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Biopesticide based sustainable pest management for safer production of vegetable legumes and brassicas in Asia and Africa

Ramasamy Srinivasan,[a*] Subramanian Sevgan,[b] Sunday Ekesi[b] and Manuele Tamò[c]

Abstract

Vegetables are one of the important crops which could alleviate poverty and malnutrition among the smallholder farmers in tropical Asia and Africa. However, a plethora of pests limit the productivity of these crops, leading to economic losses. Vegetable producers overwhelmingly rely on chemical pesticides in order to reduce pest-caused economic losses. However, over-reliance on chemical pesticides poses serious threats to human and environmental health. Hence, biopesticides offer a viable alternative to chemical pesticides in sustainable pest management programs. Baculoviruses such as nucleopolyhedrovirus (NPV) and granulovirus (GV) have been exploited as successful biological pesticides in agriculture, horticulture and forestry. *Maruca vitrata* multiple nucleocapsid NPV (MaviMNPV) was found to be a unique baculovirus specifically infecting pod borer on food legumes, and it has been successfully developed as a biopesticide in Asia and Africa. Entomopathogenic fungi also offer sustainable pest management options. Several strains of *Metarhizium anisopliae* and *Beauveria bassiana* have been tested and developed as biopesticides in Asia and Africa. This review specifically focuses on the discovery and development of entomopathogenic virus and fungi-based biopesticides against major pests of vegetable legumes and brassicas in Asia and Africa.

Keywords: vegetable legumes; brassicas; MaviMNPV; *Metarhizium anisopliae*; *Beauveria bassiana*

1 INTRODUCTION

Vegetables are high value crops with potential to offer higher economic returns per unit area. For instance, the average value of yard-long bean sales is US$ 4400/ha per cropping cycle in Lao PDR, while the value of Chinese kale in Cambodia is US$ 6900/ha.[1–3] Hence, high value vegetables, which are repeat-cycle crops could lift small-scale farmers out of poverty. Vegetable legumes and brassicas constitute an important place in the diet and livelihood of a majority of the population in tropical Asia and sub-Saharan Africa. Yard-long bean (*Vigna unguiculata sesquipedalis*) is one of the most popular leguminous vegetables in Asia. For example, it occupies 7% of the total vegetable production area in Southeast Asia.[4] Other food legumes are being cultivated in an area of 35 million ha in South and Southeast Asia.[5] Cowpea (*V. unguiculata*) and bean (*Phaseolus* spp.) are the two most important food and vegetable legumes grown in Africa, occupying a total of about 19 million ha.[5] Brassica vegetables are being cultivated in an area of 1.30 million ha in South and Southeast Asia as well as Africa.[5] Hence, their contribution to overall livelihoods is quite significant.

Pests are one of the major limiting factors, which reduce the productivity of leguminous and brassica vegetables in tropical Asia and Africa. Aphid [*Aphis craccivora* Koch (Aphididae: Hemiptera)], thrips [*Megalurothrips usitatus* Bagnall, *M. sjostedti* Trybom and *Frankliniella occidentalis* (Thripidae: Thysanoptera)], pod borers [*Maruca vitrata* Fab. (Crambidae: Lepidoptera); *Helicoverpa armigera* Hübner (Noctuidae: Lepidoptera)] are some of the major insect pests of vegetable and grain legumes in tropical Asia and sub-Saharan Africa.[6–11] *Maruca vitrata* can alone cause yield losses between 20% and 80%.[7,12] Diamondback moth [*Plutella xylostella* L. (Plutellidae: Lepidoptera)] is the predominant pest of brassicas worldwide, although *Spodoptera litura* F. (Noctuidae: Lepidoptera), *Pieris rapae* L. (Pieridae: Lepidoptera), *Crocidolomia pavonana* F. (Crambidae: Lepidoptera), *Hellula undalis* Guenée (Crambidae: Lepidoptera), *Brevicoryne brassicae* Linnaeus (Aphididae: Hemiptera), *Thrips tabaci Lindeman* and *Phyllotreta striolata* F. (Chrysomelidae: Coleoptera) cause marketable yield losses of up to 100% in brassica vegetables.[10,13–16]

In their attempt to produce blemish-free vegetables, which are preferred by consumers in the market, vegetable growers in low income countries almost exclusively rely on chemical pesticides. A recent study in Southeast Asia showed that synthetic pesticides were used by all vegetable farmers sampled in Vietnam and by 96% in Cambodia.[10] The study also found that quantity of formulated pesticides used per hectare per week and the associated expenditures were much higher in Vietnam than in Cambodia and Laos. Surprisingly, farmers in Laos sprayed more frequently than in the

* *Correspondence to: R Srinivasan, World Vegetable Center, Shanhua, Tainan, Taiwan. E-mail: srini.ramasamy@worldveg.org*

a *World Vegetable Center, Tainan, Taiwan*

b *International Centre of Insect Physiology and Ecology, Nairobi, Kenya*

c *International Institute of Tropical Agriculture, Cotonou, Benin*

other countries. An earlier study in Bangladesh showed that farmers use insecticides intensively, sometimes more than once a week during the growing season, against pests in legume crops,[17] and often still do not achieve satisfactory pest control.[18] About 45% of the cabbage growers use synthetic insecticides in controlling insect pests, including diamondback moth in Ghana.[19] Although average quantities of pesticides are not necessarily greater in Africa than in high income countries, environmental and human health risks tend to be much higher. Average pesticide use per hectare, for instance, in Africa is only 1.23 kg a.i./ha, compared with 7.17 and 3.12 kg for Latin America and Asia, respectively.[20] Inefficient pesticide use practices include inappropriate choice of products, incorrect dosages and improper timing of application, which have consequences on human and environmental health.[21,22] Hence, alternative pest management strategies are warranted to reduce the pesticide risks as well as to enhance sustainable production of safer vegetables. Microbial biopesticides especially those based on entomopathogenic fungi, bacteria and viruses offer a great scope to reduce the use of hazardous chemical pesticides in tropical vegetable production. In this review, we specifically focus on the discovery and development of entomopathogenic virus and fungi-based biopesticides against major pests of vegetable legumes and brassicas in Asia and Africa.

2 BACULOVIRUSES IN MANAGEMENT OF LEGUME AND BRASSICA PESTS

Although viruses from 15 families are known to be entomopathogenic, those belonging to family *Baculoviridae* are mostly being used as biological pesticides.[23,24] Because of their very narrow spectrum of activity (quite often limited to only one particular insect species), they are considered safe to human and other animal species. Baculoviruses belonging to two genera: *Alphabaculovirus* (lepidopteran-specific nucleopolyhedroviruses), *Betabaculovirus* (lepidopteran-specific granuloviruses) have been exploited for management of key lepidopteran pests of legumes and brassicas. These include *Maruca vitrata* multiple nucleopolyhedrovirus (MaviMNPV) infecting cowpea pod borer, *M. vitrata*; *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HearSNPV) infecting gram pod borer, *H. armigera*; *Spodoptera litura* NPV (SpltNPV), *Spodoptera littoralis* NPV (*SpliNPV*), *Spodoptera exigua* NPV (*SeMNPV*), *Spodoptera litura* granulovirus (SpltGV) infecting pests belonging to the genus, *Spodoptera* and *Plutella xylostella* nucleopolyhedrovirus (PlxyMNPV) and *Plutella xylostella* granulovirus (PlxyGV) infecting diamondback moth, *P. xylostella* (Table 1). Apart from these, other baculoviruses with a broader host range such as *Galleria mellonella* NPV (GmNPV) have also been assessed against pest of legume and brassica crops[25] (Table 1). It should also be noted that the efficacy of NPVs are synergistically enhanced when applied with neem, for instance, as demonstrated against *H. armigera* and *S. litura*.[26,27]

2.1 Discovery, characterization and development of *Maruca vitrata* multiple nucleopolyhedrovirus (MaviMNPV) as a biopesticide

Granulovirus (GV) and cypovirus (CPV) were reported infecting *M. vitrata* larvae in Africa (Kenya, Benin) and China.[42–45] However, they were not successfully exploited in biological control because of the chronic nature of those viruses, especially CPV infections.[43] Therefore, these viruses were not considered effective in controlling borer pests such as *M. vitrata*. The high virulence of NPVs has made them most appropriate pest control candidates. Although GmNPV was found to be highly infectious to *M. vitrata*,[46] there have been no reports on NPVs isolated from *M. vitrata* until 2004. In the spring of 2004, diseased *M. vitrata* larvae, which were sluggish, pinkish, not feeding and often with a fragile or ruptured larval body were found on *Sesbania cannabina* in Taiwan. The cadaver found hanging from the tops of plants with prolegs attached to the *S. cannabina* plant indicated the typical NPV infection.[47]

Subsequent characterization of the NPV-infecting *M. vitrata* showed that the occlusion bodies (OB) of this virus had almost 20 virions with up to six nucleocapsids packaged within a single viral envelope. Hence, it was named as *M. vitrata* multiple nucleocapsid nucleopolyhedrovirus (MaviMNPV).[48] The polyhedrin gene of MaviMNPV contained 735 nucleotides, and the complete genome of MaviMNPV is about 112 Kbp.[49] Based on the gene content and order, MaviMNPV has the highest similarity to AcMNPV. However, the phylogenetic analysis showed that MaviMNPV separated from AcMNPV and *Bombyx mori* NPV (BmNPV) before they diverged from each other. Thus, MaviNPV is a distinct species of the group I lepidopteran NPV, which was the first recorded instance of an NPV specifically infecting *M. vitrata* in the world. Like other NPVs, MaviMNPV formulation was found to be highly effective against early instars of *M. vitrata*.[48] Since early instars of *M. vitrata* feed on the surface of flower buds, flowers or pods in legume flowers,[50] MaviMNPV has become an ideal component for killing the larvae before entering into these reproductive organs and thus reducing the economic losses quite significantly. When MaviMNPV formulations were evaluated for their efficacy, either alone or in combination with neem and *Bacillus thuringiensis*, the pod damage in hyacinth bean was significantly reduced by MaviMNPV formulations in Taiwan.[31]

2.2 Integrating MaviMNPV as a component in sustainable pest management strategies for food legumes production and its introduction into sub-Saharan Africa

MaviMNPV was subsequently introduced into Benin in West Africa in 2006 by International Institute of Tropical Agriculture. The evaluation results from Benin also confirmed the potential of this virus as a biopesticide for the control of *M. vitrata* on cowpea. About 88% larval mortality was obtained when MaviMNPV was used at the rate of 2×10^{13} OBs/ha.[31] A subsequent study in Benin which evaluated the efficacy of a neem–MaviMNPV mixture, demonstrated the effectiveness of these biopesticides in reducing the damage of *M. vitrata* and thus increasing the grain yield.[51] Another study in Benin also showed that combinations of MaviMNPV resulted in a significantly higher *M. vitrata* larval mortality than treatment with either virus or botanical insecticide (oil from neem, *Azadirachta indica* Juss or *Jatropha curcas* L.) alone.[28] Larvae of *M. vitrata* infected with MaviMNPV and treated with botanical oils died sooner than those infected with only one control agent. Thus, combinations of MaviMNPV and botanical oils produced additive or synergistic effects. Similarly, the combination of MaviMNPV and neem was found to reduce the *M. vitrata* damage on cowpeas in field trials in Niger during 2014–2016.[29] MaviMNPV was also found effective in reducing *M. vitrata* pod borer populations on cowpea in Nigeria during 2015–2016 trials.[30] It is interesting to note that MaviMNPV was also transmitted by one of the parasitoids of *M. vitrata*, *Apanteles taragamae* Viereck (Braconidae: Hymenoptera) in Benin.[31] We consider this quite a breakthrough because the parasitoid might be able to spread the virus to *M. vitrata* populations without any further intervention once the virus is released into any new environments.[31] Thus, MaviMNPV was found to be an effective

Table 1. Baculovirus biopesticides used in the management of legume and brassica pests

Target pest	Baculovirus	Crop	Country/ Regions	Formulation and dose	Reference(s)
Maruca vitrata	*Maruca vitrata* multiple nucleopolyhedrovirus (MaviMNPV)	Cowpea	Benin, Niger, Nigeria	Aqueous suspension (1×10^5 OB/ml)	Sokame *et al.*,[28] Abdoulaye Zakari *et al.*,[29] Muhammad *et al.*[30]
		Hyacinth bean	Taiwan	Aqueous suspension with or without UV protectant (0.375×10^9 OB/l)	Srinivasan *et al.*[31]
Helicoverpa armigera	*Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HaSNPV)	Chick pea	India	Aqueous suspension (250–450 Larval Equivalents (LE); 1.5×10^{12} OB/ha)	Singh and Ali,[32] Cherry *et al.*[33]
		Chick pea	Nepal	Liquid formulation (250 LE/ha)	Rijal *et al.*[34]
		Cotton, pepper, soybean, pigeon, tomato, pea	China		Sun and Peng,[35] Yang *et al.*[36]
Mamestra brassicae	*Mamestra brassicae* multiple nucleopolyhedrovirus	Cabbage	Japan	10^5 – 10^6 OB/mL	Goto *et al.*[37]
		Chinese Cabbage	China	$1.0–3.0 \times 10^{11}$ OB/ha	Guoxun *et al.*[38]
Spodoptera litura	*Spodoptera litura nucleopolyhedrovirus*	Cabbage	India	NPV-S (500 LE/ha)	Vinod Kumari and Singh[39]
Plutella xylostella	*Plutella xylostella granulovirus*	Cauliflower	India	1.5×10^{13} OB/ha	Subramanian *et al.*[40]
		Cabbage	South Africa	2.3×10^8 OBs/ha	Hatting *et al.*[41]

component in integrated pest management packages based on other biopesticides and natural enemies in sub-Saharan Africa.

2.3 Successful local production and future prospects for MaviMNPV in sub-Saharan Africa

Although MaviMNPV has been successfully demonstrated to be effective in reducing the pod borer damage on cowpea in Benin, Niger and Nigeria, the commercial production has not yet been started in any of these countries. Hence, a community-based production model has been experimented for the pilot production of MaviMNPV. If this model is sustained, it will provide additional income to disadvantaged groups such as women and youth in sub-Saharan Africa. IITA – Benin has offered the basic training on mass-production of host insect (*M. vitrata* using the cowpea sprouts as the diet), infection of *M. vitrata* larvae with MaviMNPV, collection of dead larvae and extraction as well as formulation of virus for spraying to the selected members of the local community. Since cowpea sprout can be easily produced at household level, large scale mass-production of *M. vitrata* larvae on this diet has become possible and it keeps the MaviMNPV production costs cheaper. Currently, the quality of the formulated MaviMNPV is being tested by IITA-Benin, but this will eventually be transferred to the National University of Agriculture in Porto Novo. The business model has been validated by letting local communities produce MaviMNPV for IITA's own field trials, but they are not into full commercial production yet. Since Benin has a Social Enterprise, *Biophyto Collines*, which currently produces neem oil formulations, the MaviMNPV production technology can also be transferred to commercial firms such as this company, besides women or youth groups. This would enable the production of MaviMNPV, and possibly other NPVs including the SpfrNPV targeting the recent invasive pest species, *S. frugiperda* for the large-scale adoption by farmers in sub-Saharan Africa.

2.4 Characterization and development of effective strains of other baculoviruses for management of pests of crucifers and legumes in Africa

Compared to Asia, the development of commercial baculovirus products for management of crop pests in Africa is weak. However, there are research outcomes in terms of screening for effective strains, mass production and field use of baculoviruses that have the potential to be carried forward. Most of these research efforts are focused towards target lepidopteran pests of legumes, such as *H. armigera* and crucifers, such as *P. xylostella* and *S. exigua*.

Effective strains of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HearSNPV) have been identified in Kenya and South Africa.[52–54] Some of these isolates have been commercialized as products in South Africa[55] and Kenya. Field efficacy of commercial formulations of HearSNPV for management of *H. armigera* has been demonstrated in citrus orchards in South Africa.[55,56] Research on nucleopolyhedrovirus for several *Spodoptera* species (Noctuidae: Lepidoptera) including *S. exigua* NPV, *S. exempta* NPV and *S. littoralis* NPV were carried out and they were field tested in Africa.[57–60]

Besides the nucleopolyhedrovirus for diamondback moth, *P. xylostella* (PlxyMNPV), which is a close relative of *A. californica* NPV and *An. falcifera* NPV,[61] a most effective strain of granulovirus (PlxyGV) has also been identified for managing *P. xylostella*.[62] Later studies indicated the existence of genetically and biologically diverse isolates of *P. xylostella* granulovirus in Africa,[63–65] and some of these fast killing isolates could be further developed into effective biopesticides for the management of *P. xylostella*.[40] Like NPVs, granuloviruses can also synergistically enhance efficacy of synthetic pesticides and delay resistance development as demonstrated with *S. litura* granulovirus.[66] Thus, baculoviruses play an important role in reducing the use of chemical pesticides in pest management programs in agriculture, horticulture and forestry around the world.

3 ENTOMOPATHOGENIC FUNGI IN PEST MANAGEMENT

Entomopathogenic fungi (EPF) are nearly ubiquitous, and they are responsible for most natural epizootics regulating arthropods in nature.[67] Several favorable intrinsic characteristics of EPFs make them effective candidates for development as biopesticides for insect pest management. Ability of entomopathogenic fungi to infect hosts through the cuticle make them amenable for use against diverse group of insects, mites, ticks and even nematodes.[67–70] Further EPFs are useful to target all life stages of insects, making them better candidates in an integrated pest management framework. Recent efforts to understand the ecology and interactions of entomopathogenic fungi with the environment, especially plants have highlighted several of their additional roles. These include endophytism, plant disease antagonism, plant growth promotion and rhizosphere colonization[71] which significantly enhances the value of EPFs in integrated pest management.[72]

The majority of fungal epizootics are caused by entomopathogenic species belonging to the Orders Hypocreales and Entomophthorales. While Hypocrealean fungi such as *Beauveria* and *Metarhizium* cause host death through production of toxins, Entomophthoraleans such as *Neozygites* and *Pandora* cause host death by tissue colonization with little or no toxins involved. Entomophthorales have a biotrophic relationship with the host insect with little or no saprophytic phase. These fungi have proven difficult to be mass produced and formulated.[73] Hence they are largely considered in a conservation perspective to impact the pest population. Entomopathogenic fungi belonging to Hypocreales have a hemibiotrophic relationship with the host insects, including a well-defined saprophytic phase. Hence, they are well suited for mass production on various organic substrates.[74] Ease of mass production of EPFs through both liquid fermentation and solid-substrate production systems is a key attribute for its commercial potential, especially in the developing countries of Asia and Africa. Commercial production of biopesticides-based on EPFs such as *Beauveria*, *Metarhizium, Isaria*, and *Lecanicillium* can be undertaken at various scales from small cottage- to large-scale production.

However, weaknesses of entomopathogenic fungi such as slow speed of kill, short persistence, and relatively high cost of production limit their use by smallholders in the developing world. Further weaknesses in regulatory frameworks and policies related to access to diverse EPFs and registration of commercial biopesticides remains an impediment to their wide commercialization and scale-up in Asia and Africa.

3.1 Development of *Metarhizium anisopliae* and *Beauveria bassiana* isolates as biopesticides

Development of commercial biopesticide products involves several steps that includes bioprospecting, identification, selection of potent strains, optimization of mass-production, formulation, quality control, field efficacy and biosafety assessment and registration.[75] Effective partnership between diverse public and private sector organizations is critical to accomplish the above steps for commercialization of biopesticides. Here we review some research for development initiatives undertaken through a public-private-partnership (PPP) initiative that has led to the development of entomopathogenic fungi-based biopesticide products in Africa.

Rapid isolation of effective strains of entomopathogens to key pest targets can only be achieved if a systematic bioprospecting and curation of collections is undertaken. Several years of bioprospecting and research for development (R4D) efforts by the International Centre of Insect Physiology and Ecology's (ICIPE) Arthropod Pathology Unit (APU) has led to the establishment of a repository of over 350 strains of entomopathogens, including entomopathogenic fungi (EPF), *Bacillus thuringiensis*, baculoviruses and more recently entomopathogenic nematodes. Screening these strains for efficacy against diverse pest constraints has resulted in identification of potent fungal entomopathogens. Fungal entomopathogens effective against legume and brassica vegetable pests such as thrips,[76,77] aphids,[78,79] spider mites,[80] legume pod borer *M. vitrata*,[81] *Liriomyza* leafminers[82] and bean flies[83,84] among others have been identified (Table 2). Jointly with the private sector, some of these entomopathogens have been commercialized for the management of different pests such as *Metarhizium anisopliae* 69 for management of thrips, fruitflies and mealybugs; *M. anisopliae* 78 for management of spidermites; and *M. anisopliae* 62 for management of aphids (www.realipm.org).

3.2 Integrating entomopathogenic fungi as a component in sustainable pest management strategies for vegetable crops and food legume production in sub-Saharan Africa

Effective integration of entomopathogenic fungi as a component in vegetable and food legume IPM depends on their safety to other natural enemies and its compatibility with other IPM options including synthetic pesticides. Further strategies such as pest monitoring based timely application, innovative application strategies such as "lure and infect" and development of novel formulations can significantly enhance the efficacy and affordability of entomopathogenic fungi and its integration in legume and brassica crop IPM.

Entomopathogenic fungi are known to be compatible and largely safe to most ecosystem-service providers such as bees, earthworms, parasitoids and predatory insects.[94,95] Soil application of *M. anisopliae* for management of fruit flies was found to have no adverse effects on non-target natural enemies, as compared to insecticide-treated soils where no parasitoids emerged.[96] Predatory coccinellids, *Cheilomenes lunata* were found to avoid feeding on aphids infected with entomopathogenic fungi, while foraging adult of *C. lunata* enhanced spread of *M. anisopliae* conidia between aphids which demonstrated the compatibility between the control agents.[78] With the exception of spiders, application of *M. anisopliae* was highly compatible with non-target organisms in onion fields as compared to dimethoate-treated plots.[97]

Efficacy of entomopathogenic fungi (EPFs) varies with the resistance levels of host plants on which they are applied. It was observed that EPFs were highly compatible with moderately thrip-resistant cowpea cultivars, enhancing the mortality of bean flower thrips, *Megalurothrips sjostedti*, while highly thrips-tolerant cowpea cultivars were antagonistic to *M. anisopliae*.[98] Host plants with different resistance levels are found to influence the interaction between the insects, their parasitoids, and entomopathogens. These interactions depend on the timing of parasitoid oviposition and fungal infection.[99] Intercropping with maize is a cultural

Table 2. Screening for effective entomopathogenic fungi-based biopesticides used in the management of legume and brassica pests

Crop	Target pest	Entomopathogenic fungi	Country/ Regions	Formulation and dose	Field/Laboratory assessment	Reference
French bean	Western Flower thrips	*Metarhizium anisopliae* ICIPE 69	Kenya	Oil formulation (275 mL/ha @ 1×10^{11} Colony forming units per ml)	Field assay	Nyasani *et al.*[85]
	Pea Leaf miner	*Metarhizium anisopliae* ICIPE 20 and others	Kenya	–	Laboratory assay	Migiro *et al.*[82]
Common bean	Spider mites	*Metarhizium anisopliae* ICIPE 78	Kenya	Aqueous and emulsifiable formulation @ 1.0×10^{8} conidia/mL	Screenhouse and field assay	Bugeme *et al.*[86]
Cowpea	Bean flower thrips	*Metarhizium anisopliae* ICIPE 69	Kenya	Autodissemination	Field assay	Mfuti *et al.*[87]
	Bean flower thrips	*Metarhizium anisopliae* ICIPE 69	Kenya	Spot spray of emulsifiable formulation @ 2.0×10^{11} conidia/ha	Field assay	Mfuti *et al.*[88]
	Pod Sucking bug	*Beauveria bassiana* CPD 9 and *Metarhizium anisopliae* CPD 5 and 12	Nigeria	Aqueous formulation 1×10^{8} conidia/mL	Laboratory and field assay	Ekesi *et al.*[89]
	Pod borer	*Beauveria bassiana* CPD 9 and *Metarhizium anisopliae* CPD 5 and 12	Nigeria	Aqueous formulation 1×10^{8} conidia/mL	Laboratory and field assay	Ekesi *et al.*[89]
	Cowpea aphid	*Beauveria bassiana* CPD 11 and *Metarhizium anisopliae* CPD 4 and 5	Nigeria	–	Laboratory assay	Ekesi *et al.*[90]
Crucifer	*Plutella xylostella*	*Beauveria bassiana – Myco Jaal*	India	Emulsifiable formulation 5×10^{12} Conidia per acre (0.405 ha)	Field application	Ghosh *et al.*[91,92]
		Beauveria bassiana – Bba5653	Benin	Aqueous formulation 0.5 kg Conidia per ha	Field application	Godonou *et al.*[93]
	Pieris brassicae	*Beauveria bassiana – Myco Jaal*	India	Emulsifiable formulation 5×10^{12} Conidia per acre (0.405 ha)	Field application	Singh *et al.*[92]
	Aphis gossypii; Brevicoryne brassicae; Lipaphis pseudobrassicae	*Metarhizium anisopliae* isolate ICIPE 62	Kenya	–	Laboratory assays	Bayissa *et al.*[78]

approach to reduce thrips infestation on cowpea. Efficacy of *M. anisopliae* against bean flower thrips was significantly higher in intercropped cowpea fields as compared to monocropped cowpea.[100]

Better understanding on the interaction between the host plants, arthropod pests, their predators and EPFs can aid in development of improved application strategies for biopesticides. For instance, better understanding on the dispersal behavior of invasive, *Tetranychus evansi* and its predator, *Phytoseiulus longipes* resulted in the development of a novel foam-based application of *M. anisopliae*.[101] Similarly, understanding of thrips attraction to kairomones and its impact on the distribution of thrips resulted in the development of a 'lure and infect' spot-spray application technique for *M. anisopliae* in cowpea. Spot spray application of *M. anisopliae* was effective as a cover spray application, and could result in greater profits due to the reduced labor and fungal inoculum requirement.[88] Approaches such as 'auto-inoculation' of biopesticides in combination with insect attractants can aid in mitigating pest outbreaks and protecting the fungal inoculum in the field. Such strategies are being developed for the management of leafminers,[82] thrips[102] and fruit flies.[103]

Due to the contact infectivity of the entomopathogenic fungi, larval stages of internal feeders such as leafminers infesting legumes and brassica vegetables and bean flies on legumes is a challenge. This could be countered through exploitation of the endophytic property of the fungal biopesticides which has been found to induce systemic resistance in beans against *Liriomyza* leafminer flies in common beans and faba beans[84,104] and bean stem maggot, *Ophiomyia phaseoli* infesting common beans.[83] The role of endophytes in inducing systemic resistance to other legume and brassica pests and diseases needs to be investigated further.

Crops encounter diverse pest and disease constraints beyond those that are targets of entomopathogenic fungi. Understanding the compatibility of synthetic pesticides commonly used against these constraints with entomopathogenic fungi is critical to develop a holistic IPM package. Compatibility studies on *M. anisopliae* and commonly used synthetic pesticides in French bean indicated that fungicides were highly toxic, while azadirachtin and L-cyhalothrin were toxic, adversely affecting vegetative growth and sporulation of the fungus.[105] The insecticides abamectin and imidacloprid were highly compatible.

3.3 Successful commercial production in sub-Saharan Africa and future prospects for entomopathogenic fungi in Asia

Among the various entomopathogens, commercial production in sub-Saharan Africa is largely dominated by entomopathogenic fungi.[41] Countries such as Kenya and South Africa are in the forefront of commercial production of biopesticides. In South Africa, out of the 31 biopesticide products currently registered for use, 23 are imported, while six entomopathogenic fungi-based and two baculovirus-based biopesticide products are locally produced.[41] In Kenya, 20 microbial pesticides are registered for use out of 868 registered products. This includes nine products based on *Bacillus thuringiensis*, nine based on entomopathogenic fungi, one baculovirus and one product based on entomopathogenic nematode. Of these three EPFs, one baculovirus and one EPN are locally produced. Well defined regulatory procedures for registration of biopesticides and an extensive export horticultural sector are key drivers for the growing demand for biopesticide-based products.[41,106] Effective PPP is critical for the commercialization of biopesticide products. For instance, successful PPP between private sector partners, RealIPM and ICIPE, over the past 10 years has resulted in three *M. anisopliae*-based products. These biopesticides have been registered in six sub-Saharan African countries. Recently, some of these biopesticides, especially *M. anisopliae*-based formulations from RealIPM were successfully evaluated on vegetable legumes and brassicas in farmer participatory trials in Southeast Asia.[107,108] Similarly, *M. anisopliae* and *B. bassiana*-based formulations from the local markets were shown to be highly effective against *M. vitrata* on yard-long bean in India, Thailand and Vietnam.[109,110] Hence, harmonization of regulatory procedures for biopesticide registration across Africa and effective PPPs must be fostered to strengthen commercialization of biopesticides in Sub-Saharan Africa. Recently, significant progress has been made on the harmonization of regulatory procedures for registration of bio-control agents across Southeast Asia under the ASEAN Sustainable Agrifood Systems (SAS) project. In fact, 2500 bio-inputs have been registered for ASEAN trade (https://www.asean-agrifood.org/download/results-at-a-glance-as-of-september-2017/?wpdmdl=10508). Hence, these EPF based biopesticides will also have a great demand to reduce the misuse of chemical pesticides in Southeast Asia and beyond.

4 CONCLUSIONS

It has been clearly demonstrated in different parts of Asia and Africa that biopesticide formulations based on baculoviruses and/or entomopathogenic fungi have great potential to reduce the use of chemical pesticides in vegetable production systems. MaviMNPV, a most effective baculovirus which was identified and developed as a biopesticide in Taiwan has already been introduced into West Africa. MaviMNPV was demonstrated to be effective when used in combination with botanical pesticides on cowpea. Different strains of *B. bassiana* and *M. ansiopliae* from the collection maintained at ICIPE were found to be effective against various sucking pests as well as lepidopteran caterpillars on vegetable legumes, brassicas, *etc*. in Africa. They were also successfully commercialized through public-private partnership. However, the availability and access to biopesticides are still major limitations for large-scale adoption of biopesticides in Asia and Africa. In fact, lack of access to effective strains of entomopathogens, lack of an appropriate registration system, lack of adequate support for the small and medium sized firms which are interested to produce and/or sell the biopesticides are some of the major bottlenecks that have constrained the large-scale commercialization of biopesticides in Asia and Africa. However, the recent developments in the technological innovations in identification, development and formulation of biopesticides, improvements in registration system for bio-control agents, enabling policy environment for the production and consumption of safer food produces, and other factors have increased the availability of biopesticide products in Asian and African markets. Thus, the production and utilization of biopesticides in vegetable production will continue to grow.

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