INTERNATIONAL JOURNAL OF PLANT PROTECTION / VOLUME 6 | ISSUE 1 | APRIL, 2013 | 198-204

A CASE STUDY

Record of natural incidence of entomopathogens in Gangetic alluvial zone of West Bengal

■ LAKSHMAN CHANDRA PATEL*[1] AND ANIRUDHYA PRAMANIK[2]

[1]Divyodaya Krishi Vigyan Kendra, Chebri, KHOWAI (TRIPURA) INDIA
[2]AICRP on Plant Parasitic Nematodes, B.C. Krishi Viswavidyalaya, Kalyani, NADIA (W.B.) INDIA

ARITCLE INFO

Received : 16.08.2012
Accepted : 20.03.2013

Key Words :
Natural incidence,
Entomopathogens,
Gangetic alluvialzone,
Isolation, Pathogenicity

ABSTRACT

A survey was conducted to find out the natural incidence of entomopathogens and their pathogenicity in Gangetic alluvial zone. Three major groups of entomopathogens such as fungus, bacteria and virus have been observed and isolated from natural infected insects. Natural occurrence of viral and fungal diseases was much more pronounced, so far as field infestation and laboratory cultures were concerned. Pathogenicity of the viral isolates (V_1, V_2 and V_3) caused 83.33, 91.67 and 56.67 per cent mortality for *S. litura, H. armigera* and *S. obliqua,* respectively. Characterizations of pathogenic microbes isolated during the course of investigation were also attempted. It revealed that the entire three isolated viruses belonged to polyhedrosis type. White mascardine fungus named, *Beauveria bassiana* isolated from infected *Spilarctia obliqua* and *Amritodus atkinsoni* resulted 33.67 and 76.67 per cent mortality, respectively. Three different types of bacterial colonies were isolated from disease infected larvae of *Earias vitella*. Pathogenicity test of these isolates revealed only one isolate having 53.33 per cent mortality. But, in case of *Pieris brassicae* among two isolates, only one caused 26.67 per cent mortality. The isolates for both the insects those caused more or less pathogenicity after isolation belonged to single genus *Bacillus*.

How to view point the article : Patel, Lakshman Chandra and Pramanik, Anirudhya (2013). Record of natural incidence of entomopathogens in Gangetic alluvial zone of West Bengal. *Internat. J. Plant Protec.*, **6**(1) : 198-204.

*Corresponding author:
lakshman_patel@rediffmail.com

INTRODUCTION

In recent days the term 'Bio-intensive pest management' came in 2000 onwards and it may be a main component of second green revolution in India. One of the important ingredient of BIPM is the biological suppression of insect pests by employing pathogens like bacteria, fungus, virus, protozoa and nematodes as bio-control agents (Dutky, 1959) designated as 'microbial control' (Steinhaus, 1949).

There are so many records of natural occurrence of these entomopathogens in India. Green muscardine fungus on *Pyrilla* sp. was reported as insect pathogen in India by Kamat *et al*. (1952) and fungus on *Schistocerca gregaria* F by Misra in 1952. Subsequently, *Metarhizium anisopliae* was isolated from *Oryctes rhinoceros* (Nirula *et al*., 1955). Thereafter, several reports came in such as *Bt* on *Adisura atkinsoni* M. (Majumder *et al*. 1956), *Beauveria bassiana* Vuillemin and *Aspergillus parasiticus* on *Holotrichia consanguinea* (Rao and Vijaylakshmi, 1959), *Serratia marcescence* on *Spodoptera litura* (Pandey and Rangarajan, 1967), *Marmis* sp.on *Antigastra catalaunalis* D. (Patnaik and Das, 1969), nuclear polyhedrosis virus (NPV) on *Spodoptera litura* (Ramkrishnan and Tiwari, 1969) and *Bt* on *Spodoptera litura* (Rangaswami *et al*., 1970). Oblisami *et al*. (1969) launched systemic survey of crop pests in Chennai and Mysore to study the incidence of microbial diseases. In this direction, Battu *et al*. (1971) carried out a survey work in Punjab during 1969-70. The pathogens isolated were non-spore forming bacterium, *Streptococcus faecalis* from *Spilosoma obliqua* W, *Pieris brassicae* L and *Spodoptera litura* F., spore forming bacterium, *Bacillus*

thuringiensis from *Mythimna separata* W. and K. and *Plusia orichalcea* F., one fungus, *Aspergillus flavus* from *Spilosoma obliqua* W and *Spodoptera litura* F., respectively. Natural incidence of NPV in *Spodoptera litura* to the tune of 32-49 per cent was reported by Pawar and Ramkrishnan (1971) and that of *Beauveria bassiana* on *Nisotra orbiculata* M. was reported by Pandit *et al*. (1979). The fungus isolated from brinjal mealy bug was recorded as *Metarhizium anisopliae* . Natural occurrence of *Beauveria bassiana* on mango mealy bug was reported by Srivastava and Masarrat (1988). Following the views of survey on natural incidence of microbial diseases of *Spodoptera litura* F. in cabbage and cauliflower, Prasad and Kushwaha (1990) revealed that bacteriosis (*Bt*, *Pseudomonas aeruginosa* and *Streptococcus* sp.) among larvae, pupae and adults occurred in peak during Feb.-March and Aug.-Oct. in cauliflower. The larval mortality on account of fungal infection (*Metarhizium anisopliae* and *Entomopthora* sp.) was noticed exclusively from cauliflower from Aug.-Oct. (1975 and 1976). Viruses were used as microbial control by artificial dissemination against crop pests like cabage looper, *T. ni* (Hb.) (Hall, 1957). NPVs were recorded from cotton bollworm, *Heliothis zea* (Boddie) and tobbao budworm, *H. virescence* (F.) (Ignoffo *et al.*, 1965), Nearly 200 spp. of *NPVs* have been recorded in insects (Aizawa, 1963), majority of which under Lepidoptera such as beher hairy caterpillar, *S. obliqua* (Wlk.) by Jacob and Thomas, 1972, tobacco caterpillar, *S. litura* and American bollworm, H. *armigera* by Narayanan (1985). Battu (1982) noticed natural incidence of NPV as high as 80 per cent in the field population of *S. obliqua* (Wlk.). Incidence of nuclear polyhedrosis virus on cabbage diamond back moth, *Plutella xylostella* was noticed and recorded by Padmavathamma and Veeresh (1989). Natural occurrence of Baculovirus disease in *Oryctes rhinoceros* (L.) population in Tamil Nadu has been recorded by Rajamanickam *et al.* (1989). Certain sugarcane pests were diseased by *Fusarium subglutinans* as reported by Easwaramoorthy and Santhalakshi (1989). They also reported the occurrence of *Beauveria bassiana* on sugarcane root borer, *Emmalocera depressella.* In West Bengal natural occurrence of viral diseases of insects were much more pronounced than other organisms as found on *Spodoptera litura* and *Spilosoma obliqua* (Pramanik, 1995). A granulovirus was found to infect sugarcane top borer, *Scirpophaga excerptalis* for the first time. In the laboratory test, the virus caused mortality of final instars larvae upto 55.2 per cent in 4-8 days after infection (Singaravelu *et al*., 1999). Natural incidence of *Hirsutella thompsonii* fisher on the coconut eriophyid mite, *Aceria guerreronis Keifer* was recorded by Kumar *et al.* (2001) in certain district of Karnataka and Tamil Nadu in India. Narayanan and Veenakumari (2003) isolated and reported two NPV from the coconut blackheaded catterpiller, *Opisinia arenosella* and sorghum spotted stem borer, *Chilo partellus* for the first time in India. The pathogenicity of both the NPVs have been tested and proved along with gross pathological symptoms.

With the use of these bio-pesticides under overall ambit of IPM, the load of toxic pesticide residues in the agricultural food commodities will be significantly reduced besides the protection of environment. There has been an increasing interest in initiating a number of research projects operating in India and abroad in exploring the potentiality of these organisms in crop pest suppression. So far, more than 100 bacteria, 800 viruses, 530 fungi have been studied and described. The micro-organisms causing diseases on insect pests feeding on useful plants neither harm either man or other animals. They do not leave undesirable residues and can be used even closed to harvest. This is the most important factor that is encouraging the use of pathogens for the control of plant feeding insects. Microbial control is also compatible with other methods of pest management. Some of these microbial agents (Dhaliwal and Arora, 2000) are potential and offer an excellent alternative to chemical pesticides in the world market. Among the microbial pesticides, *Bacillus thuringiensis* represents the majority of commercial bio-pesticides. Reports, however, indicate that *Bt* total market volume represents around 1.4 per cent of total insecticides used and occupies less than 0.5 per cent of total agricultural chemical market value. A few bio-pesticides have been mass produced, patented and applied in the field. But, till now the respective data on availability and consumption of bio-pesticides in the country are very meagre.

Acknowledging the use of microbial agents as a prioritized tool in BIPM, the present investigation was laid out on the search, isolation and pathogenicity for natural incidence of entomopathogenic micro-organisms in the Gangetic Alluvial zone of West Bengal as the naturally occurring entomopathogens have a potential role to play in management of insect pests. In this connection, it should be mentioned that farmers continue to resort to insecticidal use for checking pest incidence in their fields without being aware of either the natural microbial control taking place or impact of chemicals on the natural bio-agents.

MATERIALS AND METHODS

Site of investigation :

A rapid roving survey were made to search for natural incidence with records for entomopathogens in Gangetic alluvial tracts of West Bengal during the year 2003-2006 and their isolation and pathogenicity were performed in research laboratory of AICRP on Nematology under Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia. The suspected disease infected insects of any stage were collected in a sterilized glass vial and initially the dead one was separated by its external

appearance of disease symptoms following the key described by Weiser and Briggs (1971).

Isolation of micro-organisms from infected insects :

A few disease infected larvae or adults collected from field during the survey and some laboratory cultured growth medium for pathogens were used for isolation of the micro-organisms associated with the collected dead insects.

Bacterial pathogen :

The suspected bacterial infected insects were surface sterilized by dipping in 0.1 mercuric chloride solution and then washed 3-4 times in sterile distilled water. The specimen was then opened or crushed with sterile scissors or scalpel by cutting integument along a longitudinal dorsal or lateral line. Body fluid or crushed material diluted in 2-4 ml sterile distilled water and then placed on suitable bacterial medium by the streak plate method. The agar plates or slant incubated at 25 ± 2^0C for 4-5 days and then examined for well isolated colonies. Nutrient agar medium was used for the isolation of the bacteria which was purified by single colony isolation techniques (Anonymous, 1957).

Fungal pathogen :

The suspected fungus infected insects were used for isolation of the fungus by following the same methods as mentioned above by using only PDA medium. Fungi were purified by single spore isolation techniques (Thom and Raper, 1951).

Viral pathogen :

The suspected virus infected dead larvae were collected in sterilized distilled water and allowed to putrefy for 7 days. The putrefied material was filtered through double layer muslin cloth and centrifuged for 1 min. at 500 rpm. Discarded the sediments which contained only tissue debris and the supernatant containing polyhedral inclusion bodies (PIB's) of the nuclear polyhedrosis virus (NPV) of the respective insect. The supernatant was centrifuged at 3000 rpm for 5-7 minutes. The sediments of PIB's were resuspended in sterile distilled water and differential centrifugation was repeated to collect pure PIBs as stock suspension and used for pathogenicity test.

Pathogenicity test of the isolated bacteria :

The isolated bacteria from infected insects were used through dipping of food materials in water suspension of the bacterial slant culture. Fresh tender marketable okra fruits and cabbage leaves were used for okra fruit and shoot borer (*Earias vitella*) and *Pieris brassicae,* respectively, as food material. In another treatment, tube culture isolated from dead insects was smeared on food materials and then they were fed by respective insect larvae.

Pathogenicity test of the isolated virus :

Pathogenicity of the respected isolated viruses from *Spodoptera litura* and *Spilarctia obliqua* was tested against the second instars larvae of respective insects simply by smearing isolated virus on leaf surface. For *Heliothis armigera*, cut surface of unripe tomato fruit was smeared with isolated virus. After that, virus treated food materials are subjected to feeding for 24 hours with three replications and ten insects in each replication. After 24 hours they were transferred to fresh food materials. Observations were taken on mortality at different hours of treatment.

Pathogenicity test of the isolated fungus :

For confirming pathogenicity of isolated fungus from *S. obliqua* on the same insect, 10 second instars larvae were dipped in and leaves were smeared with tube slant cultured isolated fungal suspension. For confirming pathogenicity of isolated fungus from mango leaf hopper on the same insect, mango leaf branch was smeared with isolated fungal suspension and it was kept with mango hopper in a plastic container. The observations on symptoms and mortality were taken on three replications of each.

Growth medium used :

To study the characteristics of entomopathogenic micro-organisms following media were used :

Nutrient –agar (NA) medium:

Peptone	5.0 g
Beef extract	3.0 g
Agar/agar	20.0 g
Distilled water	1000.00 ml

For preparation of NA, required quantity of ingredients were taken in a flask and were dissolved in desired volume of distilled water in hot water bath. The medium thus prepared was poured in culture tubes @ 5 ml/tube and plugged with non-absorbent cotton. The tubes with medium were then sterilized at 121^0C under 15 lbs pressure per sq. inch for 15 minutes. For slant preparation the medium was solidified by keeping the culture tubes at slanting position.

Potato dextrose agar (PDA) medium :

Peeled potato	200.0 g
Dextrose	20.0 g
Agar-agar	20.0 g
Distilled water	1000.00 ml

Requisite quantity of peeled potato was thoroughly washed and cut into pieces and boiled in 500 ml of distilled water and then decoction was filtered out. Dextrose was mixed with the extract to make the volume upto 1 litre. The medium was poured in culture tubes @ 5 ml/tube and plugged with non-absorbent cotton. The tubes with medium were then

sterilized at 121^{0}C under 15 lbs pressure per sq. inch for 15 minutes. For slant preparation the medium was solidified by keeping the culture tubes at slanting position.

Cleaning and sterilization of glass wares and other articles:

Before use all the glass wares like Petridishes, pipettes, culture tubes etc., were washed with tap water thoroughly. After proper drying the glasswares were wrapped with brown paper and sterilized at 160^0 C for 2 h in a hot air oven. The media which were prepared were also sterilized in autoclave.

Maintenance and storage of culture :

The bacterial culture were grown on NA slants and stored in refrigerator and subcultured at every 30 days interval. The transferred culture after incubation at 30^0 C ± 1^0 C for 48 h were kept in the refrigerator at 5^0 C ± 1^0 C for storage for different periods.

For manouevering of microorganisms and their successful introduction in the control of insect pests, regular survey and surveillance of naturally occurring insect pathogen is a first required need. It should be followed by isolation, identification and their evaluation under laboratory condition which is off keen interest as it is done in the present studies.

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussions have been presented under following sub heads:

Survey on the natural occurrence of microbial diseases :

During the course of investigation, a large number of larval and adult stages of important insect pests of different agricultural and horticultural crops were sampled to record the natural occurrence of entomopathogenic micro-organisms. They belonged to the 3 groups of pathogens *viz.,* bacteria, fungus and virus (Table 1). The detailed descriptions of the symptomology on the based on which the identification have been made are given below:

Viral diseases :

Two dull coloured infected larvae of Bihar hairy caterpillar, *Spilarctia obliqua* (Wlk.) hanging down the head downward from jute leaves were collected from the field. Four larvae of *Spodoptera litura* and three larvae of *Helicoverpa armigera* with the same symptoms were collected through field survey from cabbage and tomato crop, respectively. These larvae were suspected as virosed and subjected to isolation process for inclusion bodies.

Table 1: Natural occurrence of microbial diseases in some important insect pests in Gangetic alluvial region during 2003-2006

Host insect	Host plant of insects	No. of diseased insect collected	Suspected disease causing organism	Period of collection
Spodoptera litura	Cabbage	4	Virus	January,2006
Helicoverpa armigera	Tomato	3	Virus	February, 2005
Spilarctia obliqua	Jute	2	Virus	June, 2005
Spilarctia obliqua	Jute	12	Fungus (white mascardine)	July, 2004
Amritodus atkinsoni	Mango	28	Fungus(white mascardine)	April, 2005
Earias vitella	Okra	4	Bacteria	June, 2005
Pieris brassicae	Cabbage	6	Bacteria	February, 2006

Table 2: Salient features and pathogenicity of isolated micro-organisms

Host insect	Type of micro organism	Name of isolates	Salient features	Pathogenicity (%)
Spodoptera litura	Virus	V_1	Polyhedral inclusion bodies with Brownian movement	83.33
Helicoverpa armigera	Virus	V_2	Polyhedral inclusion bodies with Brownian movement	91.67
Spilarctia obliqua	Virus	V_3	Polyhedral inclusion bodies with Brownian movement	56.67
Spilarctia obliqua	Fungus	F_1	White mycelial mat grows on PDA medium	33.67
Amritodus atkinsoni	Fungus	F_2	Yellowish to whitish mycelial growth on insect body and White mycelial tuft mat grows on PDA medium	76.67
Earias vitella	Bacteria	B_1	Dry rough colony, Gram + ve,	53.33
Earias vitella	Bacteria	B_2	Smooth silmy colony, Gram –ve,	0.00
Pieris brassicae	Bacteria	B_3	Dry rough colony, Gram + ve,	26.67
Pieris brassicae	Bacteria	B_4	Dry smooth colony, Gram –ve,	0.00

Bacterial diseases :

Eight disease infected larvae of *Earias vitella* (Fig. 1) and six infected larvae of *Pieris brassicae* (Fig. 3) were collected from the field of okra and cabbage, respectively grown in the experimental field. These were sluggish in nature, blackish in colour, soft and shriveled.

Fungal diseases :

Larvae of *Spilarctia obliqua* were collected from jute field with shrinken, mummified body covered with white mycelial mat and fungal spore (Table 1 and Fig. 2). A few of them died showing the typical symptom of fungal infection in laboratory culture. Few fungal infected nymphs and adults of mango leaf hopper were collected from mango plant. Their bodies were covered with white colour cottony mycelial growth (Fig. 4).

Isolation of viral pathogen and its pathogenicity on respective insects :

Three viral isolates *i.e.* V_1, V_2 and V_3 were obtained from virus infected larvae of *Spodoptera litura, Helicoverpa armigera* and *Spilarctia obliqua,* respectively. The isolated virus showed polyhedral inclusion bodies. These isolates were tested against the larvae of respective insect species. The treated larvae showed various symptoms like sluggishness, loss of appetite and starvation after 3-4 days of exposure to these isolates. In advanced stage, they became flaccid, white fluid exuded from the ruptured skin and larvae died. In all the cases, few larvae died showing the classical symptom of hanging heads downwards with posterior prolegs fixed with top of the jar.

Pathogenicity of the viral isolates (V_1, V_2 and V_3) from respective insect hosts caused 83.33, 91.67 and 56.67 per cent mortality for *S. litura, H. armigera* and *S. obliqua,* respectively. Characterizations of pathogenic microbes isolated during the course of investigation were also attempted. It revealed that the entire three isolated virus belonged to polyhedrosis type (Table 2).

Fig 1: Bacteria infected *Earias vitella*

Fig 3: Bacteria infected *Pieris brassicae*

Fig 2: Fungus infected *Spilarctia obliqua*

Fig 4 : Bacteria infected *Amritodus atkinsonii*

Isolation of fungal pathogen and its pathogenicity on respective insects:

White mascardine fungus was isolated from both of infected *Spilarctia obliqua* and *Amritodus atkinsoni*. After isolation (Fig. 6), in pathogenicity test of these funguses on the same insects resulted 33.67 and 76.67 per cent mortality for *Spilarctia obliqua* and *Amritodus atkinsoni,* respectively (Table 2). This particular fungus was identified as *Beauveria bassiana* for both the insect pests.

Isolation of bacterial pathogens and its pathogenicity on respective insects:

Two different types of bacterial colony were isolated from disease infected larvae of *Earias vitella* (Fig. 5). Pathogenicity test of these isolates revealed only one isolate having 53.33 per cent mortality. But, in case of *Pieris brassicae* among two isolates, only one caused 26.67 per cent mortality (Table 2). The isolates for both the insects those caused more or less pathogenicity after isolation belonged to single genus *Bacillus*. So, these isolated entomopathogens could be studied in future for mass culture and field efficacy.

Fig 5: Isolation of bacteria from *Earias vitella*

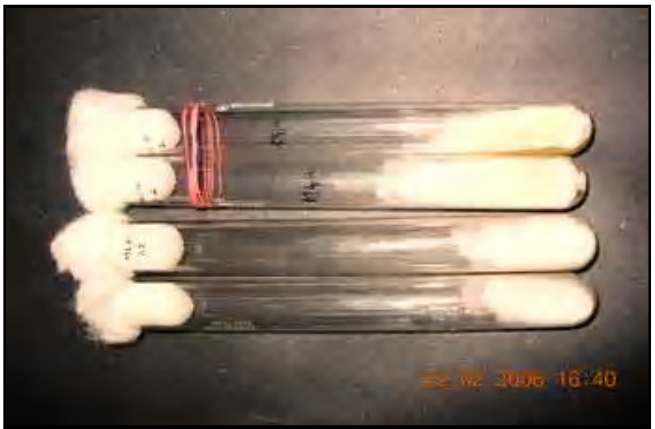

Fig 6: Isolation of fungus from *Amritodus atkinsonii*

In recent years, maximum attention has been paid to explore the potentiality of disease causing organisms of major pests' species. Unfortunately, the Eastern India is lacking far behind in the field of research of microbial control of insect pests. A perusal of available literature reveals that large number of microbial insecticides are being used worldwide to combat serious pests like *Spodoptera litura*, *Helicoverpa armigera, Plutella xylostella, Oryctes rhinoceros, Spilosoma obliqua, mango mealy bugs,* and so on. The main contributions have been made by Steinhaus (1957, 1960), Aizawa (1963) and Ignoffo *et al*. (1965). Ramkrishna and his group (1969), Jacob and Thomas (1972), Narayanan (1985) have paved the way to utilize NPV in successful control of *S. litura* and *H. armigera* under Indian situation. In spite of these, very little attention has been paid in this direction in eastern India.

A little attention has been made by Pramanik (1995) who identified fungus, bacteria, virus and nematode as naturally occurring insect pathogen and reported natural occurrence of viral diseases of insects were much more pronounced than other organisms as found in *S. litura* and S. *obliqua* in West Bengal . In the present investigation, three groups of disease causing organisms of insect pests belonging to fungus, viruses and bacteria were also identified.

REFERENCES

Aizawa, K.A. (1963). The nature of the infections caused by nuclear polyhedrosis viruses. In E. A. Steinhaus (ed.) *Insects Pathology. An advanced treatise*. Vol. **I**. Academic Press. NEW YORK, U.S.A.

Anonymous (1957). Manual of microbiological methods. *Mc Graw Hill book Co. Inc.* NEW YORK, U.S.A. 315 pp.

Battu, G.S. (1982). Studies on the baculovirus–A nuclear polyhedrosis virus of *Diacrisia obliqua* (Wlk*).* Ph. D. Thesis, IARI. NEW DELHI, INDIA.

Battu, G.S., Bindra, O.S. and Rangarajan, M. (1971). Investigations on microbial infections of insect pests in the Punjab. *Indian J. Entomol.,* **33** (3): 317- 325.

Dhaliwal, G.S. and Arora, R. (2000). *Principles of Insect pest Management.* Kalyani Pub., Ludhina (PUNJAB) INDIA. pp. 128 – 141.

Dutky, S.R. (1959). Insect microbiology. *Adv. Appl. Microbiol.*, **1**: 175 – 200.

Easwaramoorthy, S. and Santhalakshi, G. (1989). Occurrence of *Fusarium subglutinans* on certain sugarcane pests. *J. Biological Control,* **3**(2): 132.

Hall, I.M. (1957). Use of polyhedrosis virus to control the looper on lettuce in California. *J. Economic Entomol.,* **50**: 551-553.

Ignoffo, C.M., Chapman, A.J. and Martin, D.F. (1965). The nuclear polyhedrosis virus of *Heliothis zea* (Boddie) and *Heliothis virescens* (Fabr.). III Effectiveness of virus against field population of *Heliothis* on cotton, corn and grain sorghum. *J. Invertebrate Pathol.,* **7**: 227-235.

Jacob, A. and Thomas, M.J. (1972). A nuclear polyhedrosis virus of *Diacrisia obliqua* (Wlk.) (Arctidae: Lepidoptera). *Agric. Res. J. Kerala*, **10**(2): 182.

Kamat, M.N., Patel, M.K. and Dhande, G.N. (1952). Occurrence of green muscardine fungus on *Pyrilla* in Bombay. *Curr. Sci.,* **21** (11): 317.

Kumar, P.S., Singh, S.P. and Gopal, T.S. (2001). Natural incidence of *Hirsutella thompsonii* Fisher on the coconut eriphyid mite, *Aceria guerreronis* Keifer in certain districts of Karnataka and Tamil Nadu in India. *J.Biological Control,* **15**(2): 151-156.

Majumder, S.K., Mathu, M. and Pingle, S.V. (1956). Bacterial Control of insects. I-Studies on the field control of Lablab pod boring catterpiller. *Indian J. Entomol.,* **18**(4): 377-407.

Misra, AP. (1952). *Aspergillus flavus* L., a parasite of the desert locust (*Schistocerca gregaria*). *Curr. Sci.*, **21**(8): 225.

Narayana, K. (1985). Control of *Heliothis armigera* through nuclear polyhedrosis virus. In: *Microbial control and pest management* pp. 76-86. Jayraj, S. (Ed). Arul Jothi Printers, Coimbatore (T.N.) INDIA.

Narayanan, K. and Veenakumari, K. (2003). Nuclear polyhedrosis viruses from coconut blackheaded catterpiller, *Opisina arenosella* Walker and sorghum spotted stem borer, *Chilo partellus* (Swinhoe). *J. Biological Control*, **17**(1): 97-98.

Narayanan, K., Jayraj, S. and Subramanian, T.R. (1973). Control of diamond back moth caterpillar, *Plutella xylostella* (L.) and the green peach aphid, *Myzus persicae* (Sulz.) with insecticides and *Bacillus thuringiensis* Berliner. *Madras Agric. J.*, **62**(8): 498-503.

Nirula, K.K., Radha, K., Menon, K.P.V. (1955). Mucardine disease of *Oryctes rhinoceros* L. *Indian Coconut J.*, **9**: 3-10.

Oblisami, G., Ramoorthi, K. and Rangaswami, G. (1969). Studies on the pathology of some crop pests of South India. *Mysore J. Agric. Sci.,* **3** (1): 86-98.

Padmavathamma, K. and Veeresh, G.K. (1989). Record of nuclear polyhedrosis virus of cabbage diamond back moth, *Plutella xylostella* (Linnaeus). J. *Biological Control*, **3**(2): 130-131.

Pandey, S.N. and Rangarajan, M. (1967). *Prodenia litura* F., a new host of *Serratia marcescens* Bizio. *Indian J. Entomol.,* **29** (1): 14-17.

Pandit, N.C., Som, D. and Chatterjee, S.M. (1979). *Beauveria bassiana* (Bals.) Vuile as a pathogen of *Nisotra orbiculata* Mots. infesting mesta, *Hibiscus cannabinus. J.Entomol. Res.,***3**(1):111-113.

Patnaik, N.C.and Das, M.S. (1969). A new record of *Mermis* sp. (Ascardoidea, Mermithidae) as a larval parasite on *Antigastra catalaunalis* D. (Lepidoptera : Pyralidae). All India Nematolog Symposium, p. 33.

Pawar, V.M. and Ramakrishnan, N. (1971). Investigations on the nuclear polyhedrosis of *Prodenia litura* Fabricus- I. Nature of the polyhedral disease. *Indian J.Entomol.*, **33** (2): 111-122.

Pramanik, A. (1995). Studies on micro-organisms pathogenic to some insect pests of agriculture importance. Ph.D. Thesis, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, W.B. (INDIA).

Prasad, J. and Kushwaha, K.S. (1990). Role of pathogenic microbes in management of the tobacco caterpillar, *Spodopera litura* (F.) in cabbage and cauliflower crops. *Indian J.Entomol.*, **52**(4): 641-652.

Rajamanickam, K.,Raveendran, T.S. and Kennedy, F.J.S. (1989). Natural occurrence of baculovirus disease in *Oryctes rhinoceros* (I.) population in Tamil Nadu. *J.Biological Control,* **3**(2): 93-95.

Ramakrishnan, N. and Tiwari, L.D. (1969). Polyhedrosis of *Prodenia litura* Fabr. (Noctuidae: Lepidoptera). *Indian J. Entomol.*, **31**(2): 191-192.

Rangaswami, G., Ramamurthi, K. and Oblisami, G. (1970). Studies on microbiology and pathology of insect pests of crop plants, USDA PL 480 Research Project (2963 – 69). Final Rep. University Agricultural Sciences, Hebbal, Bengaluru (KARNATAKA) INDIA. pp. 94.

Rao, G.N. and Vijayalakshmi, V. (1959). A note on the occurrence of certain parasitic fungi on insect pests of sugarcane. *Curr. Sci.*, **28**: 297.

Singaravelu, B., Nirmala R. and Easwaramoorthy, S. (1999). A new record of sugarcane top borer, *Scirpophaga excerptalis* Walker (Lepidoptera: Pyralidae). *J. Biological Control,* **13**: 133-135.

Srinivasa, R.M., Raman, G.V., Srimannarayana, G. and Venkatesmarlu, B. (1999). Efficacy of botanicals against Gram Pod borer *Helicoverpa armigera* Hub. *Pestology,* **23** (1): 18.

Srivastava, R.P. and Masarrat, F. (1988). Natural occurrence of *Beauveria bassiana* an entomogenous fungus on mealybug *Drosicha mangiferae*. *Indian J.Plant Pathol.,* **6** (1): 8-10.

Steinhaus, E.A. (1949). Principles of Insect Pathology. *Mc. Graw Hill Book Cp. Inc.* NEW YORK,. 757 pp.

Steinhaus, E.A. (1957). Concerning the harmlessness of insect pathogens and the standardization of microbial control products. *J. Economic Entomol.*, **50**(6): 715-720.

Steinhaus, E.A. (1960). Notes on polyhedrosis in *Peridroma, Prodenia, Colias, Heliothis* and other Lepidoptera. *J. Insect Pathol.,* **2**: 327-333.

Thom, C. and Raper, K.B. (1951). A manual of the Aspergilli. The Williams and Wilkins Co., Baltimore, 373 pp.

Weiser, J. and Briggs, J.D. (1971). Identification of pathogens. In: *Microbial control of insect and mites* (Eds.) Burges, H. D. and Hussey, N.W., Academic Press, 13-16 pp.

****