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Integrating biopesticides in pest management strategies for tropical vegetable production

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ABSTRACT

Vegetables, cultivated on 4.65 million ha with annual production of 53.5 million t in South and Southeast Asia, are subject to severe yield losses from insect pests and diseases in the tropics. Chemical pesticides account for one-third to one-half of the total mean material input cost for vegetable production in the region. Extensive and inappropriate pesticide use has led to pests developing resistance to major groups of pesticides, resurgence of secondary pests, high pesticide residues in produce, and decimation of natural enemies. The adverse effects on human and environmental health cannot be ignored. Integrated pest management (IPM) strategies often have been suggested to mitigate such a problem. Although various IPM strategies have been developed and promoted for vegetables, adoption remains low due to IPM's limited effectiveness in managing insect pests compared with chemical pesticides. Moreover, IPM has been promoted as a combination of techniques without giving due consideration to the compatibility of each component. Biopesticides could play a crucial role in IPM strategies although they cover only about 4 percent of the global pesticide market. Biopesticides have high compatibility with other pest management techniques such as natural enemies, resistant varieties, etc. Integrating biopesticide could enhance performance of IPM strategies. For instance, with the adoption of *Bacillus thuringiensis* based biopesticides, parasitoids such as *Diadegma semiclausum, Cotesia plutellae* and *Diadromus collaris* established in several countries, and provided significant control of diamondback moth (*Plutella xylostella*) on brassicas in South- and Southeast Asia. An IPM strategy based on sex pheromone for managing the eggplant fruit and shoot borer (*Leucinodes orbonalis*) has reduced pesticide abuse and enhanced the activities of natural enemies including *Trathala flavoorbitalis* in Indo-Gangetic plains of South Asia. Thus, this paper reviews some of the most effective vegetable IPM strategies developed and/or promoted by AVRDC – The World Vegetable Center to manage insect pests on brassicas, eggplant, vegetable legumes and tomato in tropical Asia, and presents a discussion of an appropriate public – private partnership model in dissemination and adoption of vegetable IPM strategies.

Key words: Biopesticides, *Leucinodes orbonalis*, *Maruca vitrata*, *Phyllotreta striolata*, *Plutella xylostella*, vegetables

INTRODUCTION

Vegetables are high-value crops and the use of chemical pesticides is intensive due to severe yield losses by insect pests and diseases under tropical conditions in South Asia and Southeast Asia. A survey of pesticide use in Bangladesh indicated farmers sprayed up to 180 times with chemical insecticides during a year to protect their eggplant crop against the eggplant fruit and shoot borer, *Leucinodes orbonalis* (SUSVEG-Asia, 2007). Pesticide use is equally intensive in the Philippines, where spraying occurred about 56 times during a cropping season and the total quantity of pesticide used per hectare was about 41 l of different brands belonging to the four major pesticide groups (Gapud and Canapi, 1994; Orden *et al*., 1994). Pesticide application often exceeded 50 sprays per tomato crop season in south India (Nagaraju *et al*., 2002). The share of chemical pesticides can be very high in the total material input cost for certain vegetables. For instance, it was 55 percent for eggplant and ranked first when compared to tomato (31%) and cabbage (49%) in the Philippines (Orden *et al*., 1994). It was 40-50 percent in eggplant in Bangladesh (SUSVEG-Asia, 2007) and 38 percent in vegetable brassicas in parts of India (Shetty, 2004). Indiscriminate pesticide use is detrimental to the environment and human health and increases insects' resistance to pesticides. Alternative pest management strategies are warranted to reduce the misuse of chemical pesticides in vegetables. Despite several constraints, biopesticides are being used in vegetable production systems. This paper reviews some successful cases, most of which were conducted through the research and development efforts of AVRDC– The World Vegetable Center and its partners.

DEFINITION OF BIOPESTICIDES

According to the United States Environmental Protection Agency (EPA), biopesticides are pesticides derived from natural materials, such as animals, plants, bacteria, and certain minerals (www.epa.gov). The EPA places biopesticides into three major classes: microbial pesticides, plant incorporated protectants (PIPs) and biochemical pesticides. Microbial pesticides consist of a microorganism as the active ingredient; all the entomopathogenic bacteria, fungi, and viruses are under this group. PIPs refer to transgenic plant materials; they are not reviewed in this paper. Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. These include sex pheromones and scented plant extracts that attract pests. However, it is not clear from the EPA classification whether biochemical pesticides also include plant-derived (botanical) pesticides. For the purposes of this paper, botanical pesticides have been grouped as a separate class.

MICROBIAL PESTICIDES

Bacteria

Microbial pesticides based on the soil-borne bacterium *Bacillus thuringiensis* (Bt) are among the most widely used groups of biopesticides. Formulations based on Bt subsp. *kurstaki* and Bt subsp. *aizawai* have been found to be effective against several lepidopteran pests either alone or in combination with other biopesticides or biocontrol agents on vegetables.

One of the most successful examples of microbial biopesticide use is in the management of diamondback moth (*Plutella xylostella*) in tropical Asia and Africa. Diamondback moth is the most destructive insect pest on vegetable brassicas in the world, sometimes causing more than 90 percent crop losses (Iqbal *et al.*, 1996). Pesticides have been the predominant control method for several decades (Syed, 1992), although efforts to introduce biocontrol agents also have a long history. One of the earliest parasitoid introductions occurred in Indonesia in 1928 (Eveleens and Vermeulen, 1976). A similar effort was made in 1936, when *Diadegma semiclausum*, an ichneumonid larval parasitoid, was introduced from England into New Zealand (Talekar and Shelton, 1993). However, *D. semiclausum* became effective only when it was introduced from New Zealand into Indonesia in the early 1950s. Due to the intensive use of chemical pesticides on vegetable brassicas the beneficial effect of this parasitoid was not realized in tropical Asia until the mid-1980s. With the adoption of *B. thuringiensis*, *D. semiclausum* established in several countries and exerted more than 70 percent parasitism on diamondback moth (Talekar and Shelton, 1993).

AVRDC took the lead in integrated pest management (IPM) for diamondback moth in Asia. The Center implemented a brassica IPM program under the Asian Vegetable Network (AVNET) from 1989-1992. It introduced parasitoids such as *D. semiclausum*, *Cotesia plutellae*, *Diadromus collaris*, and *Trichogrammatoidea bactrae* in Indonesia, Malaysia, the Philippines, and Thailand. The biopesticide *B. thuringiensis* complemented the action of these parasitoids. Participating farmers from collaborating countries adopted IPM, resulting in a significant reduction in pesticide use that drastically reduced the cost of production and enhanced environmental health (AVRDC, 1993). After IPM adoption, insecticide application was reduced by 51 percent in Indonesia, 86 percent in Malaysia's highlands, and 61 percent in the Philippines. The spraying cost was reduced by 57 percent in the lowlands of Malaysia and 23 percent in Thailand. Apart from this initial adoption in the pilot project sites, no large-scale studies have been conducted to quantify actual adoption on the ground. Sivapragasam (2001) reported the results of a cursory survey conducted among IPM personnel in the pilot countries on the current level of adoption of brassica IPM by growers, which indicated a range of adoption between 50 to 100 percent. The current level of adoption increased by 20-30 percent from the initial adoption rate in Malaysia, Philippines, and Thailand; however, it decreased by at least 20 percent in Indonesia. It is imperative to quantify the actual adoption of IPM by growers to compare with the perception of the implementers.

Secondary lepidopterans including the cabbage head caterpillar (*Crocidolomia binotalis*) and the cabbage web worm (*Hellula undalis*) also are serious pests causing significant damage on vegetable brassicas (Smyth *et al.*, 2003). Unlike the diamondback moth, they do not have any effective biocontrol agents. However, *B. thuringiensis*-based biopesticides are an effective tool against secondary lepidopterans. For instance, the cabbage head caterpillar is quite susceptible to most of the Cry1A toxins such as Cry1Aa, Cry1Ab, and Cry1Ac (Srinivasan and Hsu, 2008) and to Bt subsp. *kurstaki*- based formulations, in which the Cry1A toxins are the major components (Ooi, 1980; Sastrosiswojo and Setiawati, 1992; Malathi and Sriramulu, 2000; Ravikumar *et al.*, 2010). Cabbage web worm is more susceptible to Cry1Ca toxin and to Bt subsp. *aizawai*-based formulations, in which Cry1Ca is a major component (Srinivasan and Hsu, 2008). Secondary lepidopterans on vegetable brassicas can be controlled by at least one of the *B. thuringiensis* formulations.

The use of Bt for this purpose is necessary to sustain biological control of diamondback moth, because the parasitoids of diamondback moth will be eliminated if growers resort to chemical pesticides targeting secondary lepidopterans on vegetable brassicas. For example, diamondback moth outbreaks occurred due to the mortality of *D. semiclausum* when insecticides were used to control cabbage head caterpillar in Indonesia (Shepard and Schellhorn, 1997). It can be concluded that *B. thuringiensis*-based biopesticides and parasitoids of diamondback moth act synergistically to suppress major lepidopteran pests on vegetable brassicas in tropical Asia.

Legume pod borer (*Maruca vitrata*) is a serious production constraint on vegetable and grain legumes in tropical Asia, sub-Saharan Africa, and Latin America. A concerted effort is in progress to develop sustainable management strategies for this pest, and *B. thuringiensis* is one of the components evaluated. Legume pod borer was found to be highly susceptible to Cry1Ab and Cry1Ca in Taiwan and West Africa (Machuka, 2002; Srinivasan, 2008). In addition, it was also susceptible to both Bt subsp. *aizawai* and Bt subsp. *kurstaki* based formulations (AVRDC, 1996; 1997). A network has been formed to develop an IPM strategy based on combinations of *B. thuringiensis* with other biopesticides to mitigate the legume pod borer menace on food legumes (Srinivasan *et al.*, 2007).

Viruses

Entomopathogenic viruses, especially nucleopolyhedrovirus (NPV) and granulovirus (GV), also are known to be effective against various insect pests on vegetables. *Helicoverpa armigera* NPV (HaNPV), *Spodoptera litura* NPV (SlNPV), and *S. exigua* NPV (SeNPV) already have been commercialized and are widely used against tomato fruit worm (*Helicoverpa armigera*), common army worm (*Spodoptera litura*) and beet army worm (*S. exigua*), respectively (Vinod Kumari and Singh, 2009).

AVRDC – The World Vegetable Center recently has identified a NPV that infects legume pod borer in Taiwan. This is the world's first recorded instance of a NPV specifically infecting legume pod borer. It was characterized based on ultra-structural morphology, restriction endonuclease (REN) cleavage patterns, and sequences of the coding region of the polyhedrin (*Polh*) gene, and named MaviMNPV (Srinivasan *et al.*, 2005). Electron microscopic studies on the ultra-structure of MaviMNPV occlusion bodies showed several virions with multiple nucleocapsids packaged within a single viral envelope. The complete sequence of the MaviMNPV *Polh* gene contained 735 nucleotides and the genome size of MaviMNPV estimated with restriction enzymes was 113.41±1.50 kbp (Lee *et al.*, 2007). The complete genome of MaviMNPV was sequenced and it was found to be 111,953 bp in length (Chen *et al.*, 2008). According to Chen *et al.* (2008), the gene content and order of MaviMNPV have the highest similarity to *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and all of its open reading frames (ORFs) have homologues in the AcMNPV genome except for one, which seems to be a mini-AcMNPV possessing a smaller genome with fewer auxiliary genes than the AcMNPV type species. However, the phylogenetic analysis of the whole genome revealed that MaviMNPV was separated from the common ancestor of AcMNPV and *Bombyx mori* NPV (BmNPV) before they diverged from each other. Thus, MaviNPV is a distinct species of the group I lepidopteran NPV.

Laboratory bioassays revealed that the first instar legume pod borer larvae were the most susceptible stage to MaviMNPV and the median lethal concentrations (LC50s) increased with increasing larval instars, like other lepidopterans (Srinivasan *et al.*, 2005). Formulations of this NPV have been found to be effective against legume pod borer on food legumes either alone or in combination with *B. thuringiensis* and neem under laboratory and field conditions in Taiwan and Benin (Tamo *et al.,* 2007; Srinivasan *et al.*, 2008). In addition, it was found that the braconid parasitoid of legume pod borer, *Apanteles taragamae,* was able to transmit MaviMNPV. When monitoring the establishment of *A. taragamae* close to the original parasitoid release site, International Institute of Tropical Agriculture (IITA) scientists came across a few legume pod borer larvae with apparent symptoms of MaviMNPV infection. This site was more than 150 km away from the location where caged experiments with the virus were conducted, and MaviMNPV never has been found or released in the open field in Benin. This made researchers suspicious about whether *A. taragamae* would be involved in the transmission of the virus, as known from literature for other *Apanteles* species (Raimo *et al.*, 1977). A series of experiments were conducted to verify this assumption and the preliminary results have supported the plausible synergism between MaviMNPV and *A. taragamae* (Srinivasan *et al*., 2009).

Fungi

Entomopathogenic fungi play a vital role in managing the insect pests in humid tropics, *Beauveria bassiana* and *Metarhizium anisopliae* constitute about 68 percent of the entomopathogenic fungi based microbial pesticides (Faria and Wraight, 2007). Masuda (1998) demonstrated that *B. bassiana* caused mortality of over 80 percent in diamondback moth at 76 percent RH or higher, but only 30 percent mortality at 52 percent RH. It was found that the optimal temperature for mycelial growth of *B. bassiana* is 24°C and the sub-optimal temperature is 24±4°C (Tsai *et al.*, 2006). However, Bugeme *et al.* (2009) documented that isolates of *B. bassiana* and *M. anisopliae* caused 71 to 100 percent mortality of *Tetranychus urticae* between 25–35°C. Hence, temperature and humidity are important factors determining the effectiveness of entomopathogenic fungi.

Several reports have confirmed the effectiveness of entomopathogenic fungi against various pests on vegetables. For instance, some of the entomopathogenic fungi isolates were known to possess ovicidal and larvicidal effects against legume pod borer (Ekesi *et al.*, 2002); larvicidal effects against diamondback moth on cabbage (AVRDC, 1999; James *et al.*, 2007) larvicidal effects against web worms (*Hymenia recurvalis* and *Psara basalis*) on amaranth (James *et al.*, 2007) and pupicidal effects against tomato fruit worm (AVRDC, 1992). About 19 different isolates of *M. anisopliae* collected throughout Taiwan were evaluated against the diamondback moth, and five were found to be most effective. However, isolates of *Fusarium* sp. and *Paecilomyces* sp. were found to be less effective than *M. anisopliae* (AVRDC, 1999). Extensive research has been carried out at IITA, Benin on the effectiveness of entomopathogenic fungi against diamondback moth. The water formulation of *B. bassiana* at 1 kg conidial powder per hectare caused 94 percent mortality of diamondback moth and performed better than the emulsion formulation (Godonou *et al.*, 2009). Shelton *et al.* (1998) also found that *B. bassiana* was as effective as *B. thuringiensis* in controlling diamondback moth, and it persisted for more than two weeks after a single application. However, they reported the ineffectiveness of *M. anisopliae*. *B. bassiana* was found to cause about 62 percent pupal mortality in tomato fruit worm when the fungus was applied to the soil. The soil application seemed more appropriate because the fungus persists in the soil and tomato fruit worm pupates in the soil. Application of fungal suspension to plants to control tomato fruit worm may not be practical because most of the larval period is spent concealed inside tomato fruit (AVRDC, 1992), unlike diamondback moth, which spend the entire larval and pupal periods on plant surfaces.

Additive effects were found on the mortality of diamondback moth when entomopathogenic fungi were combined with the parasitoid, *Oomyzus sokolowskii* (Santos Junior *et al.*, 2006a). However, the parasitism was reduced when the diamondback moth was treated with entomopathogenic fungi 24 h before the exposure to the parasitoid. The entomopathogenic fungi caused 9-21 percent confirmed mortality of the parasitoid and *S. litura* (Santos Junior *et al.*, 2006b; Sanehdeep Kaur *et al.*, 2011).

Oil-based formulations of *B. bassiana* and *M. anisopliae* reduced the population density of spider mites significantly under laboratory and greenhouse conditions at the International Centre of Insect Physiology and Ecology (*icipe*) in Kenya (Wekesa *et al.*, 2005; Wekesa *et al.*, 2006). The fungal pathogens were toxic, and also reduced the fecundity and egg viability in red spider mite, *T. evansi*. In addition, a Brazilian strain of the predatory mite *Phytoseiulus longipes* and the pathogenic fungus *Neozygites floridana* recently have shown promising results against spider mites in laboratory experiments (Furtado *et al.*, 2007; Wekesa *et al.*, 2007). Although *N. floridana* and *N. tanajoae* have been reported to be non-pathogenic to some predatory mites and several non-target natural enemies (Moraes and Delalibera 1992; Hountondji *et al.*, 2002), Furtado *et al.* (1996) reported that the fungus *N. acaricida* is pathogenic to the predatory mite, *Euseius citrifolius*. The effect of *N. floridana* was assessed on predation and oviposition of *P. longipes*, which was fed on *N. floridana*-infected *T. evansi* and *T. urticae*. No *N. floridana* hyphal bodies were found in *P. longipes*, demonstrating that *N. floridana* is not pathogenic to *P. longipes* and did not affect its oviposition (Wekesa *et al.*, 2007). Thus, it was proven that the entomopathogenic fungi did not affect the predatory mites, and that the fungi could be used synergistically in managing spider mites. AVRDC is organizing a collaborative research study with *icipe* to determine the efficiency of individual and combined effects of entomopathogenic fungi and predatory mites in reducing the damage of red spider mite on tomato under field conditions in Kenya. Although extensive research has been done on entomopathogenic fungi, they have not been widely commercialized compared with *B. thuringiensis* and NPVs.

BOTANICAL PESTICIDES

Hundreds of native plant species have been evaluated against a range of insect pests on various crops. Botanical pesticides act as a synergistic component in several IPM strategies. Among the botanical pesticides, neem (*Azadirachta indica*) is being widely used and several formulations thus containing the active component azadirachtin are commercially available. Earlier, products with lower azadirachtin concentrations were not found to be useful under field conditions. However, several formulations with azadirachtin concentrations ranging up to 65,000 ppm recently have been developed (Kumar *et al.*, 2003; Anis Joseph *et al.*, 2010). There is evidence available for the synergistic action of neem with microbial pesticides such as NPVs of tomato fruit worm (Senthilkumar *et al.*, 2008) and common army worm (Nathan and Kalaivani, 2006), and entomopathogenic fungi (*B. bassiana*) against common army worm (Mohan *et al.*, 2007). AVRDC has developed IPM strategies for tomato and vegetable soybean involving neem as an integral component with microbial pesticides such *B. thuringiensis* and NPVs in managing phytophagous insects (Srinivasan *et al.*, 2009).

In addition to neem, China berry (*Melia azedarach*) also is being used extensively, as it has several limonoids similar to neem. When extracts from *M. azedarach* were sprayed to control diamondback moth in cabbage, they enhanced the attraction of the parasitoid, *C. plutellae*. Further analysis showed that the extracts of *M. azedarach* induced the emission of cabbage volatiles, which in turn attracted the parasitoids. This is the first example of a plant extract inducing the emission of plant volatiles in another plant, which in turn attracted natural enemies (Charleston *et al.*, 2006). The extracts of *M. azedarach* not only controlled the diamondback moth, but also enhanced the activity of the parasitoid against diamondback moth. Similar synergistic activities should be explored for other species. Although the potential of various plant species in pest management has been demonstrated, the plants have not been exploited commercially. Developing a greater range of commercial botanical pesticides will enhance IPM options.

BIOCHEMICAL PESTICIDES

Sex pheromones

Insect sex pheromones are biochemical pesticides and have long been used as monitoring and mass-trapping tools in IPM strategies. Several sex pheromone lures including insects like tomato fruit worm, common army worm, beet army worm, legume pod borer and cucumber moth (*Diaphania indica*) are commercially available.

AVRDC has developed and promoted an IPM strategy based on sex pheromones for managing eggplant fruit and shoot borer in South Asia (Alam *et al.*, 2003; Alam *et al.*, 2006). The adoption of eggplant fruit and shoot borer IPM strategy led to a 70 percent reduction in pesticide use in Bangladesh (Alam *et al.*, 2006). This IPM strategy reduced pesticide abuse in eggplant production systems and enhanced the activities of natural enemies. *Trathala flavoorbitalis* has been reported to be an effective parasitoid of eggplant fruit and shoot borer and is present in India (Naresh *et al*., 1986) and Bangladesh (Alam and Sana, 1964). However, its contribution to pest control rarely was documented and does not appear to be significant. The initial results from Bangladesh have shown that the parasitism was only about 10 percent. However, the mean level of parasitism increased approximately three-fold after one year of eggplant cultivation without pesticide spraying. The parasitism rate during the intensive production period was considerably higher (39.3-48.9%). If this level of parasitism can be sustained over larger areas throughout the year, it would reduce the pest population on a sustainable basis, thus reducing the need for pesticide use in combating eggplant fruit and shoot borer (Alam *et al.*, 2003).

A synthetic sex pheromone for legume pod borer consisting of (E,E)-10,12-hexadecadienal, (E,E)-10,12-hexadecadienol, and (E)-10-hexadecenal (Downham *et al.*, 2003, 2004) was developed and attracted male moths in Benin and Ghana, while (E,E)-10,12-hexadecadienal alone was most effective in Burkina Faso (Downham, 2006). Neither pheromone was effective in Southeast Asia or Taiwan (AVRDC unpublished results), while a variant blend was effective in India (Hassan, 2007). This geographical variation in the blend of legume pod borer is an obstacle to the implementation of trap-based monitoring of the pest in some important subsistence legume crop regions of the world. A network has been formed to refine the sex pheromones of legume pod borer and develop an IPM strategy based on these pheromones (Srinivasan *et al.*, 2007).

Aggregation pheromones

Attempts are underway to develop an IPM strategy based on aggregation pheromones for managing the striped flea beetle (*Phyllotreta striolata*) on vegetable brassicas. Actively feeding striped flea beetle males produce an aggregation pheromone. Previously, a sesquiterpene was identified as male aggregation pheromone in the congeneric species *P. cruciferae* (Soroka *et al.*, 2005). Seven male-specific sesquiterpenes have been identified from the aggregation pheromone of striped flea beetle. However, the active male-specific compound was identified as (+)-(6R,7S)-himachala-9,11-diene. Under laboratory conditions, the activity of this synthetic pheromone either alone or in combination with the host plant volatile, allyl isothiocyanate attracted significantly high numbers of *P. striolata* (Beran *et al.*, 2011).

Plant volatiles

Certain secondary metabolites in plants act as deterrents for generalist feeders, and attractants for specialist feeders. For instance, glucosinolates and their metabolites act as attractants and stimulants for specialist brassica feeders such as flea beetles (*Phyllotreta* spp.) (Chew, 1988; Louda and Mole, 1991). The mustard oil allyl isothiocyanate (AITC), a glucosinolate breakdown product, is attractive to *P. cruciferae* in the field (Soroka *et al.*, 2005). In field experiments at AVRDC, we have shown that AITC at a dose of 0.8 ml per trap could significantly increase trapping of striped flea beetle (Beran *et al.*, 2008). However, the attraction of striped flea beetle may be higher when the AITC is combined with the aggregation pheromones, because combinations of the aggregation pheromone and AITC generally attracted greater numbers of *P. cruciferae* than did either component itself (Soroka *et al*., 2005). The synthesis of aggregation pheromones of striped flea beetle is in progress, and its effects in combination with AITC will be evaluated soon under field conditions for their attraction to striped flea beetle.

PROBLEMS AND PROSPECTS

Although biopesticides increasingly are being used as alternative pest management strategies, several constraints such as developing stable formulations, standardizing appropriate delivery methods, lack of registration procedures, etc. are associated with their introduction and promotion in most of the developing world.

Improving stability would enhance the performance of biopesticides under field conditions. In the case of microbial pesticides, the formulations should maintain the viability of the spores. For example, the insolubility and poor stability of the active constituent azadirachtin in water have limited the use of neem as a safe and effective insecticide for systemic application (Shivashankar *et al.*, 2000; Vasantharaj David, 2008). Even with the most common 'emulsifiable concentrate' formulations, the active ingredient of the neem pesticide is not stable in water. Stable formulations such as suspension concentrate, oil in water emulsion, microcapsules, and water dispersible granules should be considered (Hong, 2006). Thermo- and photo-stability are critical issues associated with microbial pesticides. For instance, rapid inactivation of viral particles in NPVs by sunlight or ultraviolet radiation has been reported in several cases. However, when substances like optical brighteners were included as UV protectants for entomopathogens during formulation, their effectiveness was enhanced. The enhanced infectivity due to optical brighteners increased larval mortality (reduced LD_{50} values) and acted rapidly (reduced LT_{50} values) (Monobrullah, 2003). In several myco-pesticides, oil-based formulations were found to be more effective because they enable fungal pathogens to remain active under conditions of low humidity (Bateman and Alves, 2000).

Developing appropriate delivery methods is very important to assure effectiveness of biopesticides under field conditions. Unlike chemical pesticides, in which the chemical is dissolved in a solvent, most microbial pesticides are particulate suspensions. Problems with suspensions include settling of the microbial pesticide, nozzle blockage unless the aperture size is carefully selected, stress affecting the viability of spores, inappropriate droplet size, suboptimal numbers of infective spores packed to a droplet etc. (Bateman *et al*., 2007).

More research is required to optimize the delivery systems for each group of biopesticide. For instance, production of smaller droplets would enhance the effectiveness of microbial pesticides (Bateman and Alves, 2000), whereas larger droplets are likely to be needed for entomopathogenic nematodes (Bateman *et al*., 2007). Stable, effective formulations and appropriate delivery systems are needed to convince growers to adopt biopesticides. However, the slow progress in research on formulation and delivery systems is a major issue in promoting biopesticides use (Boyetchko *et al*., 1999).

The registration procedure for biopesticides is absent or in the development stage in several developing countries in Asia. For instance, a project entitled, "Commercialization of biopesticides in Southeast Asia" recently has developed guidelines for registration of microbials and pheromones in Southeast Asia and officially submitted it to the Association of Southeast Asian Nations (ASEAN) in March 2009 (http://www.biopesticides-seasia.net/). Based on the experiences of AVRDC, public–private partnerships can help overcome constraints to biopesticide registration and commercialization. Two different models were adopted by the Center in India and Bangladesh for the commercialization and promotion of eggplant fruit and shoot borer sex pheromones.

An IPM strategy to control eggplant fruit and shoot borer with minimal use of pesticides was developed during a UK Department for International Development (DFID)-funded project in Bangladesh, India, and Sri Lanka from 2000 to 2003. During the second phase of the project, from October 2003 through January 2006, the IPM strategy was implemented on farmers' fields via pilot project demonstrations in selected areas of Bangladesh and India, and its use was promoted in both countries (Srinivasan, 2008). Project activities included working with small and medium enterprises in both countries to encourage commercialization of sex pheromone. Companies involved in commercializing the product began production only after the utility of sex pheromone in combating eggplant fruit and shoot borer was demonstrated in the early years of the first phase of this project. There are as many as nine small and medium enterprises in India currently selling sex pheromone of eggplant fruit and shoot borer throughout the country (Alam *et al*., 2006). This is largely due to the Central Insecticides Board (CIB), which has greatly helped to spread and encourage the use of biopesticides in India. The CIB simplified the registration system to allow commercial pilot production in parallel with registration. This is particularly encouraging to small and medium enterprises (RIU, 2008).

However, Bangladesh has no such registration procedure. When Bangladesh Minister for Agriculture, Mr. M.K. Anwar, attended a farmers' field day on June 5, 2005 at Kazura Bazar, Jessore, the farmers engaged in an open dialogue with the Minister and lobbied for speedy registration of eggplant fruit and shoot borer sex pheromone so that they could easily purchase and use the pheromone whenever needed. Bangladesh's Pesticide Registration Law (1980) does not cover microbial pesticides and sex pheromones. The Bangladesh Agricultural Research Institute provided all technical data and requested to register the pheromone with the Department of Agricultural Extension (DAE), the government body that handles registration of all pest control agents. In late 2005, the Bangladesh parliament passed a law to facilitate registration and use of sex pheromone and other similar chemicals for pest control in the country (Alam *et al*., 2006). A team was constituted to revise the law to accommodate microbial pesticides and sex pheromones and the revisions are ready for inclusion; the law will soon be amended in parliament. However, the Bangladesh Ministry of Agriculture (BARI) intends to continue the promotion of eggplant fruit and shoot borer IPM and to ensure the supply of sex pheromones for the eggplant growers due to the potential for reducing pesticide use on eggplant. BARI was collaborating with three private companies in Bangladesh to produce the eggplant fruit and shoot borer pheromone lures and supplied them to the growers through DAE. However, the registration system for bio-pesticides in Bangladesh has been opened from July 2010 and several companies were aiming to get registration not only for the sex pheromones but also for different other biopesticides (Dr. Syed Nurul Alam, Personal Communication).

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