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Role of Insect Pathogens in Plant Protection

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There are several insect pathogens identified in many key pests. The success of these biocontrol agents depends on the virulence of the pathogens, susceptibility of the pest species and influence of the environment for causing epizootics. Much progress has been made in the isolation, identification, establishment of pathogenicity and safety, mass production and field use of insect pathogens in the last few decades in many countries. Several insect pathogens have been used either as sole agents for insect control or as a component of insect pest management system. However, much more studies are needed on identifying newer pathogens, increasing virulence of known pathogens, better methods of augmenting and conserving pathogens, prevention of photo-inactivation of certain pathogens, and development of economic methods of mass production, storage and field use. The promising insect pathogens that could be used for biological suppression of pests have been highlighted.

Key Words: Insect pathogens in plants protection

Introduction

Insect pest control through conventional methods has often been found to be difficult under intensive cultivation of crops. Emphasis has been given in the recent past on the importance of pest management involving a sound knowledge of ecology of the key pests. One of the important components in pest management is biological suppression of insect pests employing pathogens like viruses, fungi, bacteria, protozoa and nematodes as biocontrol agents. These pathogens when conserved and augmented would keep some of the pests under check within the economic injury levels. In other cases, the pathogens cultured *in vitro* are employed either for short-term control equivalent to chemical control or long-term biological control.

Important Groups of Insect Pathogens

Insect Viruses

Viruses from more than 800 species of insects have so far been isolated and described. The insect viruses are broadly classified as inclusion and non-inclusion viruses. Certain viruses like baculoviruses have the inclusion bodies in the form of polyhedra or capsules. The viruses having polyhedra are of two kinds, viz., nuclear polyhedrosis (NPV)

and cytoplasmic polyhedrosis (CPV). The capsules are found as inclusion bodies in granulosis viruses (GV) of insects.

About 300 isolates of NPVs have been recorded in Lepidoptera, Hymenoptera and Diptera. The family Baculoviridae with a very narrow host range within the class Insecta and very safe to other invertebrates and higher animals. The reported cases of successful suppression of insect pests are mainly from the group of Baculoviruses including NPVs and GVs though a few CPVs are also known to be important in pest control.

In the USA three viruses were extensively evaluated and registered for use against certain agricultural and forest insect pests. The NPVs of *Heliothis* spp. (Elcar), *Lymantria dispar* (Gyp-check) and *Orgyia pseudosugata* (Biocontrol-1) are the viruses developed. In addition, other NPVs studied in great detail include those of *Autographa californica* on vegetables, soybean, sugarbeet and cotton in USA, *Choristoneura fumiferana* and *Neodiprion* spp. on forest trees in Canada, *Heliothis* spp. on cotton, sorghum and chickpea in Australia and India, *Trichoplusiani* on cotton in South America, *Amsacta* spp. in India, *Spodoptera* spp. on cotton and vegetables in India, China and USA, and *Mythimna separata* on various crops in India and China.

The other baculovirus on *Oryctes rhinoceros* has been intensively tested in the South Pacific Islands and now in India.

Heliothis NPV

The success of *Heliothis* control programme through the use of NPV was mainly attributed to the time of the first virus treatment and coverage of plant parts with the virus. In the case of cotton and chickpea, the first application was suggested when some eggs and few first instar larvae are found. In the last two decades, about 200 field tests have been carried out in various countries with the NPV, 60% on cotton, 30% on maize, and the rest on sorghum, soybean, tobacco, tomato etc. (Burges 1981).

It is evident that crop selection is important and success on cotton is less evident than on maize, control on sorghum is frequently better, and control on soybean and chickpea is the best of all. In the case of sorghum, a single application of the virus at 11.6×10^{10} PIB/0.4 ha showed excellent control of the pest. The success is related to the localised feeding of the larvae in the heads and to the absence of growth dilution. Virus treatment significantly increases cotton yields over untreated checks and when compared with insecticides the virus is at least as good as the insecticides in a majority of cases. The efficacy of Baculovirus against field populations of *H. armigera* on chickpea has been tested by Narayanan (1979). He reported 81.3% and 71.6% reduction in larval populations of *H. armigera* in NPV treatments using *Baculovirus heliothis* @ 250 and 125 LE/ha respectively (1 LE=6×10^9 inclusion bodies) (table 1). Endosulfan 0.07% is comparable to NPV alone applied at 250 LE during evening hours and virus at 250 LE with Sandoz Liquid Adjuvant applied during morning hours. The high reduction in the larval populations following three applications of the virus at weekly intervals is attributable to the time of first application synchronizing with the occurrence of 1st and 2nd instars of larvae predominantly together with some egg population. Chickpea has been suggested to be an ideal crop for suppression of *H. armigera* populations with the virus as the pest occurs even before flower and pod set.

Jayaraj (1981) observed that the virus at 125 LE/ha along with endosulfan at half the recommended dose (0.035%) significantly reduced the pest damage caused by *H. armigera* to both pods and grains of lablab and chickpea. This is on par with endosulfan 0.07% spray. According to Santharam and

Table 1 *Effect of NPV of* Heliothis armigera *on larval population, pod damage and yield of chickpea*

Treatments	No. of larvae/ 10 plants	No. of pods formed	% pod infestation	Seed yield kg/ha.
Virus 250 LE/ha	5.15[a]	372.60[b]	6.51[a]	696.60[b]
Virus 125 LE/ha	7.85[b]	305.40[b]	8.87[a]	642.90[b]
Endosulfan 0.07%	23.75[c]	351.40[b]	22.82[b]	659.60[b]
Control	27.65[d]	163.80[a]	53.69[c]	377.80[a]

Means followed by similar letters are not different statistically ($P=0.05$)

Balasubramanian (1981), two applications of the NPV @ 375 LE/ha result in maximum protection and yield of chickpea. A single round of the combination spray at reduced doses of carbaryl (0.05%) and NPV (125 LE/ha) is on par with three applications of NPV at 375 LE/ha or one application of carbaryl 0.1%. The pest on pigeonpea is, however, not checked effectively by the virus (Santharam et al. 1981). Bhatnagar et al. (1981) have reported that in field tests of sprays containing NPV, originating from a culture supplied by the Tamil Nadu Agricultural University, Coimbatore, considerable mortality (up to 60%) of *H. armigera* larvae on chickpea could be obtained.

A minimum of 24 plant species, 36 invertebrate species, 4 avian species, 7 piscian species and 6 mammalian species including man have been exposed to heavy doses of *Heliothis* NPV without any adverse reaction (see Ignoffo 1973). In no instance, toxicity or pathogenicity in any organism other than target *Heliothis* species was noticed. No deleterious effects attributable to the NPV of *H. armigera* have been observed in white rats, poultry birds and fishes (Narayanan 1979).

Spodoptera NPV

An instance of natural epizootic of polyhedrosis in *S. litura* on Egyptian clover, wiping out completely the larval population, was reported by Abul-Nasr (1959). Recently, Dhandapani et al. (1982) have noticed an epizootic form of natural occurrence of the NPV of *S. litura* on *daincha* in Tamil Nadu, inflicting 90% mortality of larvae. These observations indicated that the virus can persist in soil and/or other substrates and induce infection on a field scale.

Santharam et al. (1978b) reported that the virus suspension @ 1.2, 2.4 and 3.6×10^{12} PIB/ha along with an adjuvant Triton E 100 (0.05%) during evening hours against *S. litura* larval population on banana inflicted 80.1–90.4% larval mortality. Considering the recovery rate of PIB from individual larvae (8.62×10^{8}), an estimated 1400 LE will be required to treat one hectare of banana plantation. The virus at 93×10^{8} PIB/ml and at half dose along with carbaryl 0.02% or activated charcoal 1% reduces significantly the leaf damage in tobacco, and the number of larvae recovered from treated plots is significantly lower than those from control plots (Ramakrishnan 1976, Ramakrishnan et al. 1981). The effectiveness of NPV against *S. litura* on tobacco was confirmed by Santharam and Balasubramanian (1980) : virus at 250 LE/ha (15×10^{11} PIB) and 125 LE/ha (7.5×10^{11} PIB) with Teepol 0.05% caused 86.4% and 62.8% reduction respectively, while a combination of the virus at the latter dose and chlorpyrifos 0.04% resulted in

100% reduction in larval population 7 days after treatment. The virus at the higher dose is on par with diflubenzuron (0.01%) but inferior to chlorpyrifos (0.08% and 0.04%), fenvalerate (0.01%) and dichlorvos (0.1%).

Jayaraj et al. (1981) have studied the efficacy of NPV in the suppression of *S. litura* on cotton in four locations. In two locations, where the larvae were mostly 2nd and 3rd instars, high mortalities (80.6–89.7%) were recorded at 250–500 LE/ha doses (1 LE=6×10^9 PIB) when applied after 4PM as HV using 1000 litres spray fluid/ha with Triton X-100 (0.05%). In another trial, where 4th and 5th instars were predominant, the virus at 250 LE/ha could cause only 58.5% mortality which was on a par with chlorpyriphos (2.5 l/ha) resulting in 51.4% larval mortality. Combination sprays on NPV 125 LE/ha+methamidophos 1.25 l/ha and NPV 125 LE/ha+molasses (5%) are equal in effect to methamidophos 2.5 l/ha causing 67.4–72.0% mortality. The leaf damage index (grades 0–5) is reduced from 3.84 in control to 2.03 in NPV 250 LE/ha treatment and the number of larvae from 75.7 to 21.6/5 plants in the 4th location (tables 2–5). Application in the evening hours has helped to minimise photo-inactivation of the virus and for its greater ingestion, for the larvae show greater feeding activity only during night (Jayaraj 1981).

The safety of *Spodoptera* NPV to non-target organisms has been tested in several cases. The PIBs administered to white mice intraperitoneally do not produce toxic or pathogenic symptoms. Both virus-injected and tissue-injected mice exhibit comparable, normal weight gains. The safety of *S. litura* NPV to poultry birds has been shown by Regupathy et al. (1978). All birds fed with the virus have been found to be normal in their general behaviour, body temperature, body weight gain, and feed-conversion ratio.

Table 2 *Effect of NPV on early instars of* Spodoptera litura *on suvin cotton under field conditions in Kottur, Madurai District*

Treatments	Percentage mean mortality
50 LE/ac	64.25 (53.37)
100 LE/ac	76.50 (61.06)
150 LE/ac	84.00 (66.47)
200 LE/ac	89.75 (71.37)
Control	12.50 (20.62)
CD (P=0.05)	3.68 **

** Significant at 1% level

Table 3 *Mortality of early instars of* Spodoptera litura *on MCU5 cotton due to NPV (Uppukottai)*

Treatments LE/ac	% mortality
25	52.03 (46.17)
50	67.42 (55.23)
75	77.16 (61.48)
100	80.63 (63.99)
125	82.96 (65.67)
150	88.59 (69.71)
Control	11.26 (19.51)
CD (P=0.05)	4.42**

** Significant at 1% level
(Figures in parenthesis are transformed values)

There is no difference in blood sugar and serum alkaline and acid phosphatase activity due to virus treatment. No gross and histopathological changes are detected. Among the beneficial insects, the parasitoids, *Parasarcophaga misera* (Walker), *Trichogramma australicum* and *Chelonus blackburni* and honeybee, *Apis cerana indica*, have been used as test organisms for the safety of *S. litura* NPV, and in all the cases the virus has been found to be absolutely safe. The virus is also safe to silkworm and white mice.

Table 4 *Effect of NPV alone and in combination with insecticides on grown-up caterpillars of* S. litura *on Varalaxmi cotton (Vilampatti)*

Treatments	Cumulative mortality (%)
50 LE	53.85 (47.26)
100 LE	58.54 (49.24)
Chlorpyriphos 1 lit	51.43 (45.79)
50 LE+Chlorpyriphos 0.5 lit	52.43 (46.35)
Methamidophos 1 lit	72.00 (58.10)
50 LE+Methamidophos 0.5 lit	72.00 (58.12)
50 LE+Molasses spray	67.44 (55.20)
50 LE in rice bran+molasses bait	63.44 (52.76)
Chlorpyriphos 0.5 lit+Fenitrothion 0.25 lit.	60.61 (51.06)
Phoxim 1 lit.	56.67 (48.90)
Control	0.00 (1.81)
CD (P=0.05)	5.34**

** Significant at 1% level

Table 5 *Effect of NPV and insecticides on grown-up caterpillars of* Spodoptera litura *on MCU 5 cotton (Kodangipatti)*

Treatments	Larval population per 5 plants	Leaf damage index (grades 0 to 5)
Virus 100 LE	21.6 (3.96)	2.03
Virus 50 LE+Chlorpyriphos 0.25 lit+Fenitrothion 0.125 lit	27.5 (5.00)	2.24
Chlorpyriphos 0.5 lit+Fenitrothion 0.25 lit	35.7 (5.86)	2.38
Control	75.7 (8.65)	3.84
CD (P=0.05)	1.60**	0.44**

** Significant at 1% level
(Figures in parentheses are transformed values)

Amsacta NPV

An induced epizootic of NPV in *A. albistriga* was observed by Rabindra and Balasubramanian (1980). About 19% of the larvae on groundnut plants, 52% crawling in between plants, and 94% migrated to fence plants were found diseased. There is a tendency for initial spurt in restlessness and migratory activity following infection before becoming sluggish. Observations made in 1974-75 (Jayaraj & Narayanan unpub.) reveal that the infected 4th and 5th instar larvae migrate to *Calotropis gigantea* L. and other fence plants 500 m away from the field site of treatment and die in large numbers hanging upside down.

Jayaraj et al. (1977) made an attempt to suppress the field population of *A. albistriga* with NPV and *B. thuringiensis*. The bacterial preparation, Biotrol at 4 kg/ha is at par with endrin 600 ml/ha in reducing the population of grown-up larvae, and mortality occurs in 2–3 days in both treatments. The NPV at a low dose of 2×10^5 PIB/l is statistically at par with Biotrol, but larval mortality is delayed to 7–9 days. The efficacy of NPV in combination with 1/10th recommended dose of insecticides has been tested by Narayanan (unpub.) against 5th and 6th instar larvae. NPV combined with quinalphos (0.0075%), endrin (0.009%), fenitrothion (0.005%), or dichlorvos (0.01%) inflicted 60–68% mortality while the insecticides at these sub-lethal doses caused only 10.3–21.3% mortality indicating a physiological stress induced by the chemicals. He has also tried the NPV at 7 doses, viz. $16\times10^{5-11}$ PIBs/0.4 ha and observed that the larval mortality was directly related to the doses; earlier instars were more susceptible than middle and late instars. Maximum mortality was caused 6 days after treatment.

Interestingly, Rabindra and Balasubramanian (1980) have reported persistence

of NPV applied to groundnut in September 1978 causing very high mortality in the next year in September 1979. The severe epizootic induced resulted in disease in 94.5% larvae migrated to fence plants, 52% crawling larvae, and 18.7% feeding larvae. The authors have suggested that transmission from generation to generation either through infected eggs, accumulation and persistence in soil, or through the remnants of infected cadavers might have carried virus from the previous season. The gregarious and migratory habits of the larvae might have also exerted an influence in spreading the disease.

The NPV of *A. albistriga* has been found to be absolutely safe to white mice, poultry birds, and common carp fish. No death occurs in any of the white mice and poultry birds in the test period of 21 and 42 days, respectively (Narayanan et al. 1977d, 1978a). There is no change in their bodily appearance, behaviour, and feeding activity. Body temperature, body weight gains, and feed efficiency of virus-treated animals are essentially similar to those of the control. No difference in blood sugar, blood protein and serum enzymes is detected. Post-mortem examinations indicate no gross pathology in either group. In the case of common carp, *Cyprinus carpis* L., all fishes are found healthy showing normal weight gains. The swimming pattern and feeding behaviour are also unaltered (Narayanan et al. 1977e).

Baculovirus of Oryctes

An investigation into the diseases of *Oryctes rhinoceros* conducted in 1963, led to the discovery of a virus disease in Malaysia caused by *Oryctes* baculovirus, referred to as 'Malaya disease'. From 1967 onwards this virus was intentionally introduced into several of the South Pacific Islands, in an attempt to biologically suppress the pest population. The virus disease became established and spread rapidly throughout the islands and as a consequence pest damage to fronds fell drastically by as much as 80% in some islands in about 40 months. This successful control is being acclaimed to be a land-mark in biological control of crop pests (Bedford 1980, Caltagirone 1981).

The most economical, effective and simple method adopted for the dissemination of *Oryctes* baculovirus in wild beetle populations is to release infected beetles. Laboratory-reared healthy beetles/field-collected beetles are allowed to swim partially submerged in a suspension of macerated diseased grubs containing 5% sucrose for 10 min and later confined together in a container having sawdust mixed with virus suspension for 24 hr. This kind of treatment ensures 90–95% infection as inferred by diagnostic tests. The treated beetles are let free at night at various locations. Dispersing widely before death, the beetle spread the disease in the natural population by contaminating the breeding sites containing larval broods and to other beetles.

Introduced into artificial log traps in Western Samoa in 1967, the virus became established in the beetle population in one year and spread widely. Studies in 1970-71 showed that 3% of larvae and 35% of adults were infected. Breeding sites with infected larvae fluctuated between 5.6 and 11.2%.

Granulosis Viruses

Granulosis viruses affecting *Cydia pomonella, Pieris rapae, Agrotis* spp. and *Chilo* spp. have been found to be promising based on studies made in several countries. In a recent study in Tamil Nadu, the granulosis virus of sugarcane early shoot borer, *Chilo infuscatellus* was found to be quite effective in checking the pest population under field conditions. The virus was also found to be safe to the non-target species of organisms tested (Easwaramoorthy 1984).

Fungal Pathogens

This is the largest group of insect pathogens having more than 500 species reported from different insects. Though all the four classes of fungi, viz. Phycomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes (Fungi imperfecti) include organisms causing mycoses in insects, the classes Phycomycetes and Deuteromycetes are the most important ones. The genera *Entomophthora*, *Coelomomyces* and *Massospora* of Phycomycetes and *Beauveria*, *Metarhizium*, *Verticillium*, *Spicaria*, *Aspergillus*, *Isaria* and *Hirsutella* of Deuteromycetes are important insect pathogens. The genus *Cordyceps* of Ascomycetes and *Septobasidium* of Basidiomycetes also contain a few species of fungi infecting insect pests.

Several attempts have been made to use the entomogenous fungi for the control of insect pests since 1950. The pests belonging to insect orders, viz. Lepidoptera, Coleoptera, Homoptera, Hymenoptera and Diptera are susceptible to different fungal pathogens. Successful cases of pest suppression using entomopathogenic fungi have been reported in the case of 28 species of insects. Several fungi are also known to produce toxins which are lethal to insects.

Entomophthora spp.

Mathur and Srivastava (1966) recorded *Entomophthora aphidis* on *Therioaphis trifolii*. *Myzus persicae*, *Lipaphis erysimi* and *Brevicoryne brassicae* were infected by *E. coronata* (Ramaseshiah 1967). *E. exitialis* was noted on *Rhopalosiphum maidis* (Sahni 1969).

The rice brown planthopper, *Nilaparvata lugens* Stal., has been found to be infected by *E. delphacis* Hori, *E. apiculata* var. *major* and *E. coronata* (Samal et al. 1978). *E. thripiolum* causes epizootics in *Thrips tabaci*. Ramaseshiah (1971) noticed epizootics of *Entomophthora* sp. nr. *floridana* in *Tetranychus ludeni*, *T. telarius* and *T. cinnabarinus*. As high as 80–100% mortality was observed in *T. ludeni*.

Some of the problems encountered with *Entomophthora* are: they are difficult to culture, conidia are short-lived, the pathogenicity for man and other vertebrates is unknown. In addition, the fungi need a water-saturated atmosphere for several hours to infect their hosts and very little is known about the specificity of strains or species or of the effect of pesticides on fungal activity.

Beauveria spp.

Beauveria sp. was found to cause an epizootic infection in *Spodoptera litura* and *Utethesia pulchella* (Rangaswami et al. 1968). Ayala and Monzon (1977) reported the effectiveness of *B. bassiana* at 10^5 spores/mg at a dosage of 4–16g/tree against the larvae of banana rhizome borer, *Cosmopolites sordidus*.

In USSR, an industrial preparation of *B. bassiana* called 'Beauverin' or 'Boverin' with 2×10^9 conidia/g in an inert substrate of kaolin is used. For the control of Colorado beetle, *Leptinotarsa decemlineata*, 2 kg of Boverin at 6×10^9 conidia/g and 1/5th of the quantity of normally applied DDT or polychloropinene/ha is found effective. Boverin alone at 1 to 1.5 kg at 30×10^1 conidia/g is also effective. In general, two successive treatments at intervals of 2 or 3 weeks are needed. In France, similar results have been reported (Ferron 1978).

By the use of Boverin at 1 kg, satisfactory protection of fruits from the codling moth (*Laspeyresia pomonella*) damage was obtained in Ukraine. But wherever fungicide applications intervened the results were less satisfactory. In France and Canada too good results were reported.

B. brongniartii when infected into soil as a spore suspension (20×10^9) conidia/m^2 against *Melolontha melolontha* in meadows, an epizootic developed 16 months after

soil inoculation and the disease persisted into the next generation (Ferron 1978). Jayaramiah (1981) found that application of *B. brongniartii* at 10^5 spores/g of soil along with 50 g of HCH 50%/m^2 or 10^9 spores alone suppressed the population of *Holotrichia serrata*.

Metarhizium spp.

Rangaswamy et al. (1968) reported that *Metarhizium* sp. was found to cause 100% mortality of *Pyrilla perpusilla* in field. *M. anisopliae* was used to control spittle bugs, *Mahanarva posticata* on sugarcane. Aerial sprays at 50 l/ha (6×10^{11} to 1.2×10^{12} conidia) were followed (Ferron 1978).

Recently, Sundara Babu et al. (1983) studied in detail the culturing, dosage, role of enzymes in disease production, influence of ecological factors and control of *O. rhinoceros* at the breeding site with *M. anisopliae*. They reported carrot broth with a *p*H of 6.5 to 7 was suitable for culturing the fungus when incubated for 25 to 30 days at 25 to 30°C and a RH of 92% and above.

The fungal enzymes chitinase and lipase played an important role in the pathogenicity of the fungus. Germination of the fungal spores was inhibited by HCH and carbaryl. *M. anisopliae*, when applied at 34.4×10^4 spores/g of farmyard manure when it is partially decomposed, infected eggs, grubs and adults of *O. rhinoceros* and effected 100% mortality of grubs (Sundara Babu et al. 1983) (table 6).

Fusarium spp.

Fusarium oxysporum Schlecht was reported on *Coccus viridis*, and it was found to cause 90% mortality within 10 days when fungal spore suspension was applied on the plants and kept in natural condition (Kasiviswanathan 1972). *F. oxysporum* when applied at 6.25×10^6 spores/ml on BPH on rice, 100% mortality was observed within 3 days. The first and second instar nymphs were more susceptible than the 3, 4 and 5th instar nymphs and adults (Kuruvilla & Jacob 1979).

Gopinathan et al. (1982) reported 100% mortality of brinjal mealybug *Coceidohystrix insolita* Green in 9 days after spraying with *F. equiseti*. *F. moniliforme* var. *subglutinans* Walloenw and Reink was reported to be pathogenic to spotted beetle, *Henosepilachna vigintioctopunctata* F. and this pathogen was effective in controlling the pest on bittergourd and safe to cotton, tomato, bittergourd, snakegourd and brinjal plants. the pathogen was also reported infecting *Aulacophora* sp. and *Mylabris pustulata* (Beevi & Jacob 1982 a,b).

Table 6 *Control of* Oryctes rhinoceros *Grubs with* Metarhizium anisopliae

Dose (Spores/5 of FYM)	% mortality after six weeks of application (Mean of eight replications)	
	I Trial	II Trial
1.72×10^4	17.50 (24.53)^a	18.75 (25.55)^a
8.60×10^4	26.25 (30.72)^{ab}	32.50 (34.72)^{ab}
17.20×10^4	35.00 (36.22)^b	40.00 (39.20)^b
34.40×10^4	100.00 (90.00)^c	100.00 (90.00)^c
C.D. at 5% level	(11.48)	(12.37)

Values in the parenthesis are arc/sin $\sqrt{P}$. Means followed by same letter are not significantly different at 5% level

Nagalingam (1983) observed peak incidence of *M. persicae* and *F. semitectum* during October on cauliflower, rainfall being important requisite for the induction of fungal epizootics. The incidence of the fungus was greater on the aphids on lower leaves in cabbage. For the mass production of the fungus maize broken grain+blackgram husk or redgram husk at 4:1 (W/W) was highly suitable.

The fungus was responsible for epizootics causing 100% mortality of *M. persicae*. It was inhibited by the insecticides heptachlor, monocrotophos and endosulfan and fungicides carbendazim, benomyl and thiram while wettable sulphur and quintozone were innocuous.

The fungus was safe to all instars of mulberry silkworm, adult honey bees, hymenopterous parasitoids and coccinellid predators. The fungus was also safe to plants like chilli, cabbage, brinjal and tobacco.

Verticillium lecanii

Easwaramoorthy and co-workers made detailed studies on this fungus with reference to culture, host insects, ecological factors influencing infection, compatibility with insecticides and fungicides and the utilization of this fungus for the control of the coffee green bug, *Coccus viridis* (tables 7–9).

Pearl millet extract was the most suitable medium for radial growth and dry weight of *V. lecanii* while for mass production sorghum grains were suitable at *p*H-6, temperature=25–30°C and incubation= 2 weeks. The pathogenic effect of the fungus on coffee green bug, *Coccus viridis* was studied at Lower Palney Hills, Madurai, which showed that the fungus was highly effective against the pest under field conditions. With two fortnightly applications of 16×10^8 spores/ml it caused 73% mortality of the bug 2 weeks after the 2nd application. Increased mortality of the pest (97.6%) was observed when Tween 20 or Teepol was added to the fungus during winter months. The fungus in combination with fenthion resulted in highest mortality of the pest. The fungus was more effective as high volume spray than as low volume spray. A greater inhibition of the fungus was observed with dichlorvos, carbaryl and monocrotophos

Table 7 *Effect of* Verticillium lecanii *with certain surfactants on the percentage mortality of coffee green bug*

Treatments	Period in weeks after spraying		
	1	2	3
Fungus alone	49.2	68.1	67.1
Fungus+Tween 20 0.05%	88.0	96.7	95.4
Fungus+Methyl cellulose 0.05%	40.0	80.8	72.9
Fungus+Starch 0.05%	60.8	94.2	92.8
Fungus+Lerolat 0.05%	71.5	96.6	92.0
Fungus+Erkentrol 0.05%	62.7	85.1	74.3
Fungus+Wannin 0.05%	50.4	77.8	81.2
	SE	LSD (P=0.05)[a]	
Between treatments	0.4**	1.3	
Interaction between treatments and periods	1.2**	3.4	

[a] For data transformed by arc sin (percentage) $\frac{1}{2}$

** Significant at 1% level

Table 8 *Effect of Fungus and certain insecticides on the percentage mortality of coffee green bug*

Treatments	Period in weeks after spraying		
	1	2	3
Fungus alone	49.2	68.1	67.1
Fungus+Tween 20, 0.05%	88.0	96.7	95.4
Fungus+Tween 20, 0.05%+DDT 0.1%	80.6	92.0	89.9
Fungus+Tween 20, 0.05%+B.H.C. 0.1%	45.9	66.5	67.3
Fungus+Tween 20, 0.05%+Acephate 0.225%	74.6	93.1	98.5
DDT 0.1%	46.4	44.3	41.5
B.H.C. 0.1%	27.9	38.5	29.1
Acephate 0.225%	75.0	97.7	98.1
	SE		LSD (P=0.05)[a]
Between treatments	1.4*		4.6
Interaction between treatments and periods	2.8*		8.8

[a] For data transformed by arc sin (percentage) ($\frac{1}{2}$)
* Significant at 5% level

Table 9 *Effect of* Verticillium lecanii *and insecticides on the mortality of coffee green bug (Mean of 3 observations)*

Treatment	Mortality*	
	7 days	14 days
Fungus (Spores/ml) 16×10^6	24.6 (27.4)	28.4 (31.0)
Fenthion 0.05%	48.1 (43.5)	49.9 (45.2)
Phosphamidon 0.05%	13.5 (21.4)	28.6 (32.0)
Fungus +Phosphamidon 0.05%	85.3 (67.8)	91.7 (73.9)
Fungus+Fenthion 0.05%	83.3 (66.2)	88.8 (72.0)
S.E.	(6.0)**	(5.1)**
C.D. (P=0.05)	(17.4)	(14.7)

* Percent of dead scales P., in parenthesis are the values of arc sin/$\sqrt{P}$
** Significant at 1% level

whereas acephate, DDT and phosphamidon were least inhibitive.

Boredeaux mixture, Dithane M-45 and Z-78 inhibited the fungal growth while sulphur was innocuous. The fungus was effective against guava scale and chilli aphid also (Easwaramoorthy & Jayaraj 1976, 1977 a,b, 1978, Easwaramoorthy et al. 1978, Santharam et al. 1978a).

Bacterial Pathogens

The bacteria which affect insects are broadly classified as spore-formers and non-spore-formers. There are both obligate spore-formers like milky disease organisms viz. *Bacillus popilliae* and *B. lentimorbus* and facultative spore-formers such as *B. thuringiensis* and *B. cereus*. *B. cereus* is known to mutate into *B. anthracis* which causes anthrax disease in cattle. The non-spore former bacteria like species of *Serratia, Pseudomonas, aerobacter* and *Streptococcus* are not of considerable importance as they are not able to thrive under adverse conditions to cause repeated infections in insects.

Bacillus thuringiensis

There are about 25 varieties of *B. thuringiensis*. These produce several insecticidal toxins, viz. α-exotoxin (heat-labile exotoxin), β-exotoxin (fly factor or heat stable exotoxin), δ-endotoxin (crystalline toxin or crystal) and 'louse factor'. Two of these are used in agriculture viz., the δ-endotoxin and the β-exotoxin. The insecticidal actions of isolates of *B. t.* are complex. The relative activity of each isolate against different insect species, i.e. the 'spectrum of activity' arises partly from the combined effects of potencies of the varying concentrations of the different insecticidal materials that it produces. The primary cause of the spectral differences lies in the spectrum of activity of the δ-endotoxin itself. Different isolates can produce more of the same δ-endotoxin than others.

Nearly 525 insects belonging to various orders have been found to be infected by *B. thuringiensis* as detailed below:

Order	No. of species
Coleoptera	34
Diptera	59
Ephemeroptera	5
Heteroptera	7
Homoptera	4
Hymenoptera	57
Isoptera	5
Lepidoptera	318
Mallophaga	7
Neuroptera	2
Orthoptera	12
Thysanoptera	1
Trichoptera	3

The very wide host range which includes a large number of injurious species among Lepidoptera, the relative stability of the toxic crystals and the spores and the ease with which *B. t.* can be produced in quantity on a wide variety of media, have led to its commercial exploitation as the basis of a number of microbial insecticides.

In crucifers, spray and dust formulations of *B.t.* gave generally good to excellent control of the cabbage pests, *Pieris brassicae, P. rapae* and *Plutella xylostella*. Results with *Trichoplusia ni* have been poor to erratic. In recent years *B.t.* products have been extensively used for the control of foliage chewing species of Lepidoptera. Control of *Heliothis zea* in sweet corn has been reported with heavy dosages of *B. t.* An encapsulated formulation and clay granular and emulsifiable formulation of *B. t.* were effective against the European

corn-borer *Ostrinia nubilalis*. Tobacco hornworm *Manduca sexta* and tobacco budworm, *H. virescens* were successfully controlled. Good suppression of cotton leafworm, *Alabama argillacea* has been reported. Thuricide is registered against *H. zea* on cotton. Numerous other lepidopteran larvae have been found to be controlled by *B. t.* formulations to a great extent.

Bacillus popilliae and Bacillus lentimorbus

B. popilliae and *B. lentimorbus* cause, respectively, milky disease A and B. These two bacteria multiply and sporulate readily in the haemolymph of the grubs. The turbidity due to accumulating spores leads to milkiness of the haemolymph and hence the name 'milky disease'.

Milky disease organisms are able to infect only certain closely related beetles of the family Scarabaeidae. About 62 species of insects have been found to pick up infection by injection or soil inoculation.

Protozoan Pathogens

There are four important classes which include pathogens of insects. The class Mastigophora (Flagellates) includes species of *Leptomonas*, *Herpetomonas*, and *Crithidia*. *Tetrahymena* is the important genus in the class Ciliophora (Ciliates). Among the amoebae (Sarcodina), *Malpighamoeba mellificae* infecting honey bees and *Malamoeba locustae* infecting grasshoppers and locusts are important. The class Sporozoa (Coccidia) includes several important protozoan pathogens like gregarines, schizogregarines, coccidia, Haplosporidia and Microsporidia. Most of the protozoa are debilitative pathogens. The two groups Sporozoa and Conidospora include large number of protozoan pathogens of insects some of which have great potential in pest control. The protozoan pathogens infect insects of orders like Lepidoptera, Coleoptera, Orthoptera, Hemiptera and Diptera.

Mattesia grandis

Significant progress has been made in the control of the boll weevil with *Mattesia grandis*. Since the adults feed within the cotton boll avoiding infection even under complete spray coverage, McLaughlin et al. (1969) used a bait containing a feeding stimulant and the protozoan. Subsequently better feeding stimulants and formulations improved the effectiveness. The use of feeding stimulant in large viscous drops of bait was extremely effective in the spring against the over-wintered adults and again in the fall against weevils entering diapause in preparation for over-wintering. At these times 50–70% infection was frequently attained and sometime 80–98% in the fall in open field tests.

Nosema pyrausta

Lewis and Lynch (1978) demonstrated that the European cornborer. *Ostrinia nubilalis* could be effectively suppressed with *Nosema pyrausta*. However, the success of control often depends on the interaction with additional stress factors such as crowding or the resistance of maize plants to corn borer feeding (Lewis & Lynch 1976, Lynch & Lewis 1976). Lublinkhof et al. (1979) integrated *N. pyrausta* with insecticides in control of the corn borer. For two seasons, artificially infested corn plants were first treated with 1.2×10^7 spores; the next day they were treated with *Bacillus thuringiensis* var. *kurstaki*, carbaryl or carbofuran. In each instance, larval densities were reduced following application of *N. pyrausta* and an insecticide. Combination of microsporidium with *Bacillus thuringiensis* or carbaryl was more effective than its combination with carbofuran. Generally these mortality factors acted independently

with additive effects. This study indicates a good potential for developing an integrated approach using various combinations of mortality factors including *N. pyrausta* for control of European corn borer.

Farinocystis tribolii

The red flour beetle is affected by the schizogregarine, *Farinocystis tribolii.* The red flour beetle is highly susceptible to this sporozoan and its occurrence has been reported in India by Rabindra and Subramanian (1974). Studying the susceptibility of *T. castaneum* to *F. tribolii*, Rabindra et al. (1981, 1983) found that larvae of all instars were equally susceptible and a dose of 10^8 spores/g of diet with a 24 hr inoculation feeding resulted in 100% mortality in all the instars. The LT_{50} increased with age of the insect indicating that older larvae took more time to succumb to the infection. Adults were less susceptible than larvae and between sexes, females were more susceptible than males. Mortality time due to *F. tribolii* was shorter at 35°C than at 25°C.

Rabindra (1979) conducted two experiments to find out the effectiveness of the protozoan in the control of the flour beetles (tables 10, 11). In one, infected cadavers were inoculated at 10, 50 and 100 cadavers (either whole or powdered) per container carrying 500 g of wheat flour. Counts with haemocytometer revealed that 10, 50 and 100 cadavers carried Ca 0.7×10^7, 3.61×10^7 and 7.57×10^7 spores respectively. Initially 50 second instar larvae were inoculated into each container. In the second experiment, the efficacy of cellulose-based spore powder was tested in different concentrations viz. 5×10^8, 10×10^8, 15×10^8, 20×10^8 and 25×10^8 spores per container carrying 500 g of wheat flour. Then each container was inoculated with 50 second instar larvae. In both experiments, suitable controls with the spores were maintained. The population was recorded at 15 days interval up to 90 days. The results showed that infected cadavers as well as purified spores were highly effective in checking the population. When the different cadaver treatments were compared, powdered cadavers were more effective, higher dose recording lower population.

Table 10 *Effect of* Farinocystis tribolii *on* Tribolium *population (Cadaver Treatment)*

Treatment	Mean population Percontainer after treatment Days	
	75*	135**
Powdered cadavers 50	490	159[a]
Powdered cadavers 100	350	106[a]
Whole cadavers 50	901	414[b]
Whole cadavers 100	556	295[b]
Control	15371	9006[b]

* Means significantly different (P=0.05)
** Means followed by similar letters are not statistically different

Table 11 *Effect of purified spores of* F. tribolii *on the total population development of* T. castaneum

Dose/500 flour	Total population days after* inoculation (Mean of three replications)	
	45	75
25×10^8 spores	84.33[a]	94.00[a]
20×10^8	104.67[a]	172.33[ab]
15×10^8	154.00[a]	224.33[b]
10×10^8	119.67[a]	339.33[bc]
5×10^8	436.33[b]	598.33[c]
Control	3282.33[c]	8613.33[d]

Means in a column followed by same letters are not significantly different at 5% by LSD

In another study, the susceptibility of healthy and *Farinocystis* infected larvae to malathion was studied. The dosage-mortality response showed that infected larvae were more susceptible to malathion than healthy larvae (table 12). The above studies indicate that *Farinocystis tribolii* has a great potential in the integrated pest control of the red flour beetle. Adults being less susceptible to any attempt to use this pathogen in the control of this pest should be directed against the larvae.

Advantages and Disadvantages of Microbial Control

Microbial control as a method is harmless and non-toxic to many forms of life. There is relatively high degree of specificity in most cases which assists to protect non-target organisms. Many insect pathogens are compatible with insecticides and can be easily incorporated in the insect pest management system. Their large scale culture and application is relatively easy and inexpensive in most cases. Permanent control of insects could be achieved in some cases and in others fairly a good check of the pest has been established. Generally, resistance in insects to pathogens is not developed in most cases or may develop slowly compared to insecticides. In view of the above advantages, microbial control has been given importance in pest management in many countries.

There are certain disadvantages also in microbial control of insects. It is necessary for the correct timing of applications as most pathogens caused disease after a definite incubation period which will vary in different stages of insects. In view of the marked specificity of certain insect pathogens, spectrum of effectiveness is narrowed down. It is also necessary to maintain the virulence and viability of the pathogens till its use, and some pathogens like protozoa are difficult to be mass produced. There are also objections in using the pathogens because of the tendency of insect cadavers remaining on the edible parts of plants. Above all, the success of microbial control largely depends on climatic conditions.

Suggestions for Future Work

1. Systematic surveys should be undertaken to identify naturally occurring insect pathogens under varied agro-ecological conditions in the country.
2. Detailed studies are necessary on the properties, mode of action and pathogenicity of insect pathogens.
3. Basic studies on the isolation and characterisation of toxins produced by different insect pathogens.
4. Ecological studies on the dynamics of the diseases in insect populations are necessary as the environment plays an important role in the successful development of diseases and ultimate control of insect

Table 12 *Profit analysis of dosage—mortality response of healthy and* F. tribolii-*infected larvae of* T. castanum *to malathion*

Treatment	Slope 'b'	+2 (n–2)	LC 50	Fiducial limits
Healthy	2.85	1.61	0.1852	0.2291–0.1497
F. tribolii-infected	2.68	4.78	0.1059	0.1351–0.0831

pests. Loss in infectivity of certain pathogens due to photo-inactivation should be minimised.

5. Development of cheap and effective methods of mass culturing and formulation of biocides and better methods of storage and field use including bait preparations. Studies on cell-line cultures for the production of certain obligate pathogens.
6. Developing better methods of augmenting and conserving the naturally occurring insect pathogens and those introduced under the applied microbial control.
7. Interaction of the use of insect pathogens with other methods of pest control to develop better IPM strategy.
8. Studies on safety of the insect pathogens to higher animals, beneficial insects and plants.
9. Experimental Pilot Plants should be set up for mass production of insect pathogens.

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