (pH) on the infectivity of NPV had been reported by Ignoffo and Garcia (1966) and Gudauskas and Canerday (1968). Recently, effect of pH of soil and cotton leaf surfaces on NPV of *Trichoplusia ni* and *Heliothis zea* were reported (Thomas *et a/.,* 1973; Andrews and Sikorowski, 1973). Pawar and Ramakrishnan (1977) reported that NPV of *Spodoprera litura* was stable at pH 2,5,7 and 9. At pH 11 virions were liberated from PIB by carbonate buffers and are considerably inactivated by prolonged exposure to high alkaline condition. Shapiro and Ignoffo (1969) also observed that infectivity of virions of *Heliothis* NPV was reduced by about 99.99% after carbonate dissolution of PIB, suggesting that the usual method of treating PIB to release occluded virions has its shortcomings.

Table 3. Effect of pH on the infectivity of NPV to *H. puera*

pH	Treatment time	Initial mortality(%)	Final mortality* (%)	LT_{50} (in h)
4.0	30 min	60.0 (3)	100.0 (4)	68.0
	6 h	53.3 (3)	90.0 (4)	70.7
7.0	30 min	90.0 (3)	100.0 (4)	65.1
	6 h	90.0 (3)	100.0 (4)	65.1
9.2	30 min	60.0 (3)	93.3 (4)	68.0
	6 h	60.0 (3)	90.0 (4)	70.7
Control	30 min	90.0 (3)	100.0 (4)	65.1
	6 h	90.0 (3)	100.0(4)	65.1

N = 30 larvae. PIB concentration 14×10^6 PIB/ml. Figures in parentheses indicate the day on which mortality occurred.

2.3.4 *Effect of temperature on the infectivity of NPV*

Polyhedral inclusion bodies were held at 40°C upto 7 days (168 h) in order to test the infectivity of virus and results are presented in Table 4. Heating of NPV for 168 h at 40°C did not affect the mortality percentage of larvae. The LT_{50} values also did not change appreciably at different exposure periods.

Table 4. Effect of temperature on the infectivity of NPV to *H. puera*

Treatment time (h)	Concentration of PIB/ml x 10^6	(%)Mortality * at 96 h	LT_{50} (in h)
24	14.0	100.0	61.4
	1.4	91.1	68.0
48	14.0	100.0	65.1
	1.4	91.1	68.0
72	14.0	91.1	68.0
	1.4	88.8	70.7
120	14.0	91.1	68.0
	1.4	88.8	70.7
168	14.0	91.1	68.0
	1.4	86.6	70.7
Control	14.0	100.0	61.5
	1.4	93.3	68.0

* N = 45 larvae.

Generally, NPVs are inactivated only at abnormally high temperatures (Aizawa, 1963). The NPV of *Spodoptera litura* was inactivated by heating at 95°C for 10 min. (Pawar and Ramakrishnan, 1977). In preliminary studies, we also found that heating of NPV samples for 30 min at 80°C and 100°C completely inactivated the virus. Kislev *et al.,* (1969) observed that heating the virus at higher temperature caused projections in PIB as observed after alkaline treatment. It is, therefore, likely that infective virus particles leave the inclusion body through such holes and are inactivated.

Morris (1971) reported that heating of the virus at 45°C for upto 200 h did not affect the final percentage of mortality in the case of NPV against Western hemlock looper. However, both the mean time to death and LT_{50} decreased with exposures upto 5 h and thereafter increased. Gudauskas and Canerday (1968) obtained no loss of activity at 70-75°C after 10 min,. 40% loss at 75°C and complete loss of infectivitity at 80°C. Since there was no significant inactivation observed at 40°C for 168 h, NPV of *H. puera* could be stable in the field.

2.3.5 *Effect of ultraviolet light on the infectivity of NPV*

Table 5 gives an account of percent mortality of larvae of *H. puera* after feeding the contaminated leaves with virus suspension exposed to ultraviolet light (UV). It is evident that the virus was progressively inactivated with prolonged exposure to UV light. There was an inactivation of about 35% at a distance of 15cm from UV source. As the distance was increased to 30 cm about 25% inactivation occurred after 15 min of exposure, but there was no complete inactivation in either of the treatments. The LT_{50} values were also higher than the LT_{50} values of 65.1 h for the "unexposed" virus control.

Table 5. Effect of ultraviolet light on the infectivity of NPV to *H. puera*

Distance from UV source (cm)	Exposure time (min)	%Mortality* at 96 h	LT_{50} (in h)
15	2	100.0	72.1
	5	86.6	85.9
	10	74.3	88.4
	15	63.3	91.9
30	2	100.0	72.1
	5	93.3	84.9
	10	90.0	85.4
	15	76.7	87.7
Unexposed virus	0	100.0	65.1

* N = 30 larvae. PIB concentration 14 x 106

The nuclear polyhedral viruses within their inclusion bodies are sufficiently protected and can withstand different environmental conditions (Aizawa, 1963; Smith, 1967). Most viruses are inactivated by radiation of wave lengths in the range of maximum absorption of nucleic acid (i. e , 254-260 nm) and such irradiations result in formation of pyramidine dimers in DNA (Harm, 1980). Radiation beyond 300 nm is relatively less effective

In the case of NPV of *Lampdina fiscellaria lugubrosa* (Western Oak looper) the ultraviolet radiation for a period of 100 h produced little variation in mortality data. However, both the average time to death and LT_{50} increased with prolonged exposures (Morris, 1971). Pawar and Ramakrishnan (1977) have reported complete inactivation of NPV of S. *litura* at 15 min exposure to UV from a distance of 10 cm and at 30 cm only 70% activity of NPV was lost However, in our studies ca. 35% infectivity was lost after 15 min exposure to UV at 15 cm distance, stressing the need for using some adjuvants 'like charcoal or Indian ink while spraying the NPV in the field.

2.3.6 *Production of virus by infected larvae* : A fourth instar larva yielded an average of 38.72×10^7 PIB (range of 22.65 to 58.50×10^7) after 4 to 5 days *of* incubation. The average weight of *a* dead larva was 175mg, with a range from 150 to 230 mg.

A benefit of quantifying polyhedral production is the consequent ability to predict the potential number of polyhedra present from a given larval weight. This has implications in improving harvesting techniques for virus propagation. The productivity ratio of NPV of *H. puera* for a fourth instar larva which is defined by the ratio of polyhedra produced to dose ingested is 5530 (range 3235 to 8357). Productivity ratio even though gives information on available source of inoculum, a compromise has to be reached between the great yield from larger larvae (fifth instar) and the low doses that may be required for younger larvae. But it would be worth to study the virus growth curve data, which can be used to predict the densities of PIB in a population of insects in the field. Given the host population, age, structure and knowledge of infection levels, it should be possible to quantify the build up of polyhedra in time (Evans, 1981).

2 3.7 *Cross infectivity studies* : It is evident that NPV of *H. puera* is host specific with respect to 3 forest insect pests tested. When sprayed on host leaves @ 14×10^6 PIB/ml. no mortality could be observed even after 96 hours of incubation. All the treated larvae of *E. machaeralis, E. narcissus* and *A fabriciella.* pupated and normal adults emerged.

Baculoviruses are generally thought to have very restricted host range, until recently. But MNVP of *Autographs californica* can infect insects from

several genera (Hunter et *a/.,* 1984). Baculovirus of *Heliothis* sp, can infect seven species of Heliothis. viz.. armigera. peradoxa. peltigera, prloxiphaga. punctigera, virescens and *zea* and in contrast it could not be transmitted to 37 other insects, spiders and mites (Ignoffo and Couch, 1981). Gypchek, NPV of gypsy moth, *Lymantria dispar,* a significant forest defoliator in Central Europe and USA is selective against gypsy moth larvae (Lewis, 1981). Although sawfly NPVs are considered highly host specific, there are a few reports of cross infectivity (Cunhingham and Entwistle, 1981). Cytoplasmic polyhedral virus (CPV) on the other hand have a wide host range, wider than NPV or Granulosis viruses (Aruga, 1973). Although NPV of *H.puera* didnot infect three other forest insect pests tested, extensive tests are to be conducted with other agricultural ahd forest insect pests.

3. FUNGAL PATHOGENS

3.1. Introduction

Fungal pathogens are the largest group of insect pathogens comprising more than 500 species reported from different insects. Though all the four classes of fungi viz., Phycomycetes. Ascomycetes, Basidiomycetes and Deuteromycetes include organisms causing mycoses, Phycomycetes and Deuteromycetes are the classes of fungi from where maximum number of species are included (Brady. 1981). In Phycomycetes. Mastigornycotina comprising genera Entomophthora, Massospora and Conidiobolus are important against mosquitoes and flies. It has been proved that Cordyceps belonging to Pyrenomycetes of Ascomycetes is an important entomopathogen, the host range of the genus being wide including Diptera, Hymenoptera, Coleoptera, Lepidoptera. Hemiptera, lsoptera and Orthoptera and most species are either tropical or sub tropical (Willis, 1959). From Basidiomycetes the highly specialized genus Septobasidium is the only genus included as an entomopathogen. Deuterornycetes or fungi imperfecti encompass the maximum genera of entomogenous fungi and Samson (1974) keyed fifteen of the commoner genera and gave references to a further sixteen.

The taxonomy of Beauveria was discussed in detail by de Hoog (1972) who recognised two species, B. *bassiana* having globose to sub globose conidia and *B.* brongniartii with ellipsoidal conidia. The host range of bath species is very wide, although there is some evidence that strains with different pathogenic potential exist in B. brongniartii (Ferron, 1978). Large scale use of B. bassiana to control the colarado beetle, Leptinotarsa decemlineata (say) was started in early 1970s in USSR.Steinhaus; (1949) reported at length on

the considerable efforts in the American Midwest to utilise *B. bassiana* to control corn chinchbug, *Blissus leucopterus.* Control of corn borer *Ostirinia furnacalis* has been achieved by placing the fungus in the apices of young corn plants (Roberts and Wraight, 1986) Soper (1982) also cited reports of its effectiveness against Pinus caterpillar *Dendrolimus pynactarus* in China.

The genus *Paecilomyces* is divided into two sub sections with *Paecilomyces* (species) and *Isarioidea.* a heterogenus section with 22 species (Samson, 1974). Both the species viz. *P. farinosus* and *P. fumasoroseus* encountered in the study are well established insect parasites of wide host range (Leatherdale, 1970). *P. farinosus* has been used for biological control experiments with *Leptinotarsa decemlineata* (Bajan and Kmitowa, 1974). Generally infection by fungal pathogens results from contact between virulent infectious inoculum and a susceptible host insect. Humid tropical forests have a rich and varied entomopathogenic rnycoflora (Evans, 1982) which are an integral factor within these habitats contributing to the stabilization of arthropod populations (Evans, 1974). The forest canopy buffers the understorey from extremes of temperature and humidity, creating a stable microclimate conducive to continued fungal activity even during dry seasons. Noval and ambitious techniques have also been perfected in the absence of natural fungal epizootics, like impregnation of spores in fireworks, mortar shells and land mines with fungal inoculum. During the present investigation one synnematous fungus viz , *Hirsutella* and other non-synnematous hyphomycetes viz., *Beauveria bassiana. Paecilomyces farinosus* and *P. fumasoroseus. P lilacianus* and *Tolypocladium niveum* were recorded.

3.2. Materials and Methods

3.2.1 *Search for disease incidence* : Various teak plantations in Nilambur, Ranni, Konni,, Parambikulam. Punalur, Malayatoor ond Thenmala Divisions and *Ailanthus triphysa* plantations in Pothuchady (Trichur Division), Mularingad and Kottappara (Malayatbor Division), and Erurneli (Kottayam Division), were visited from 1986 - 1988 during peak incidence of insect infestation. Infectad insects, either larvae or pupae were collected and brought to laboratory under aseptic conditions. In the case of fungal parasitization, the diseased sample was surface Sterilized in 0.01% mercuric chloride for 1-2 min, washed in several changes of sterile water and plated on patato dextrose agar (PDA) medium. Most consistently growing fungal colonies were subcultured and identified. For confirming the identity, isolates were sent to CAB International Mycological Institute, Kew, England. Photomicrographs of different species of fungi were taken using Leitz Dialux camera attachment.

3.2.2 *Bioassay studies* : For bioes say, third to fourth instar larvae collected from field were used. Fungal pathogens, were grown on PDA for 7 to 10 days and spore suspensions prepared. The quantity of spores/ml

was determined through a haemocytometer and serial dilutions were made. For confirming the pathogenicity, larvae were sprayed directly with a concentration of 1×10^6 spores/ml. Once the pathogenicity was confirmed. subsequent tests were carried out with decreasing concentrations employing both direct and indirect application methods. In direct application method the spore suspension was directly sprayed on test larvae, which were air dried. and carefully transferred into plastic jars containing surface sterilized host leaves. The indirect method involved spraying of spore suspension on leaves and allowing the test larvae to feed on the treated leaves. Controls were sprayed with distilled water. All the treated larvae were maintained at 28 $\pm$2°C and ambient relative humidity of ca. 75% The experiments were replicated thrice with 10 larvae each and observation on mortality was recorded every 24 h and expressed in percentage. The data were probit analysed (Finney, 1977) and median lethal concentration 50 (LC_{50})was estimated.

3.2.3 *Cross infectivity and comparative efficacy tests* : Cross infectivity tests were carried out using *B. bassiana (Atteva* strain) against *E.narcissus* and *P. farinosus (Eligma* strain) against *A. fabriciella.* Initially a spore concentration of 1×10^5 spores/ml was used and when the strain was found pathogenic, direct and indirect application methods were followed with spore concentration ranging from 1×10^5 to 1×10^2 spores/ml. Other parameters were kept constant as described in item 3.2.2.

3.2.4 *Leaf surface treatment bioassay* : In order to estimate the conidial activity on a known area of leaf surface, a bioassay was standardized using P. *farinosus* and *P. fumasoroseus* against *E. narcissus.* The area of the leaves was determined by Licor Model 3100 area meter. Leaf discs of 20 $\pm$2 cm^2 were used in the study. The dorsal side of each leaf disc was treated with 0.2 ml of spore suspension prepared from a 7-day-old fungal culture in 0.05% Tween 20. The concentration of spores varied from 10-100000 conidia/cm^2. There were two leaf discs for every five larvae and four replicates were maintained. The leaf discs were kept on moist filter paper in a petri dish (90 x 15 mm) and larvae released. After 48h of incubation (25 +2°C) the larvae from each petridish were transferred to a plastic jar and fresh untreated leaves were placed at 1-2 day interval until the bioassay was terminated by 96 h.

3.2.5 *Effect of relative humidity on the growth of the pathogens* : Dry spores of *Paecilomyces farinosus, P. fumasoroseus (Eligma* strain) and *B. bassiana (Atteva* strain) were placed on each of a series of glass slides. The slides were incubated in dark at 25°C in various controlled humidities (O'Brien, 1958). Mean percentage conidia germinated was calculated after 24 h from about of 500 conidia observed.

3.2.6 *Aerospora studies* : To study the status of spores in the air especially with *Paecilomyces spp.* a 4-year-old *Ailanthus triphysa* plantation at Kottappara was selected. To standardize the best medium and time of exposure of plates for fungal growth, tests were conducted with Rose bengal agar (RBA), potato dextrose agar and malt extract agar. When the best medium for growth and time of exposure of plates were confirmed, 10 plates of 9 cm diameter were exposed at different places in the plantation and incubated for 5 to 7 days and organisms enumerated, over a period of one year.

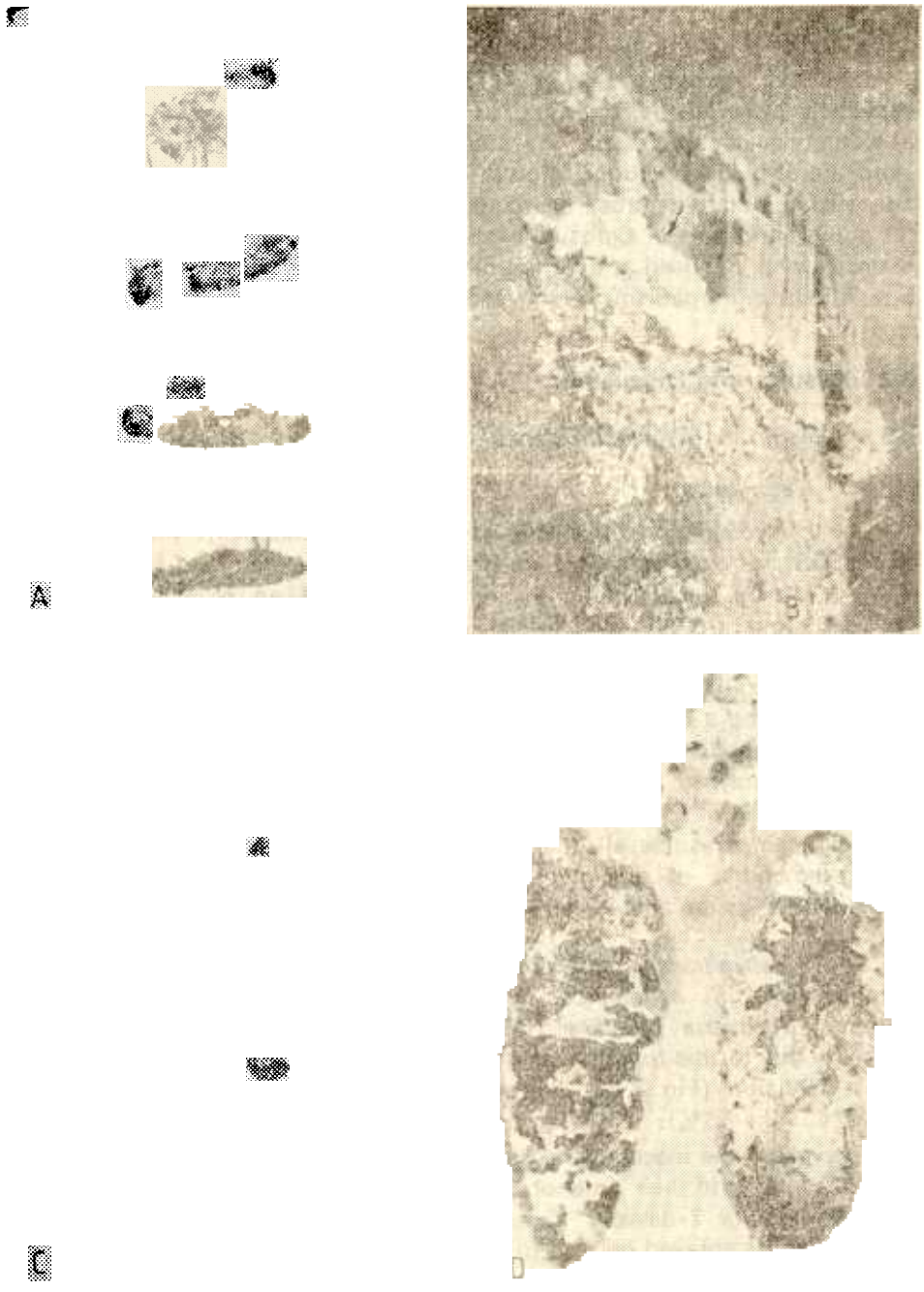
3.3 Results and Discussion

3.3.1 *Search for disease incidence*

3.3.1.I *Teak pests*

a) *Hyblaea puera* Cramer (Lepidoptera : Hyblaeidae) : From *H. puera.* a new species of *Hirsutella* (IMI No 328626) was isolated and identified (Fig. 3 A). It is a synnematous fungus and a new record on *H. puera.* Because of its very slow growth on artificial media, its efficacy was not evaluated. Other than this, no other fungal pathogens could be isolated from *H. puera* during the course of the study. However, Agarwal, *et a/,* (1985) isolated *B. bassiana* and a *Fusarium* sp from field collected diseased larvae of *H. puera.* and *E. machaeralis* in Jabalpur. The pathogenicity tests using three methods like. direct spraying, brushing of conidia on to the body with a hair brush and forced crawling of larvae on a heavy sporulating medium, indicated that both the fungi can parasitize all the six larval stages (Agarwal *et a/.,* 1985). But during the course of our investigation, we did not come across any larval mortality due to the above mentioned pathogens.

b) *Sahyadrassus malabaricus* Moore (Lepidoptera : Noctuidae): Sapling borer of teak, S. *malabaricus* was seen infected by the white muscardine fungus, *Beauveria bassiana* (Balsamo) Vuill. (IMI No 313438). Larval mortality due to this fungus was noticed in a few teak saplings in Chalakudy Forest Division (Fig. 3 B), during September - October 1986 (temperature $28\pm2^{o}C$, r.h> 85%). During this period when the insect infestation was prevalent, the insects were found underneath the frass covering the tunnel entry, usually with their anterior end protruding outside. Spore suspension prepared from a 7-day-old culture having a concentration of 1.0×10^{5} spores/ml was sprayed on to 3 to 4 month old field collected larvae maintained on artificial diet. Among the treated larvae, 80-90% died due to fungal infection after 96 h; no mortality occurred in control. The infected larvae were found covered with white mycelial growth on which profuse sporulation was noticed from 120 h onwards. From the results of laboratory studies, *S. bassiana* appeared to be a very effective pathogen of S. *malabaricus.*



ig. 3 A. Larvae of *H. puera* infected by *Hirsutella* sp.
B. Larva of *S. malabaricus* affected by *B. bassiana*
C. Pupa of *E. narcissus* infected by *P. farinosus*
D Pupae of *E. narcissus* infected by *P. fumosoroseus*

In India, *B. bassiana* has been reported from Karnataka to cause mortality of teak skeletonizer *Eutectona machaeralis* (Wlk.) (Patil and Thontadarya. 1981). This pathogen is a new record on S. *malabaricus* (Mohamed Ali and Mathew, 1989). However, field trials using this pathogen could not be undertaken.

3.3.1.2. *Ailanthus pests*

(a) *Eligma narcissus* Roth. (Lepidoptera : Noctuidae) :

During the course of this study, a few *Ailanthus* plantations were visited during August-December every year and fungal pathogens collected, isolated and identified. *Paecilomyces farinosus* (Holm) Brown & Smith (IMI No, 319337), *Paecilomyces fumosoroseus* (Wize) Brown & Smith (IMI No. 319935, 319936, 326304). *Paecilomyces lilacinus* (Thorn) Samson (IMI No. 326304) and *Tolypocladium niveum* (Rostrup) Bisset (IMI No. 326301, 326302j were the entomopathogenic fungi encountered. However, the last two mentioned were not commonly recorded and they were isolated only in two stray cases of one each from Mularingad and Kottappara. Moreover. they were not utilised for further bioassay studies due to their poor efficacy under laboratory conditions (Table 6). In the preliminary pathogenicity trials, *P. farinosus* (Fig. 3 C) and *P. furnosoroseus* (Fig,. 3 D) were found highly effective and caused 100% mortality, when used at $I X I 0^6$ spores/ml and hence were further evaluated.

Table 6. List of fungal pathogens isolated from different insect pests

Sl. No.	Insects	Location, month and year	Pathogen and %mortality	Nature of inciderice
1	*Hyblaea puera*	Nilambur Aug. 1987 Sept-Oct. 1988	*Hirsutella* sp. (not tested)	scattered, rare
2	*Sahyadrassus malabaricus*	Chalakudy Sept-Oct 1986	*Beauveria bassiana* (80-90%)	
3	*Eutectona machaeralis*	NIL	NIL	NIL
4	*Eligma narcissus*	Erumeli Sept-Dec (1986. 87)	*Paecilomyces farinosus* (100%)	epizootic
		Kottappara Sept - Dec. 1986, 87, 88	*P. fumosoroseus* (100%)	epizootic
		Mularingad Sept-Dec 1987	*P. lilacinus* (< 20%)	scattered, rare
		Kottappara Sept-Dec 1987	*Tolypocladium niveum* (<20%)	
5	*Atteva fabriciella*	Kottappara Sept-Dec (1987)	*Beauveria bassiana* (80%)	moderate, but not epizootic

*N = 30 larvae. Concentration of 1×10^6

Paecilomyces parasitize, a wide range of insect hosts, often causing epizootics (Brown and Smith, 1957; Doberski, 1978). *P. farinosus* is a ubiquitous insect parasite, common in temperate and tropical zones with a wide host range (Letherdale. 1970). The potential of *P. farinosus* as a microbial insect pathogen has been tested against several insect pests both abroad and in India, including Colorado bgetle *Leptinotarsa decemlineata* (Samsinakova and Kalalova, 1978). elm bark beetle. *Scolytus scolytus* (Doberski, 1981). apple sawfly *Hiplocampa testudinea* (Jaworska, 1979). *Malacosoma neustria* (Machowicz - Stefaniak, 1978) and various other insects (Kuruvilla and Jacob 1980; Balakrishnan and Nene. 1980). Natural mortality of current clearwing, *Synanrhedon sslmachus* was recently reported by Baker (1981). Varma and Mohanan (1984) reported the occurrence of *P. farinosus* on *E. narcissus* and compared its efficacy on *Atteva fabriciella,* another insect pest of *A. triphysa. P. fumosoroseus* is also known mainly as an insect pathogen in Lepidoptera. Diptera, Hemiptera, Isoptera, etc. (Samson, 1974). Maize pyralid, *Ostrinia nubilalis* has been found to be highly susceptible to this pathogen (Riba *et a/.,* 1983). Susceptibility of *Spodoptera littoralis* to *P. fumosoroseus* was worked out by Fargues and Rudriguez (1980). From *E. narcissus* this is the first report of *P. fumosoroseus* causing pupal mortality. Other two fungi reported in the study viz., *P. lilacinus* and *Tolypocladium niveum* are also new records of this insect.

In the field only pupae infected with fungi could be located and larval infection was not usually found. However, in the laboratory experiments. when directly sprayed, infection was noticed on larvae, (Fig. 4 A & B) pupae and as well as on adults. Field survey during 1986 - 88 in a selected *Ailanthus* plantation at Kottappara indicated that the epizootics of *Paecilomyces* infection was very high during October - November which coincides with the peak incidence of the insect. The pupal infection was as high as 89.3% during 1987, whereas it was 37.8% during 1988, obviously the percent infection is directly related to the incidence of insects, greater the infestation of insects, greater the infection record. When infected pupae collected from Kottappara were examined, it was found that ca. 15 - 25% of them were affected by *P. farinosus,* while the rest were infected by *P. fumosoroseus,* thereby indicating a mixed infection by both the species of *Paecilomyces.* However, this mixed infection could not be observed in other *Ailanthus* plantations surveyed. It is evident that both the species of *Paecilomyces* showed high potential as biocontrol agents against of *E. narcissus* and they were capable of causing significant natural epizootics during every season.

b) *Atteva fabriciella* Swed. (Lepidoptera : Yponomeutidae) : Instances of larval mortality of *A. fabriciella* was noticed during 1986-1987 season in an *Ailanthus* plantation at Kottappara. Infected larvae appeared dark white in

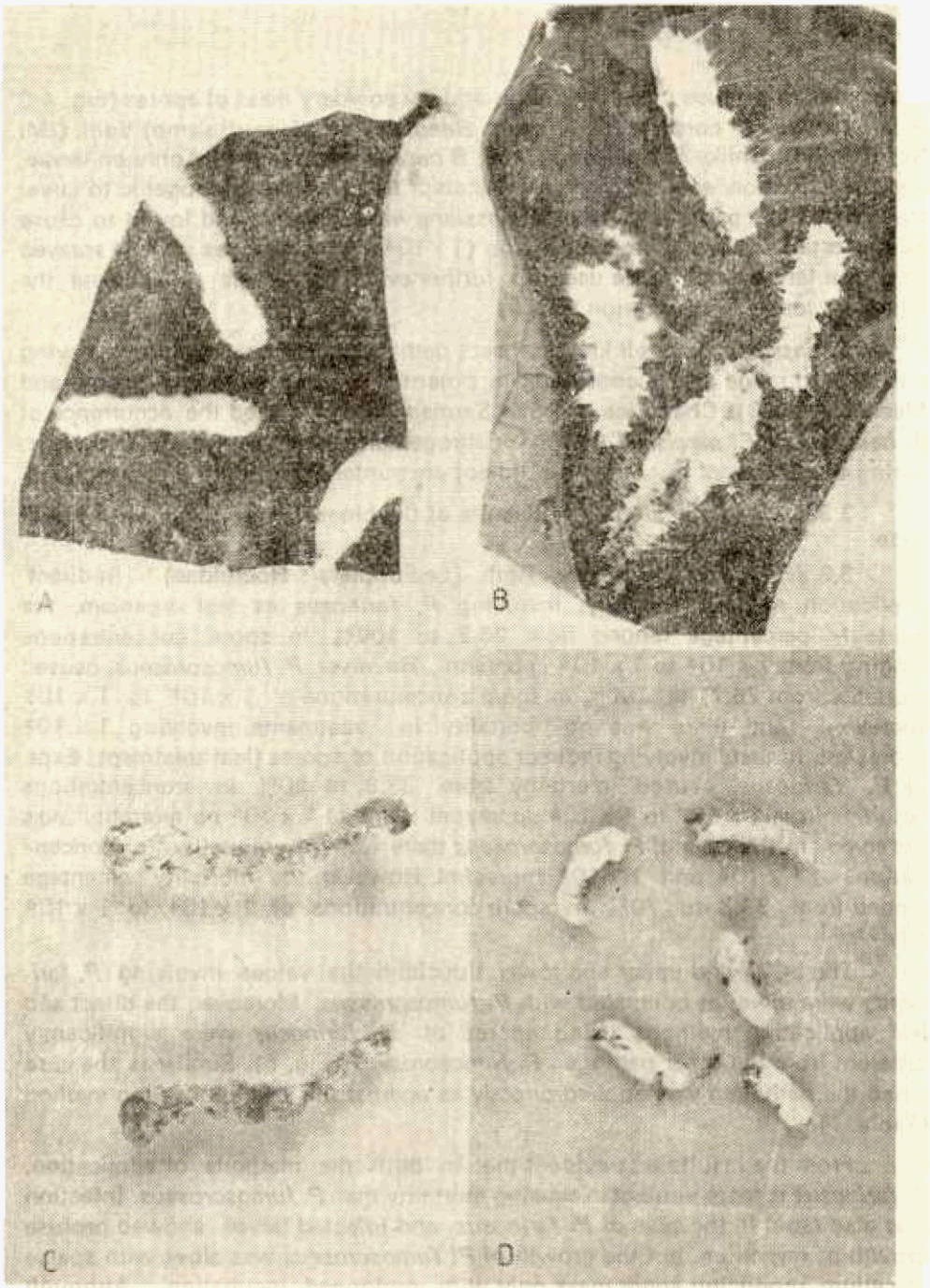


Fig. 4. Larvae of *E. narcissus* artificially inoculated by *P. farinosus* - A; and *P. fumosoroseus* - B; *B. bassiana* affected larvae of *A. fabricieilla* - field infection - C; Artificially inoculated-D.

colour due to profuse mycelial growth and dry powdery mass of spores (Fig. 4 C & D). Isolations consistently yielded *Beauveria bassiana* (Balamo) Vuill. (IMI No. 319938). Unlike *Paecilornyces* spp, *B bassiana* was recorded only on larvae, and the laboratory evaluation also indicated that it was pathogenic to larval stage only. The pathogenicity of *B. bassiana* was evaluated and found to cause 80% mortality when spore suspension ($1 \times 10_5$ spores/ml) was directly sprayed on to the larvae, and hence used in further evaluation trials to estimate the minimum lethal concentration (LC_{50})

B. bassiana is a well known insect pathogen in several countries having a wide host range and is considered a potential bio-control agent (Dunn and Mechalas, 1963). Chatterjee and Sen-Sarma (1968) reported the occurrence of ***B.*** *bassiana* on *E. narcissus* but its pathogenicity was not tested. However, during the course *of* our study we did not encounter *B. bassiana* on *E. narcissus.*

3.3.2 *Bioassay studies* : Results of the bioassay are dealt with insect wise.

3.3.2.1 *E/igma narcissus* Roth (Lepidoptera : Noctuidae) : In direct, application method (Expt. i), involving *P. farinosus* as test organism, the mortality percentage ranged from 23.3 to 100% in spore concentrations ranging from 1×10^2 to 1×10^6 spores/ml. However, *P. furnosoroseus.* caused mortality from 26.7 to 100% in spore concentrations of 1×10^3 to 1×10^6 spores/ml, and there was no mortality in treatments involving 1×10^2 spores/ml. In tests involving indirect application of spores (leaf treatment, Expt. ii) *P. farinosus* caused mortality from 33.3 to 90% in concentrations ranging from 1×10^3 to 1×10^6 spores/ml while at 1×10^2 **no** mortality was observed. In the case of *P. furnosoroseus* there was no mortality in concentrations of 1×10^3 and 1×10^2 spores/ml. However, the mortality percentage ranged from 33.3 to 70% in spore concentrations of 1×10^4 to 1×10^6 spores/ml.

The LC_{50} and upper and lower fiducial limits values involving *P. farinosus* were lower as compared with *P. fumosoroseus.* Moreover, the direct and leaf application methods using spores of *P. farinosus* were significantly different from the other pathogen *P. fumosoroseus* (Fig. 5). Similar is the case when the pathogen was applied directly as against the leaf application method (Table. 7).

From the results it is evident that in both the methods of application, *P. farinosus* is more virulent in causing mortality than *P. fumosoroseus.* Infection was also rapid in the case of *P. farinosus* and infected larvae showed profuse growth of mycelium, but the growth of *P. fumosoroseus* was slow with sparse mycelium, producing spore mass only with prolonged incubation. Although *P. farinosus* has been reported earlier (Varma and Mohanan 1984). this is the first report of *P. fumosoroseus* on *E. narcissus.* Bioassay tests indicated that under laboratory conditions, *P. farinosus* was more effective than *P. fumosoroseus.*

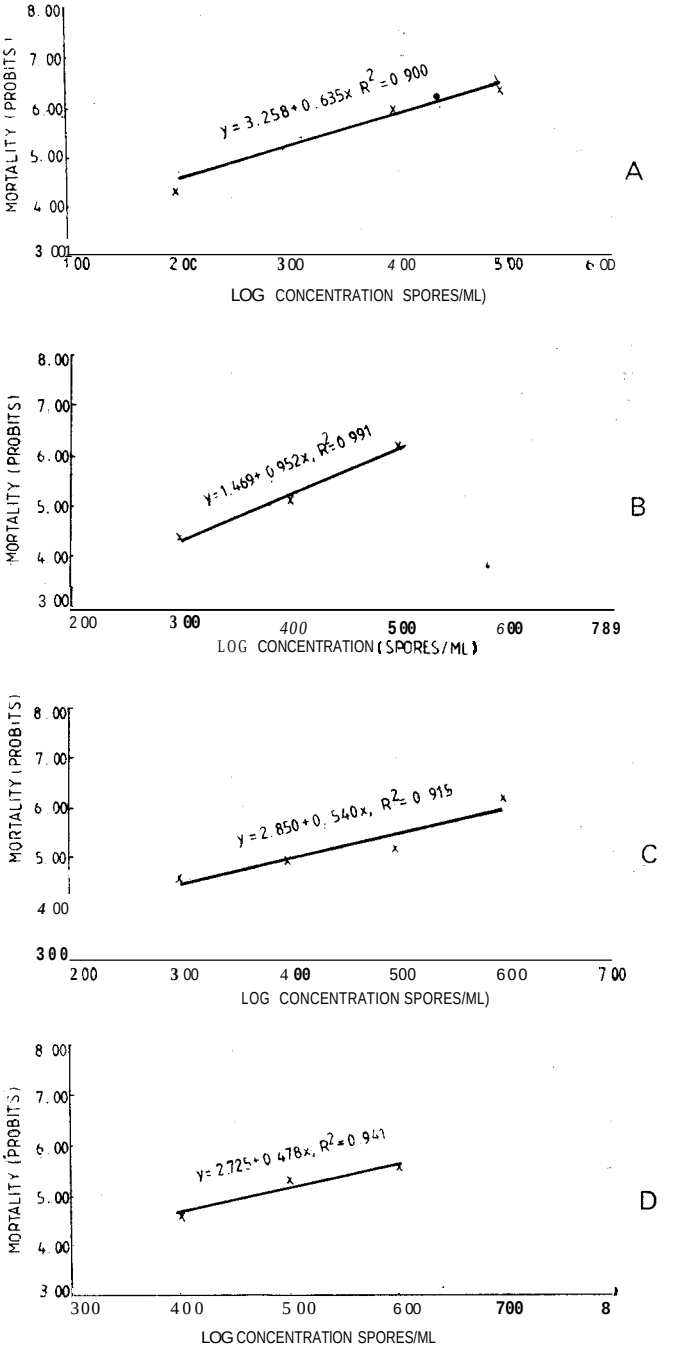


Fig. 5. Concentration - mortality curves of *E. narcissus* in direct application method involving *P. farinosus* (A) and *P. fumosoroseus* (B) and in indirect application method involving *P. farinosus (C)* and *P. fumosoroesos* (D)

Table 7. Efficacy of *P. fumosoroseus* and *P. farinosus* in different spore concentrations and treatments against *E. narcissus*

Treatment (spores/ml)	*P. fumosoroseus*				*P. farinosus*			
	% mortalityt	LC_{50}	Fiducial limit upper (spores / ml)	lower	%mortality+	LC_{50}	Finducial limit upper (spores / ml)	lower
Direct application (Expt. i)								
$1x10^2$	NIL				23.3			
$1x10^3$	26.7	$5.2x10^3$*	$10.7x10^3$	$2.1x10^3$	70.0	$5.4x10^2$*	$13.1x10^2$	$1.5x10^2$
$1x10^4$	56.7				80.0			
$1x10^5$	90.0				90.0			
$1x10^6$	100.0				100.0			
Leaf application (Expt. ii)								
$1x10^2$	NIL				NIL			
$1x10^3$	NIL	$5.8x10^4$*	$23.8x10^4$	$5.7x10^3$	33.3	$10.4x10^3$*	$30.9x10^3$	$2.3x10^3$
$1x10^4$	33.3				50.0			
$1x10^5$	60.0				60.0			
$1x10^6$	70.0				90.0			

* Significant at $p = 0.05$

† At 96 h

3.3.2.2 *Atteva fabriciella:* Results of the bioassay using various spore concentration of *B. bassiana* against shoot webber *A. fabriciella* are given in Table 8. In direct application of spores of *B. bassiana* (Expt. i) ca. 80% mortality was observed in highest concentration of 1×10^5 spores / ml after 72 h. However, profuse mycelial growth was observed only by 96 h and the whole larvae became a mass of spore powder by 120 h. The percent mortality rate came down with the reduction in spore concentration. In the second experiment (Expt. ii.) where the larvae were exposed to leaves treated with *B. bassiana,* only 40% of larvae were killed at 1×10^5 spores / ml and 10% in 1×10^2 spores/ml. In both the sets of experiments, uninfected larvae pupated normally and adults emerged.

It is evident from Table 8 that the pathogen ***B. bassiana*** is highly virulent in causing mortality and best results were obtained in direct application method, The lethal concentration 50 value was 3.1×10^3 spores/ml in direct application method, as compared to 3.8×10^5 spores/mi in leaf application method. The fiducial limits values were also high in method involving indirect application of spores and both the application methods were significantly different from one another, inferring that *B. bassiana* is most effective in direct application method against *A. fabriciella* (Fig. 6). But this method of application may not be practical in the field situation, because the larvae of *A. fabriciella* web the leaves and feed from within and the chances of spores infecting the target are low.

Table 8. Efficacy of *B. bassiana* in different spore concentrations and treatments against *A. fabriciella*

	Direct application (Expt. i)				Indirect application (Expt. ii)			
	Concentration of spores/ml							
	1×10^5	1×10^4	1×10^3	1×10^2	1×10^5	1×10^4	1×10^3	1×10^2
Percent mortality at 96 h	80.0	53.3	40.0	26.6	40.0	33.3	26.6	10.0
LC_{50}	3.1×10^3* spores/ml				3.8×10^5 NS spores/ml			
Fiducial limit upper	11.6×10^3 spores/ml				16.2×10^6 spores/ml			
lower	8.3×10^2 spores/ml				9.0×10^3 spores/ml			

* = Significant at $p = 0.05$ NS = Non significant

3.3.3 *Cross infectivity and comparative efficacy tests* : When the cross infective nature of *P. farinosus* was proved, further trials were undertaken to study the comparative efficacy of *B. bassiana* and *P. farinosus* against *A. fabriciella.* In direct application of spores (Expt. **i)** involving *B. bassiana,* ca. 80% mortality was observed in highest concentration tested and the mortality rate came down with the reduction in spore concentration; there was no mortality at 1×10^2 spores / ml. In the second experiment (Expt. **ii)** where larvae were exposed to treated leaves, *B. bassiana* caused 40% mortality at $1x10^5$ spores / ml, followed by 33.3 and 26.6% in $1x10^4$ and 10^3 spores/ml respectively, whereas 40 to 20% larvae were killed when they consumed leaves treated with *P. farinosus.* In both the cases uninfected larvae pupated and normal adults emerged. Statistical analysis of the bioassay indicated that all the methods excepting indirect application involving *B. bassiana* and *P. farinosus* were significantly different from one another (Table 9). However, in direct application of spores involving *B. bassiana,* a low LC_{50} viz. $3.1x10^3$ spores/ml was observed as against $3.8x10^5$ in lead application method, indicating the effectiveness of *B. bassiana* in direct application method.

Table 9. Comparative efficacy of *B. bassiana* and *P. farinosus* against *A. fabriciella*

Pathogen	Direct application				Indirect application			
B. bassiana	Concentration (spores/ml)							
	$1x10^5$	$1x10^4$	$1x10^3$	$1X10^2$	$1x10^5$	$1x10^4$	$1x10^3$	$1x10^2$
% mortality+	80.0	53.3	40.0	26.6	40.0	33.3	26.6	10.0
LC_{50}	$3.1x10^{3*}$ spores/ml				$3.8x10^{5NS}$ spores/ml			
Fiducial limit								
upper	$11.6x10^3$ spores/ml				$16.2x10^6$ spores/ml			
lower	$8.3x10^2$ spores/ml				$9.0x10^3$ spores/ml			
P. farinosus								
%mortality+	60.0	40.0	23.3	10.0	40.0	26.6	20.0	10.0
LC_{50}	$3.3x10^{4*}$ spores/ml				$6.0x10^{5NS}$ spores/ml			
Fiducial limit								
upper	$25.1x10^4$ spores/ml				$25.8x10^6$ spores/ml			
lower	$10.5x10^3$ spores/ml				$1.4x10^4$ spores/ml			

* = Significant at p = 0.05 NS Non significant
t=At 96 h

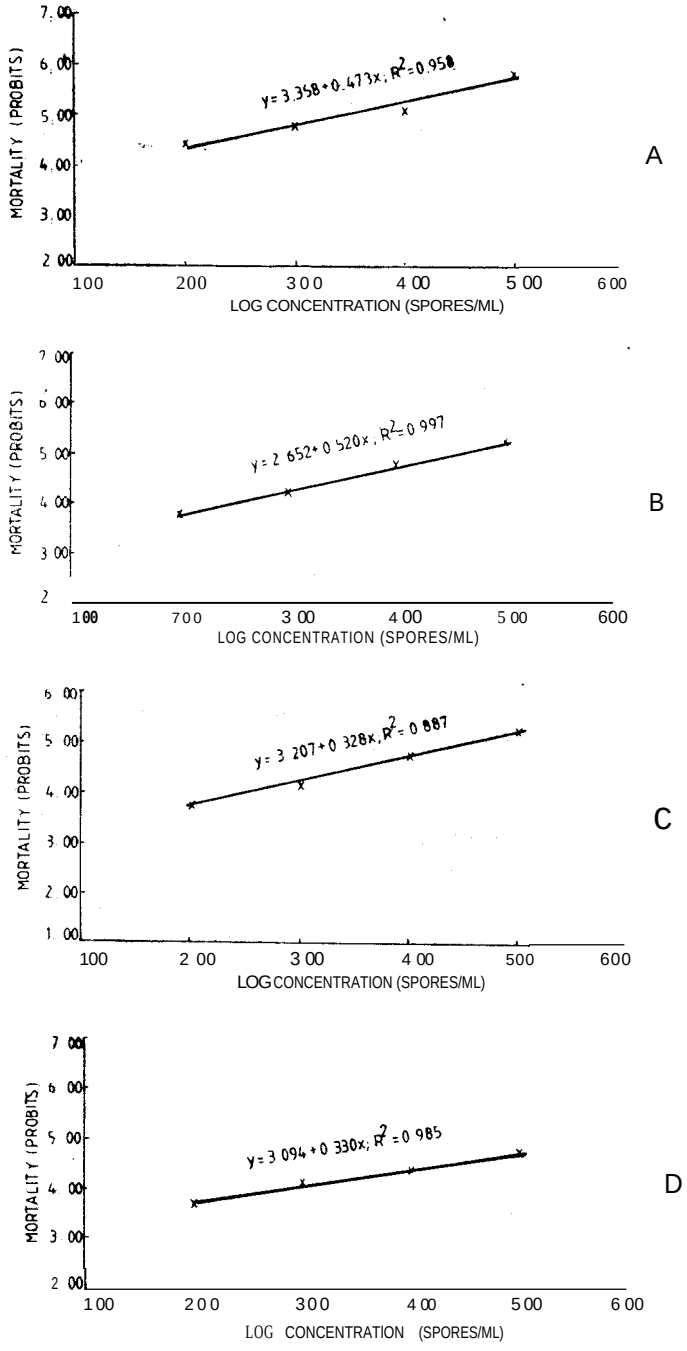


Fig. 6. Concentration - mortality curves of *A. fabricie/la* in direct application method involving *B. bassiana* (A) and *P. farinosus* (B) and in indirect application method involving *B. bassiana* (C) and *P. farinosus* (D)

Results available from this study indicated that *P. farinosus* isolated from *E. narcissus* is cross infective to *A. fabriciella.* When compared, the native pathogen *B. bassiana* was more effective than *P. farinosus* and best in direct application of spores.

3.3.4 *Leaf surface treatment bioassay:* No mortality was observed in the first 24 hours. But the treated larvae were sluggish and consumption of leaves was very poor i. e., only 10 to 50% area of the discs were consumed in 100000 spores/cm^2 to 1000 spores/cm^2 (Table 10), whereas 80-100% area of the leaf discs were consumed in treatment of 100 spores to 10 spores/cm^2. The entire leaf discs were eaten away by larvae maintained in the control experiment by 24h. The infecrion and spread of fungus were complete by 72-96 h. In all the infected larvae, subsequent feeding after 48 h was nil in 100000, 10000 and 1000 spores/cm^2 and very poor (20-30%) in 100 spores/cm^2. Infection and spread were rapid in higher concentrations and the whole larvae were covered by the fast growing mycelium by 72 h and the spore mass was produced in 7 days. Pupation was normal and adults ernerged normally in control larvae, as well as the larvae which escaped infection. Less than 65% of the larvae survived in doses of 1000 conidia/cm^2 in the case of *P. farinosus.* However, 90% larvae survived the application of *P. fumosoroseus* at the same dose. The lethal time also increased from 48 h as in higher dose, to more than 72 h in lower doses (Table 10).

Table 10. Percent mortality of *E. narcissus* to *P. farinosus (P. far)* and *P. fumosoroseus (P. fum)* in a leaf area bioassay

Treatment conidia/cm^2	%mortality at 72 h		%leaf area consumed		Lethal time (h)
	P. far	*P. fum*	*P. far*	*P. fum*	
100000	80.0	65.0	12.5	25.0	48
10000	70.0	40.0	25.0	50.0	48
1000	35.0	10.0	50.0	80 0	72
100	25.0	5.0	80.0	90.0	72
10	5 .0	Nil	90.0	100.0	>72
0	Nil	Nil	100.0	100.0	—

Although speculative it may be possible to calculate a field rate based upon the number of conidia/cm^2 required to provide various levels of infection. To estimate these values, one has to calculate a total leaf surface area of an *Ailanthus* field per hectare. From this value it would be possible to calculate the total amount of spores necessary to initiate an infection of 25, 50,

75% and so on. Earlier reports indicated that to attain 99, 90 or 50% mortality of an incipient destructive field population of colorado beetle larvae would require 200, 16.25 or 0.76 kg of conidia / hectare, assumlng a formulation of *B.bassiana* containing 10^9 conidia / g. However ca. 25 g of the formulation was needed to attain 10% mortality, a dose probably sufficient if applied early in the season to induce an epizootic in a potato field (Ignoffo *et. al.,* 1983).

In our experiments, a spore concentration of ca. 1000 spores/cm^2 of *P. farinosus* may be sufficient to induce an infection of ca. 35% or more, a dosage probably sufficient to induce an epizootic in an *Ailanthus* plantation. However, elaborate field tests are necessary to confirm this assumption.

3.3.5 *Effect of relative humidity on the growth of the pathogens*

Conidia germinated after 24 h in various relative humidities at 25 ± 2°C. indicated that r.h <50% had negative effect on the germination. All the three pathogens tested viz.. *P. farinosus P. fumosoroseus* and *B. bassiana* which showed a germination percentage of ca. 65% at r.h of 100% reduced to <20% at 43% r.h and ≤ 15% at r.h of 31% (Table 11).

Table 11. Percent conidial germination at different r.h levels

	Relative humidity (%)					
Pathogen	100	85	75	51	43	31
	condial germination %					
P. farinosus	*65.0*	60.0	58.0	28.0	15.0	12.0
P. fumosoroseus	66.5	66.0	60.0	26.0	17.0	12.0
B bassiana	65.0	62.0	60.0	28.0	18.0	15.0

Humidity can be a limiting factor at two periods, and high humidity is necessary for most fungi to germinate and cause disease, Secondly the new spores needed for the spread of infection are usually produced on cadavers only at very high humidities. Germination of fungal spores begin at high r.h usually at 90% or more. This could be possible when acqueous suspenion of fungal spores is sprayed. The results indicated that ca. 60% conidial germmination is affected when r.h is reduced to less than 50%. This may affect the field trials, as at low r.h (<50%) there will not be a good conidial germination

Table 12. List of fungi (aerospora) observed in Kottappara *Ailanthus* plantation during 1987-1988

Organism	Incidence											
	Jan.	Feb.	Mar.	Apr.	May.	Jun.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Aspergillus flavus	++	++	++	++	++	++	++	++	+++	++	++	++
A. fumigatus	+	+	+	+	+	–	–	–	–	–	–	–
A. niger	++	++	++	++	++	++	++	++	++	++	++	++
Penicillium sp.	+	+	+	+	+	+	+	+	+	+	+	+
Rhizopus sp.	+	+	+	+	+	+	+	–	–	–	–	–
Cylindrocarpon sp.	+	+	–	–	–	–	–	–	–	–	–	–
Trichoderma sp.	+	+	+	+	+	–	–	+	+	–	–	–
Paecilomyces farinosus	–	–	–	–	–	–	–	–	+	+	+	+
P. fumosoroseus	+	–	–	–	–		–		++	+++	+++	++
Others(non sporulating)	+	+	+	+		+	+	+	+	+	+	+

+++ = abundant; ++ = moderate; + = less; – = not present

and subsequent production of spores on the cadavers also will be seriously affected, which in turn affect the spread of the pathogen. This could be overcome in microbial control by introducing large quantities of spores in the environment (Dunn and Mechalas, 1963). Epizootics are usually associated with periods of high humidity, particularly rainy periods and microbial control is usually "successful" when the application takes place shortly before wet weather. This observation has to be carefully looked into before any field trials are carried out using the fungal pathogens.

3.3.6 *Aerospora studies*: The best medium for growth was confirmed as Rose Bengal agar and the time of exposure of plates was 10-15 minutes. Results of the aerospora studies conducted during 1987-88 season at Kottappara *Ai/anthus* plantation are given in Table 12. It is evident that *Paecilomyces* spp. which could not be observed frotn January-August has been observed from September onwards, which coincides with the peak incidence of insect infestation. Isolations from the bark samples of *A. triphysa* also did not yield *Paecilomyces* spp. Experiments conducted to' study the persistence of *Paecilomyces* spp, in soil could not be quantified. as other saprophytic fungi interfered with the growth of *Paecilomyces.* So it would be worth studying the persistence and transmission of these fungi in detail, the results of which could be effectively used when planning large scale field trials. This will also help to provide inoculum in new areas where natural epizootics of these pathogens are not observed.

4. BACTERIAL PATHOGENS

4.1. Introduction

Entornopathogenic bacteria are potential alternatives to chemical pesticides in biocontrol programmes. They are usually naturally occurring pathogens which have been isolated from a variety of agricultural and forest insect pests (Morris, 1982). But epizootics of naturally occurring bacterial pathogens are relatively infrequent. *Bacillus thuringiensis* belongs to a group of crystalliiferous, sporeforming, Gram-positive bacteria of the Family *Bacillaceae* which was isolated from silk worm larvae in Japan and *B. thuringiensis* vat *sotto* (B. t) was later described as the disease agent in silk worm. The sporeformers produce endospores which survive under inimical environmental conditions outside the host. They also produce paraspora1 toxic protein crystal enclosed within the sporangium (Berliher, 1915). Angus (1956) demonstrated association of toxiability of the cultures to silk worm with these crystals, and about 200 species of lepidoptera were sucesptible to these toxins; and other orders of insects were unaffected by them making *B. t.*, a relatively specific

In the past a few non-crystalliferous and non-spore formers, have been used with some success (Bucher, 1960). but commercially they have not been exploited. At present the most promising bacterial insecticides for forest insect pest control are different varieties of *B. thuringiensis.*

4.2 Materials and methods

4.2.1 *Search for disease incidence* : Various teak plantations in Nilambur. Parambikulam, Ranni, Konni, Punalur, Malayattoor, Trivandrum and Trichur Divisions and *Ailanthus triphysa* plantations in Pothuchady (Trichur Division), Kottappara (Malayattoor Division) and Erumeli (Kottayam Division) were visited from 1986-1988 during the season of peak incidence of insect pests and infected insects either larvae or pupae collected and brought to laboratory under aseptic conditions. In the case of suspected bacterial disease, the diseased sample was surface sterilized in 0.01% $HgCl_2$ for 1-2minutes. washed in several changes of sterile water, and the larvae slightly teased to expose the gut. The gut contents and haemolymph were diluted and streaked on nutrient agar plates. Bacterial colonies growing most cons istently, were sub cultured and their identification attempted; and confirmed identity was sought from CAB International Mycological Institute, Kew, England for Gram (-) bacteria and Institut Pasteur, Paris for Gram (+)bacteria.

4.2.2 *Bioassay studies* : Bacterial pathogens, Gram (+) spore formers and Gram (-) non spore formers were grown on Nutrient agar for 48-72h and bacterial suspensions prepared to give an absorbance of 1 OD at 470 nm (with a viable count of ca. 1×10^{10} CFU/ml). Healthy larvae of third-fourth instars were used in the pathogenicity studies. The bacterial suspension was sprayed on to healthy leaves using an atomiser with a fine nozzle, the leaves were air dried and test larvae allowed to feed on treated leaves. In the control experiments, leaves were sprayed with sterile water. There were three replicates each with ten larvae and observations on mortality were recorded every 24 h and expressed in percentage. All the treated larvae were kept at 28 ± 2°C and ambient r. h of > 75%

4.2.3 *Cross infectivity tests* : Cross infectivity tests were carried out using *B. thuringiensis* (HD 1. teak strain) against *E. narcissus* and *A. fabriciella* as described in item 4.2.2.

4.2.4 *Effect of various serovars of B.thuringiensis against E. narcissus* : In the present study, different strains of *B. thuringiensis* (Table 15)were obtained from Dr. O. N. Morris of the Forest Pest Management Institute, Canadian Forestry Service, Ontario, Canada. The inoculum was prepared @ 500 ng spores/ml of sterile water. One millilitre of inoculum was applied on *A. triphysa* leaves. These treated leaves were provided to test larvae. In control, the leaves were treated with distilled water. There were two replicates with 20 larvae each. The mortality was recorded at the end of 48 h.

4.3 Results and Discussion

4.3.1 *Search for disease incidence* : Results of bacterial infection of various insect pests are dealt with separately.

4.3.1.1 Teak pests

a) *Hyblaea puera:* The diseased larvae collected from teak plantations in Nilambur yielded consistently a short rod, gram negative, non-sporulating bacterium *Enterobacter aerogenes* (Kurse) Hormaeche & Edwards (IMI B.10740). Endospore forming, gram positive bacterium was isolated from some dead larvae collected in Peechi (Trichur Forest Division) which was identified as *Bacillus thuringiensis* H. 1. var *thuringiensis* (K F R I 1294). *Bacillus cereus* (KFRI 1396) another gram positive sporulating bacterium, was also isolated from dead larvae collected from Nilambur.

Dead larvae, available from laboratory cultures .yielded two gram negative, non-sporulating bacteria which were identified as *Pseudomonas aeruginosa* (Schnoeter) Migula (IMI 8.10976) and *Serratiamarcescens* Bizio(IMI B.11386). Among these S. *marcescens* was the most common one which produced bright red colonies on nutrient agar (Table 13).

Table 13. List of bacterial pathogens isolated from different insect pests :

S. No.	Insects	Location & year	Pathogen	Nature of incidence
1.	*H. puera*	Nilambur 1986 - 1987	*Enterobacter aerogenes*	Moderate
	"	Peechi 1986	*Bacillus thuringiensis*	Rare
		Nilambur 1987-1988	*Bacillus cereus*	Rare
		Laboratory infection	*Serratia marcescens*	Moderate
		,,	*Pseudomonas aeruginosa*	
2.	*E. narcissus*	Kottappara	*Bacillus firmus*	Scattered
	"		*Serratia marcescens*	Rare
3.	*A. fabriciella*	"	NIL	NIL

4.3.1.2 *Ailanthus pests*

a) *Eligma narcissus* ; During September - December 1983-1986 when there was a pest build up in an *Ailanthus triphysa* plantation, several dead larvae of *E. narcissus* were found hanging from the leaves. The gut contents of the infected larvae consistently showed the presence of a rod sahped bacterium which was identified as *Bacillus firmus.* (IMI B. 10737) (Varma and Mohamed Ali, 1988). This, a gram positive. long, rod shaped and sporulating bacterium, was morphologically very much similar to *B.subtilis.*

Stray cases of infection of *E. narcissus* by gram-negative *Serratia marcescens* was also observed during 1986-1988 in Kottappara plantation. Affected larvae looked bright red in colour, (Table 13).

b) *Atteva fabriciella* : From *A.fabriciella* no bacterial pathogens causing mortality could be observed.

4.3 2 *Bioassay studies*

4.3.2.1 *Teak Pests*

Hyblaea puera : Inoculation experiments confirmed the pathogenicity of various bacterial isolates. *B. thuringiensis* caused 100% mortality by 72 h. while it was only 70 and 60% in the case of S. *marcescens* and *E. aerogenes* respectively (Table 4) *P. aeruginosa* was found to be the least effective causing only 33.3% mortality. In all the cases, the pathogen could be reisolated.

Table 14. Effect of different bacterial pathogens on mortality of larvae of *H. puera* in artificial inoculation trials

Bacterium	Percent mortality at 72 h
Bacillus thuringiensis	100.0
Serratia rnarcescens	70.0
Enterobacter aerogenes	60.0
Pseudomonas aeruginosa	33.3

N = 30 larvae.

Among the spore forming bacteria, the genus *Bacillus* has been extensively studied. Many species like *B. thuringiensis. B, popilliae. B. cereus* and *B.sphaerieus* (Burges, 1981) are entornopathogenic. *B.thuringiensis* has been reported from nearly 525 insects belonging to various orders. However, it is for the first time this isolate is being reported from teak defoliator, *H.puera* (Sudheendrakurnar *et. al.,* 1990). Various varieties of *B. thuringiensis* has

been used extensively against a large number of lepidopterous forest pests, the success rate has been higher against broad leaf defoliators than against coniferous ones, the possible explanation being the superior deposit collecting surface of broad leaved trees compared to coniferous needles and deciduous defoliators being open feeders. Although various formulations of *B. thuringiensis* viz., Dipel, Thuricide, Biotral and Bactospeine, are to be applied aerially and the over all short term efficacy has been unpredictable and success record not as good as chemical insecticides. but this may be more than balanced by the low level of environmental risk and longterm effectiveness.

The non-sporeformers like *S. marcescens. P. aeruginosa* and *E. aerogenes* are also new records from *H. puera* (Sudheendrakurnar *et a/.,* 1990). Earlier they have been reported from a number of other insects particularly Lepidoptera (Raun and Brooks, 1963; Bucher, 1967; Patil and Thontadarya, 1983). According to Falcon (1971) these bacteria can cause mortality when the host insects are stressed by factors such as starvation, ingestion of contaminated food and mechanical rupturing of gut. But the scope of using non-spore forming bacteria in biological control is very limited, as they are not able to thrive under adverse conditions to cause repeated infections. Further, the bacteria belonging to genera *Serratia, Pseudomonas* and *Enterobacter* are not considered safe to human beings and domestic animals. However, the consistent isolation of *E. aerogenes* from the field infected larvae suggests that this bacterium could possibly be playing an important role in the natural reduction of population of *H. puera.*

4.3.3 *Cross infectivity tests* : Cross infectivity tests were positive on both the species tested. Cent percent mortality was observed by 72 h in *E. narcissus* and *A. fabriciella* when *B. thuringiensis* (HD 1, teak strain) treated leaves were fed. The treated larvae became sluggish and subsequent feeding had come down. The results indicated that *B. thuringiensis* (teak strain) could also be effectively used against *E. narcisuss* and *A. fabriciella.*

4.3.4 *Effect of various crystovars of B.thuringiensis against E.narcissus* : Based on the results, with regard to their pathogenicity, different strains tested in this experiment can be grouped into three groups (Table 15) Treatment with crystovars *aizawai* (HD 133, 122) and *Kenyae* (HD 551) showed 50.60% mortality, whereas crystovars *aizawai* (HD 854). *kurstaki* (HD 262) gave 30-35% mortality; other varieties were not effective (Varma *et a/.,* 1987). During the initial 24 h period, less than 30% of the treated leaves were consumed by the treated larvae. But when fresh untreated leaves were provided, upto 75% was consumed in the next 24 h. From the results it is evident that HD 133. 112 and 551 are effective in causing more than 50% mortality to *E. narcissus* under laboratory conditions.

Table 15. Effect of various crystovars of *B. thuringiensis* against *E. narcissus*

HD No.	Variety	Crystovar	%of leaf area consumed		mortality at
			24 h	48 h	48 h
133	aizawai	ai 2	14.00	37.20	60 0
112	"	ai 2	14.80	57.80	50.0
551	kenyae	NI	19.30	37.90	50.0
582	NI	NI	27.50	53.40	35.0
854	aizawai	NI	18.10	24.90	35.0
262	kurstaki	K - I	18.80	56.60	35.0
337		K - I	27.10	9.50	30.0
198	entomocidus	ent	22.20	75.80	30.0
282	aizawai	ai 2	27.00	30.00	20.0
562	kurstaki	NI	23.70	18.80	10.0
52	aizawai	ai 3	6.65	84.30	5.0
	control	—	100.00	100.00	NIL

5. GENERAL DISCUSSION AND CONCLUSIONS

The present investigation has shown the occurrence of a potential Nuclear Polyheral Virus (NPV) on *Hyblaea puera.*one of the two well known insect pests of teak *Tectona grandis* (Linn), commonly called the teak defoliator. This virus is highly pathogenic in the laboratory experiments with an LD50 and LC50 values of 1427 PIB/larva and 796 PlB/ml respectively. Lethal time 50 values decreased with increasing concentrations/doses of polyhedral inclusion bodies, which is in agreement with the studies related to NPV of various other insect pests. Though NPV and GV are sufficiently protected within the inclusion bodies. exposure to UV light of radiation < 300nm is injurious to NPV and ca. 35% infectivity was lost due to exposure to UV light of ca. 250 nm at 15 cm distance, indicating the necessity of using the NPV in the field with some adjuvants like charcoal, Indian ink etc. Heating of PIB solution at 40°C for 168 h did not cause any significant inactivation. NPV of *H. puera* could be stable in the field as the maximum temperature encountered in the field during peak incidence of the pest does not usually rise beyond 40°C. As the PIB usually persist in soil, this stability at 40°C could well be considered *as* a plus point for field use. The adverse effect of Hydrogen ion concentration on NPV is well known. However, in our study at pH 4.0 and 9.2 the virus activity was reduced only slightly (<10%) and at pH 7.0 the activity was not affected. Usually pH of soils under teak has never been reported to be less than 4.0 or more than 9.0; soil persistence of PIB could well be

maintained without much loss in virulence. Cross infectivity studies indicated that the NPV was not cross infective atleast to three other forest insect pests viz., *Eutectona machaeralis, Eligma narcissus* and *Atteva fabriciella* indicating the host specificity of this virus. However, it warrants checking of cross infectivity with other known forest and agricultural insect pests.

As compared to *Enterobacter aerogenes* a gram (+)ve bacterium observed in the field, which could well be a limiting factor while the insects are under stress, a gram (+) ve spore former, producing crystal toxin, *Bacillus thruringiensis* var *thuringiensis* (HD 1) was isolated in a stray case, which is well known for its pathogenicity against Lepidopteran larvae. This is also a potential candidate for biocontrol against *H. puera* as well as *E. machaeralis.*

Other than a new *Hirsutella* sp, no fungal pathogens from *H. puera* was observed. Due to the slow growing nature, its role in bio-control couldnot be ascertained.

From *Eligma narcissus* one of the two important insects of *Ailanthus triphysa,* two prominent fungal pathogens viz., *Paecilomyces farinosus* and *P. fumosoroseus* were observed. Pathogenicity trials in the laboratory using direct and indirect application method indicated that *P. farinosus* was more effective than *P. fumosoroseus* and caused higher mortality. A leaf surface bioassay method was standardized using the pathogens, and although speculative, it may be possible to calculate a possible field application rate based on the number of conidia/cm^2 required to provide various levels of infection. Relative humidity certainly has a say over conidial germination and at r. h <50% ca. 30% spores only germinated. But the peak incidence of these insects coincide with monsoon period and a high humidity prevails in the field.

Bacillus firmus a gram (+) ve bacterial pathogen showed its virulence in the initial laboratory trials against *E. narcissus.* But in further trials, in the laboratory and the field, the virulence of this bacterium has come down and further evaluation under field conditions will be required to assess the potential of this bacterium against *E. narcissus.*

White muscardine fungus *B. bassiana* was observed on shoot webber *A. fabriciella* and teak sapling borer *S. malabaricus* and proved its virulence in the laboratory trials.

In conclusion, the present investigation has revealed some potential biocontrol candidates. Nuclear Polyhedral Virus against teak defoliator *H. puera* is worth trying along with *B. thuringiensis* var *thuringiensis.* in large scale field trials. The fungal pathogens viz. *P. farinosus* and *P. fumosoroseus* have potential for the control of *E. narcissus;* and *B. bassiana* for *A. fabriciella. B. thuringiensis* which showed its cros infectivity to *E. narcissus* and *A. fabriciella* could also be a potential organism against all the three insects.

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