http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Role of Entomopathogens/Nematodes in Insect Pest Management

Mohammad Irshad
Integrated Pest Management Institute,
National Agricultural Research Centre Islamabad, Pakistan

Abstract

Insect damages the agricultural crops enormously. Heavy reliance has been placed on pesticidal control with the result th ecosystems have been disturbed. Entomopathogens whether occurring in nature or manufactured can play significant r in pest management. In Pakistan bacteria, fungi, viruses and nematodes have been recorded from some important pe in different ecologies. Similarly microbial pesticides have also been tried with beneficial results.

Introduction

Reduction of insect populations is essential for higher yields of agricultural commodities because these agents cause extensive damage, Traditionally emphasis had been laid on insecticides to achieve these objectives but due to their known adverse effects on the environment, now, lesser use of chemical pesticides is recommended. The extension entomologist tries to manage the insect populations in such a way that environment is least disturbed with economic benefits. In this context, biotic agents fit in the whole scheme. Use of insect parasites and arthropod predators has been practiced. The microbes have been less exploited although they are capable of killing the insect pests and thus reducing their population. Introduction of an insect pathogen into an insect population can bring about more or less permanent control, if the economic level of the host density is higher than the threshold level of the disease (Steinhaus, 1954). Microbial control was defined by Falcon (1971) as "including all aspects of the utilization of microorganisms or their by-products in the control of insect species". Insect diseases are often important natural regulators of insect populations (Maddox, 1982). More than 1500 insect pathogens (bacteria, fungi, rickettsia, viruses, nematodes and protozoans) are known.

Pathogens

Bacteria

Non sporing: As long as these bacteria are in the midgut of the host, they are relatively harmless but when they travel into the insect blood then they are deadly. Less effort has been made to utilize them due to their little invasive power.

Spore forming: These belong to genus *Bacillus*. of these, *B. papillae* infects beetles and is transmitted orally by ingestion. In the host, these spores germinate and penetrate into the alimentary canal. Vegetative rods multiply rapidly and sporulation occurs. After 7-12 days 2-5 billion spores/larva are formed. Larval blood appears milky and they die. Spores of this species cannot be produced on artificial media. *B. thuringiensis* (BT) during sporulation produces crystals of toxic protein. When they multiply in host body the host is paralysed and dies within few hours to 5 days. There are 14 stereotypes of *B. thuringiensis*. In Pakistan, infections caused by *B. cereus* were detect in bollworm larvae collected from bolls on the soil and stacked cotton sticks at Multan in negligible number a from flowers of *Althea rosea* at Khanewal (percen Mortality of the pink bollworm caused by bacteria duri the non-cotton season (Feb-May) was 2.1-47.8 per ce (CIBC, 1975). About per cent larvae of *Pieris brassic* were also killed by *B.thuringiensis*. at Rawalpindi duri 1981. Same bacillus disease was also detected in per c larvae at Peshawar in April and per cent at Hairpur in M Infections occurred mostly in older larvae. Mortality w higher in the crowded conditions. In the laboratory 1, 8 a 16 days old larvae died in 6-7, 2-5 and 1-2 da respectively when fed on the cauliflower leaves spray with suspension of infected larvae in sterile water (CI 1986).

Shaikh (1982) collected different strains of BT from lar of *Bombyx mori* and *Cydia pomonella* from various parts Pakistan and compared 31 indigenous strains with US standard strains (HD-241, HD-244, HD-263 and HD-1 1980) on *Heliothis armigera*. Indigenous strains provi mortality of 1-22.2 per cent Khalique *et al.* (1982) studied effect of endotoxin on *H. armigera* and its fecun was affected. According to Shakoori *et al.* (1991) heptachlor treated *B. thrungiensis* spores and untreated not produce any mortality in the larvae of fly *Zaprin indiana*.

Viruses

More than 700 viruses have been isolated from insects mites in the world. These belong to baculovirid reoviridae, poxviridae, iridoviridae, parvovirid picornavirindae and rhabdoviridae.

Nuclear polyhedrosis virus (NPV): Of all the reported vir from insects (lepidoptera, hymenoptera and diptera), 4 belong to this group. NPV viruses are rod sha measuring 20-50 nm in diameter and 200-400 nm in len These are transmitted by oral ingestion. These enter the midgut and replicate. Mortality of host is caused in 4 days. Some of the viruses are host specific (Ignoffo, 19

A polyhedral virus was recorded as an important mortality factor of willow sawfly (*Nematis melanspis*) in Himalayan forests of Pakistan. Diseased larvae were collected in small numbers (0.4%) in June while infection increased in July (15 %) and was maximum in August. The viral infected larvae which formed cocoons could not develop to adult stage and died. Mortality in cocoon was 1.1. in June, 13.6 in July and 43.6 per cent in August (Ghani and Cheema, 1973). NPV infected larvae of *Gilpinia pindrowi* (pine sawfly) became softer and whitish when dead and hung from needles. In Murree this disease caused 74.1, 48.4, 30.6, 9.6 and 7 per cent mortality in first, second, third, fourth and final instar larvae respectively showing early instars to be more susceptible (Ghani and Cheema, 1973). Disease decreased by end of September. At Murree 11 per cent cocoon of the first, 22.6 per cent of second, 28 per cent of third and 30 per cent of the fourth generation died while in Kashmir there was 18.2 per cent mortality in first, 38.7 per cent in third 9.3 per cent in the fourth generation due to same disease. Polyhedral virus also killed 3.1, 2.8, 3.4 per cent larvae of *Gilipina polytoma* in Kaghan, Kashmir and Swat respectively (Ghani and Cheema, 1973). NPV caused mortality of 0.4 per cent in June, 15 per cent in July and 35 per cent in August during 1977 in *Nematus melanaspis* larvae in Murree hills. (CIBC, 1982). The diseased bodies of *Cynosarga chrysolopha* larvae by NPV shranked, compressed and yellowish brown. It caused mortality of 54 and 85 per cent in Kashmir and Murree respectively. In artificial contamination disease started 21 days afterwards.

Granulosis Viruses (GV)

These have only been recorded from lepidoptera. GV particles range from 36-80 nm in width and 245-411 nm in length. These are transmitted orally and through egg. Mortality of host occurs between 4-25 days. In the later stages of infection larva develop a light color.

Granulosis infected larvae of *Dioryctria abietella* in Pakistan became sluggish. The body contents were liquified into brownish fluid which oozed out from the ruptured skin. Dead infected larvae were cut open and kept in distil water for 15-20 days (23 ± 1 °C). After polyhedral settlement the supernatant fluid containing larval debris was decanted. Laboratory spray on healthy larvae showed mortality after 2-3 days. The disease was more prevalent in rainy season (Ghani and Cheema, 1973).

Fungi

More than 750 fungal species are infesting insects (Maddox, 1982). About 60 species have an intermediate crustacean host. Genera like *Beauveria, Nomuraea* and *Metarhizium* are transmitted from host to host by conidia. Infection mostly occurs through body wall or cuticle. Fungus enter into hemocoel and grows until the insect is filled with mycelia and insect dies. In the sub coastal area of Pakistan, *Aspergillus* sp. attacked 42.8-50 per cent *Centrococcus insolitus* (Pseudococcidae) (Ghani and Muzaffar, 1974). Two fungi *Beauveria bassiana* and *Aspergillus ochraceus* attacked prepupae and pupue of *G. polytoma*. Its incidence was highest in Kashmir (55.5 per cent) and 35.5 per cent in Kaghan and 22.5 per cent in Swat. *Paeciliomyces fairinosus* destroyed 50 per cent cocoon of *Gilipinia indica* (Ghani and Cheema, 1973). *B. basiana* was recorded from *Mythimna separata* Wlk. (CIBC, 1986a). Some mortality of eonymphs of *Nematis melanspis* also occurred due to fungus infection (CIBC, 1982). Three fungi *Aspergillus flavus, A. fumigatus* and *Scepluriceis* sp. were recorded from different Pentatamids. It could not be transmitted from one bug to another in the laboratory (Cheema, *et al.*, 1973).

Khalil *et al.* (1985) found that spore germination of *Verticillium lecanii* was little effected by benomyl, cypermethrin, fentrothion oxide, formothion, mevinphos, copper oxychloride, oxyamyl, permethrin, primicarb and ethiophanate-methyl. Khalil *et al.* (1990) also found that entomopathogenic fungi *V. lecanii* was effective against *Myzus persicae* at 104, 105 and 107 spores/ml. Its 50 per cent conidial spores were viable upto 6 months at 2-10 °C and for 3 month at 20-25 °C.

Protozoa

Most of the entomopathogenic protozoan belongs to microsporida. More than 250 species have been described. They are commonly transmitted orally. These are obligate parasites and do not complete development outside a living host cell. *Streptococcus* sp. was detected from pink bollworm larvae collected from bolls on the soil and on stacked cotton sticks at Multan and from flowers of *Althea rosea* at Khanewal (CIBC 1975).

Nematodes

Facultative parasitic nematodes can parasitize healthy insects but can reproduce outside the host. Obligate nematodes do not have free living stages. The largest insect parasitic nematode family is mermithidae containing about 50 genera and 200 species. After penetrating the host body they remain in the hemocoele for 10 days to several months. The host usually dies before exist of the parasite. *Mermis* sp. nov. was reared parasitizing 1.6-50 per cent adults of 10 species of grasshoppers in various parts of Pakistan (CIBC 1982a). Its attack was comparatively higher in monsoon period in rice habitat. Adults survived in soil for a month but reinfestation of grasshopper was not achieved. Nematodes commonly emerged from membranous thoracic part of grasshopper. If the host was parasitized by one nematode the host could live but when more than one nematodes were present the grasshopper died.It could not be artificially reared. *Mermis nigriscense* parasitized 1-10 per cent grasshoppers in hilly and semi hilly areas. Grasshoppers get infection by feeding on nematode spores which were present on the host plant. Development of immature stages takes place inside the host body (Irshad, 1977 and CIBC, 1982a).

Hexamermis sp. parasitsed 0.3-66.6 per cent larvae of *Chilo partellus, Heliothis* sp. in hilly areas (CIBC, 1982a).

Neoplectana sp. was only reared from *Heliothis* sp. from Swat (CIBC, 1982a). Mermithids were also reared from *Adria parvula* (2-15%), *Acrosternum granarium* (0.4%), *Carpocoris purpurepennis* (1%), *Dolycoris indicus* (0.8%), *Euroderma festivum* (0.7%), *Eysarcoris guttiger* (5.0%) and *Eurygaster integriceps* (0.2%). They were active during the monsoon period during heavy rain of 1967 (Cheema, *et al.*, 1973).

Two species of nematodes *Delandenus siricidicola* and *D. wilsoni* were recovered from wood wasp (*Sirex imperialis*) on *Abies pindrow* (Ghani and Cheema, 1973). *B. thuringiensis* is produced commercially by a fermentation process. Living hosts are used for the production of large quantities of virus. Adoption of most appropriate unit for measurement of insecticidal potency of a pathogenic preparation is standardization. For BT. it is expressed in units per milligram. Various registered pesticides are given below.

Pathogens	Basic use pattern	Date registered
Bacillus popilliae	Japanese beetle larvae	20.9.48
B. lentimorbus	on turf	
B. thuringiensis	Many crops and sites	26.9.61
Heliothis nuclear polyhedrosis	Cotton bollworm and tobacco budworm on cotton	02.12.75
Douglas fur tussock moth nuclear polyhedrosis	Douglas fur tussock moth	11.8.76
Gypsy moth nuclear poly hedrosis	Gypsy moth on forest shade and ornamental trees	13.4.78
Nasema locustae	Grasshoppers	09.5.80

Modified from Maddox, 1982

Toxin of *Bacillus* was first isolated in 1902 but only in 1956 paraspirial bodies were recognized in killing insect larvae. The interest in microbial insecticides has been created by large scale production of bacterial insecticides like dipel, thuricide, bactospeine,. vectobac, tecnar, bactimos, baturin 82 and myco insecticides like mycotal, vertalex.

In Pakistan bactospeine 500 international unit granules were tested in potted plants of maize against *Chilo partellus*. Immature larvae (1-4 days old) were more susceptible (82-56 per cent mortality). This could be effective control tool for maize borer (Irshad, 1978). Sevin, mitac and bactospeine gave almost identical control of sugarcane borer *Acigonia steniellus* (Irshad *et al*, 1982) in the field trials. *Chilo infuscatellus* and *Tryporyza nivella* the sugarcane borers were controlled by bactospeine in Mardan. Mean Joint infestation was 7, 10 and 17 per cent in the plots treated with granules, wettable powder of bacospeine and control respectively (Irshad *et al.*, 1992).

Alsystin and dimilin @ 0.016 and 0.002 per cent gave 100 per cent mortality of *Euproctis lunata* on *Acacia* within 20 days and bactospeine @ 8,6,4 mg/liter covered 100, 90 an 80 per cent larval mortality respectively in 20 days (Rahman and Chaudhry, 1987).

One application of *Agrotis segtum* virus (ASGV) did no reduce cutworm damage on tobacco seedlings and potat plant while two application reduced cutworm damage b 64-82 per cent on tobacco, 85 per cent on okra, 77 pe cent in potato and 78 per cent on sugarbeet. Thre treatments were not more effective than two. (Zethner *al.* 1987). It was as effective as chemical pesticides lik tamaron, methamiodophos, dieldrin and microbial *Bacill thuringiensis*. Standard USDA strain 1+D-1-5-/980 *thrungiensis* was significantly more effective tha bactospeine 16000 i.u./mg F.C. 8500/i.u/mg an bactospeine F.C. 7500 i.u./mg. against *Diacrisia obliq* after 3 and 4 days of exposure (Ahmad *et al.*, 198 Kalique *et al.* (1989) found that LC_{50} of bactospeine w 63.5 and 177.6 1mu/ml to Ist and 3rd instar larvae *Heliocorpa armigera* at 26°C and 65-80 per cent relati humidity. LC_{50} was 56.1 and 126.4 1mu/ml by *Bacill thuringensis* standard strain (HDI, 1980).

Discussion

Bacillus compounds when applied are not capable to susta in the field and have to be applied repeatedly. These a selective and do not harm the beneficial organisms. *Bacill* is only effective against lepidoptera and diptera larva Pathogenic fungi can control aphids, whiteflies and thrip The principal advantages in using microbial insecticides a safety and host specificity. Baculovirus and *B. thuringen* have secured comparatively higher attention as they do n effect warm blooded animals. Microbial agents someti are host specific and do not disturb the biotic systems a outbreak of pests does not take place. According Thomas *et al.* (1996) the residue of fungal entomopatho remained infective for several days and grasshop population was significantly reduced in treated plots. T microbial control of invertbrate pests is reviewed a strategies for using insect pathogen is listed by Riba a Ferron (1987).

Microbial control of insects has a big potential in reduc the pest populations. Maddox, 1982). In some natu habitats of Pakistan like forest ecology or vegeta ecosystem where lepidopterous pests are problems th agents are playing significant role. It is quite an open f and the collaborative work of entomologists and ins pathologists is required. After a detailed survey potential agents may be picked up and exploited redistribution or augmentation. These agents may not able to give economic control as a single component they can be integrated with other control measures. T they can become an essential tool of Integrated appro This type of control is compatible with other co methods.

Now comparatively more pathogens are available commercial preparations are used in the USA, Austr Russia and Western Europe. Pakistan must also contri more in this researchable area. Formulation plants for manufacture of bacterial insecticides needs to be undertaken. The higher use of microbial pesticides will release pressure on synthetic pesticides.

References

Ahmad, K., N. U. Raja, M. Afzal, F. Khalique and B. A. Malik, 1988. Bio-efficacy of four strains of *Bacillus thuringiensis* Berlin. against hairy caterpillar *Diacrisia obliqua* Wlk. Pak. J. Sci. Ind. Res., 31: 637-639.

Cheema, M. A., M. Irshad, M. Murtaza and M. A. Ghani, 1973. Pentatomids associated with graminae and their natural enemies in Pakistan. Tech. Bull. Commonw. Inst. Biol. Control, 16:47-67.

CIBC, 1975. Search in Pakistan for parasites, predators and pathogens of pink bollworm *Pectinophora gossypiella* and their introduction into infested areas of the USA. Final report, Commonwealth Institute of Biological Control (CIBC) Rawalpindi, pp: 19.

CIBC, 1982. Investigations on primary mortality-causing agents affecting sawfly population in high altitude forests in Pakistan. Final Report, CIBC, pp: 113.

CIBC, 1982a. Investigations on nematodes parasitic on insect pests. Annual Report, CIBC, pp: 21.

CIBC, 1986. Investigations on the natural enemies of selected lepidopterous pests of crucifers and feasibility studies of mass rearing and releases of promising species for the control of crucifers. Annual report, CIBC. pp: 93.

CIBC, 1986a. Survey and evaluation of effectiveness of the parasites, predators and pathogens of cutworms affecting fruits, vegetables, field crops and grasses. Final report CIBC, pp: 103.

Falcon, L. A. 1971. Microbial control as a tool in integrated control programs. *In*. C. B. Huffaker *ed*. Biological control, Plenum Press, Newyork, pp: 346-364.

Ghani, M. A. and M. A. Cheema, 1973. Biology and ecology of principal natural enemies of major insect pests of forest trees in Pakistan. Misc. Publ., No. 4, pp:100.

Ghani, M. A. and N. Muzaffar, 1974. Relations between the parasite predator complex and the host plants of scale insects in Pakistan. Misc. Publ., No. 5. CIBC pp: 92.

Ignoffo, C. M., 1968. Specificity of insect viruses. Bull. Entomol. Soc. Am., 14:265-276.

Irshad, M., 1977. Notes on the occurrence of nematodes as internal parasites of grasshoppers in Pakistan. Agric. Pakistan, 28: 285-287.

Irshad, M., 1978. Efficacy of bactospeine a microbial pesticide against maize borer (*Chilo partellus* Swinh.) (Lepidoptera: Pyralidae). Biologia, 24: 53-57.

Irshad, M., S. Mirza and Rahtullah, 1992. Control of the sugar cane borer *Chilo infuscatellus* and *Tryporyzae nivella* by bactospeine a microbial pesticide. Pertanika, 5: 263-264.

Irshad, M., I. Shah and M. N. Beg, 1982. Chemical control of the gurdaspur borer *Acigona steniellus* (Hamps). (Lepidoptera : Pyralidae) in Pakistan. Intern. Pest Control, 4: 108.

Khalil, S. K., Farmanullah and M. Naeem, 1990. Studies on the entomopathogenic fungus *Verticillium lecanii* (Zimm.) for the control of green peach aphid *Myzus persicae* Sulz. Sarhad J. Agric, 6:597-600.

Khalil, S. K., M. A. Shah and M. Naeem. 1985. Laboratory studies on the compatibility of entomopathogenic fungus *Verticillium lecanii* with certain pesticides. Agriculture Ecosystems and Environment, 13:329-334.

Khalique. F., K. Ahmad and M. Afzal, 1989. Evaluation of *Bacillus thuringiensis* Berliner against chickpea pod borer. Pak. J. Sci. Ind. Res., 32:114-116.

Khalique, F., A. Khalique, A. K. Farid, S. Rafi and D. Shaikh, 1982. Effect of endotoxin of *Bacillus thuringensis* on the fecundity and longevity of bollworm *Heliothis armigera* (Hubn.). Pak. J. Sci. Ind. Res., 25:180-183.

Maddox, J. V., 1982. Use of insect pathogens in Pest Management *In*: Metcalf R. L. and Luckmann *ed*: Introduction to insect pest management, W. H. John Wiley & Sons, pp: 175-215.

Rahman, W.U. and M.I. Chaudhry, 1987. Efficacy of alsystin, dimilin and bactospeine against babul defoliator, *Euproctis lunata* Walk. Pak. J. Zool., 19: 307-311.

Riba, G., P. Ferron, 1987. {Prospects for the utilization of pathogens of invertebrates}. *In* Integrated Pest Management Protection integree, quo vades *ed*. Delluchi, V., pp: 193-215.

Shaikh, M.R., 1982. Investigations on new strains of *Bacillus thuringensis* (BT) affecting lepidopterous pests. Final report, Department of Microbiology, University of Karachi, pp: 102.

Shakoori, R. A., A. Iftikhar and S. Majeed, 1991. Effect of heptachlor, MNNG and thioactemide on the growth of *Bacillus thuringiensis* Berliner, strain HD-1 (Kurshakl.) and its toxicity to dipterous fly *Zaprionus indiana*. Proc. 11th Cong. Zool., 11: 57-73.

Steinhaus, E. A., 1954. The effect of diseases on insect populations. Hilgardia, 23: 197-261.

Thomas, M. R., J., Langewald, J. and S. N. Wood, 1996. Evaluating the affects of a biopesticide on populations of the varigated grasshopper, *Zonocerrus variegatus*. J. App. Ecol. 33:1509-1516.

Zethner, O., B. M. Khan, M.I. Chaudhry, B. Bolet, S. Khan, H. Khan, H. Gul, L. Ogaard, M., Zaman and G., Nawaz, 1987. *Agrotis segetum granulosis* virus as a control agent against field populations of *Agrotis ipsilon*, *A. segetium* (Lep. Noctuidae) on tobacco, okra, potato and sugarbeet in northern Pakistan. Entomophaga, 32: 409-415.