KAVAKA 41: 87-91 (2013)

Promotion of mycoinsecticides for agriculturaly important pests and diseases in Maharashtra

Chandele, A.G.*, Kharbade, S.B., Nakat, R.V., Jadhav, S.S., Pokharkar, D.S., Smita Bayas and Hajare, A.R.
Department of Agril. Entomology, Mahatma Phule Krishi Vidyapeeth,College of Agriculture, Pune-411 005, Maharashtra, India
(Received on 23 November 2013; Accepted on 31 December 2013)

INTRODUCTION

The pressing need for sustainable agricultural production in India has established the significance of Entomophages and Biopesticide for management of crop pests and diseases. The pests of crops are considered to be the key factor in governing crop yield and they are the limiting factors in increasing food and fiber production in agriculture. The chemical pesticides become popular among the end users owing to their quick and visible results. However, their injudicious use posed several problems like pesticide resistance, pest resurgence, residue in food stuff, health hazard, environmental pollution, destruction of beneficial fauna, etc. This has altered the pest control scenario and led to the acceptance of the Integrated Pest Management (IPM) strategies. In recent years, crop protection has been trending towards integration of IPM practices in general and using bioagents / biopesticides like bacteria, fungi, viruses, botanicals as insecticides in particular. Amongst them, entomopathogenic fungi are widely used biopesticide for the suppression of insect pests in agricultural and horticultural ecosystem. About 750 species of fungi are pathogenic to insects but only 12 of them have been focused and utilized for pest control. The fungal pathogens employed as insecticides are *Beauveria bassiana, Metarhizium anisopliae, Verticillum lecanii, Nomuraea rileyi, Trichoderma viride, T. harzianum* and *Paecilomyces lilacinus.*

The attempts have been made in the Biocontrol Research Laboratory of the Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar Maharashtra) to develop biocontrol agents and biopesticides preparations such as entomopathogenic, antagonistic and nematophagous natures' fungi (Verticillum lecanii, Beauveria bassiana, Metarhizium anisopliae, Nomuraea rileyi, Trichoderma harzianum, Paecilomyces lilacinus) and Nucleopolyhydrosis virus for the management of insect pests and diseases. Under Rashtriya Krishi Vikas Yojana (RKVY), the project on "Promotion and Large Scale Production of Biocontrol Agents Developed by MPKV, Rahuri" worth Rs. 2 cores is sanctioned by the Department of Agriculture and Co-operation, Ministry of Agriculture, Government of India, to this University. In this context, the large scale production of biopesticides was undertaken in the four biocontrol laboratories in MPKV, and the demonstrations were conducted on University farms as well as farmers' fields in ten districts of western Maharashtra covering five agro-climatic zones of the state. It is being mainly implemented on farmers' fields as action research programme and the results are focused in popularizing and encouraging the Biointensive Pest Management (BIPM) practice among end-users in important field and orchard crops.

*Corresponding Author. Email: ajitchandele@yahoo.co.in

Objectives of project

- Strengthening of existing biocontrol laboratory and to undertake mass production of the promising biopesticides / bioagents.
- Isolation, evaluation, maintenance and multiplication of local isolates / strains of effective entomopathogenic micro-organisms.
- Provide hands on training to students, entrepreneurs and farmers.
- Large scale testing of Biopesticides on farmer's fields under Action Research Programme.

The project RKVY started in 2011 and strengthening of biocontrol laboratories at Rahuri, Pune, Dhule and Kolhapur centers have been carried out to up-date necessary infrastructure as well as equipment facilities for mass production of biopesticides.

Training programme under RKVY

The Department of Agricultural Entomology, MPKV, Rahuri is actively engaged in leading the project and functioning at four Biocontrol laboratories located at Rahuri, Pune, Kolhapur and Dhule under the University jurisdiction. This project deals with organization of awareness trainings and campaigns for farmers, field demonstrations using biopesticides and large scale production of biopesticides for supply to farmers, with a view to minimize the application of hazardous plant protection chemicals and develop eco-friendly insect pests management practices (Table 1). The awareness training programmes and large scale field trials under 'Action Research' were conducted over 1130 ha for the control of important insect pests of different crop *viz., Spodoptera litura* on lucerne (Rahuri) and soybean (Pune, Nashik, Rahuri, Sangali), white grub, *Leucopholis lepidophora,*

Table 1. Details of awareness trainings of biopesticides conducted by MPKV

Sr. No.	Training & Location	Biopesticide used	Month / Year	No. of farmers participated
1	Management of root-knot nematode in pomegranate (Sangamner, Ahmednagar)	*Paecilomyces lilacinus*	14/02/2012	30
2	Biological suppression of leaf eating caterpillars in Lucerne (Digraj,Ahmednagar)	*Verticillum lecanii*	19/04/2012	50
3	Biological control of white grub in sugarcane (Gondhavani Ahmednagar)	*Metarhizium anisopliae*	27/09/2012	50
4	Management of *Helicoverpa armigera* on chickpea (ChinchodiPatil- Ahmednagar)	*Ha*NPV	31/10/2012	50
5	Biological control of white grub in potato (Rajgurunagar- Pune)	*Metarhizium anisopliae*	17/04/2013	60
6	Control of white grub in sugarcane (Radhanagari- Kolhapur)	*Metarhizium anisopliae*	20/06/2013	100

Holotricha serrata in sugarcane (Kolhapur, Satara, Sangali), safflower aphids (Solapur), mango hoppers (Pune), root-knot nematodes in pomegranate (Sangamner), *Helicoverpa armigera* on chickpea (Ahmednagar, Dhule), sucking pest complex on Cotton (Jalgaon, Dhule, Nandurbar), wheat aphids (Nashik), thrips on onion (Nashik), brinjal shoot and fruit borer, jassids, white flies and aphids (Ahmednagar).

culturing and mass production of biopesticides in Laboratory

The local isolates of entomopathogens under study at MPKV were initially isolated from the infected cadavers collected from different crops grown in various regions of western Maharashtra and maintained under laboratory conditions. The virulent spores of the fungal pathogens were produced in the laboratory by surface culture and submerged methods. In submerged culture, the liquid media was inoculated with fungal culture, whereas in surface culture technique, the fungal spores were allowed to produce on solid media (cereal grains). For mass production of *Beauveria bassiana, Verticillium lecanii, Trichoderma harzianum* and *Paecilomyces lilacinus,* the liquid media with potato dextrose broth was used, whereas for *Metarhizium anisopliae* and *Nomuraea rileyi*, various cereal grains were tested as substrates for efficiently supporting their growth. The counting of spores was made after serial dilution of suspension using hemocytometer. Among all the substrates tested, the combination of jowar and soybean has shown highest spore count. For solid media, whole grains of jowar mixed with crushed grains of soybean were washed under running water to remove any traces of dust particles and placed in a large tray. About 1 lit. distilled water + 1% yeast extract was added into 1 kg solid substrate, and allowed to soak overnight. Then, the substrate was distributed into saline bottles (100 g dry solid substrate per bottle). The bottles containing substrate were autoclaved and after cooling, the bottles were inoculated with 5 ml liquid inoculum of the fungal isolate and incubated at room temperature (25-30ºC) for 15 days. After incubation, 1 g homogenous grain sample was drawn from each batch and transferred to series of dilutions of 10 ml sterilized distilled water containing Tween 80 (0.05%). It was shaken for 10 min. and counting of spores was made by the hemocytometer. Once the fungus sporulated, the spores were harvested from the inoculated bottles after 2-3 weeks.

Results

The production of eight biopesticides (mycoinsecticides and NPV) was undertaken in four biocontrol laboratories of MPKV (Rahuri, Pune, Dhule, Kolhapur) with the aid of RKVY (Table 2). The large scale demonstrations for evaluating the effectiveness of these biopesticides on farmers' fields under action research were carried out in ten districts of western Maharashtra covering five agro-climatic zones of the state. The action Research Programmes were conducted in field crops (cotton, sugarcane, wheat, sorghum), pulse (chickpea), oilseeds (safflower,soybean), vegetables (potato, tomato, onion, brinjal), fruit crops (mango, pomegranate), forage crop (lucerne) and polyhouse crops (rose, gerbera) at 33 locations over 1130 ha cropped area (Table 3). The observations recorded on target pests of crops revealed that NPVs of *H. armigera* and *S. litura* showed 80 to 92 per cent infections the population of caterpillars of the respective pests in chickpea, lucerne and soybean crops.

Table 2. Production of biopesticides in different biocontrol laboratories of MPKV under RKVY

Centre	Quantity of biopesticides production (kg/L)from April 2012 to March 2013							
	***Ha*NPV**	***Sl*NPV**	***Trichod-erma***	***Metarhi-zium***	***Verticillium***	***Beauveria***	***Pseudo-monas***	***Nomuraea***
Rahuri	150	140	410	890	150	65	50	500
Pune	Nil	Nil	Nil	1180	50	Nil	Nil	180
Dhule	Nil	Nil	341	181	1260	34	Nil	79
Kolhapur	Nil	Nil	Nil	3728	Nil	Nil	Nil	Nil
Total	**150**	**140**	**751**	**5979**	**1460**	**99**	**50**	**759**

The myco-insecticides like *Metarhizium anisopliae* (50 to 78%), *Beauveria bassiana* (47 to 70%), *Nomuraea rileyi* (52 to 80%) and *Verticillium lecanii* (56 to 80%) showed varying degree of pathogenicity against target pests like leaf eating caterpillars, tissue borers, sap sucking pests and soil inhibiting pests (Table 4). The fungal and bacterial antagonists Trichoderma+ (*Trichderma harzianum, Pseudomonas fluorescence*) proved their effectiveness in controlling the soil dwelling fungal diseases and root-knot nematodes in pomegranate orchards.

The results of these biopesticides were shown to the host farmers on their fields through large scale action research programme and the group discussions with farmers were organized at village levels which have created awareness and confidence among the farmers for use of microbial insecticides in agricultural and horticultural ecosystems for pest management which has showed effective control of agriculturally important pests as compared to chemical pesticides.

Conclusion

Thus, the project on 'Promotion and large scale production of biopesticides developed by MPKV, Rahuri' through the action research on farmers' fields convinced the farmers and created awareness for use of biopesticides in crop pests control which is eco-friendly and safe from consumer's health hazards point of view. Further, it has shown impact in bringing down the dependence upon synthetic chemical pesticides and established sustainable pest management system. At present, there is an increasing demand from the farmers for procuring the biopesticides at various production units of the University as well as private entrepreneurs.

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Table 3. Action Research / Demonstrations carried out under RKVY during 2011-12 and 2012-13

Sr. No.	District	Month/ Year	Crop / Target pest	Biopesticides used	Area (ha)
1	Dhule	Dec., 2011	Chickpea - gram pod borer	*Ha*NPV	50
2	Kolhapur	Jan., 2012	Sugarcane - white grub	*M. anisopliae*	40
3	Pune	Jan., 2012	Mango hoppers	*M. anisopliae*	15
4	Ahmednagar	Feb., 2012	Pomegranate - root-knot nematodes	*Trichoderma* + *Paecilomyces* *Pseudomonas*	30
5	Pune	March, 2012	Polyhouse floriculture Sucking pest complex , Lepidoptera pest	*M. anisopliae*	3
6	Ahmednagar	April- May, 2012	Lucerne - leaf eating caterpillar	*Sl*NPV	40
7	Kolhapur	July, 2012	Sugarcane - white grub	*M. anisopliae*	10
8	Nashik	July-August, 2012	Soybean - leaf eating caterpillar	*Sl*NPV *N. rileyi*	50
9	Kolhapur	August-Sept., 2012	Soybean, Sugarcane - White grub	*M. anisopliae*	20
10	Pune	Sept., 2012	Soybean - leaf eating caterpillar	*N. rileyi*	25
11	Dhule	Sept., 2012	Cotton - sap sucking pests	*V. lecanii*	50
12	Jalgaon	Sept., 2012	Cotton- sap sucking pests	*M. anisopliae*	50
13	Ahmednagar	Sept., 2012	Sugarcane- white grub	*M. anisopliae*	50
14	Ahmednagar	Sept., 2012	Soybean- leaf eating caterpillar	*Sl*NPV	25
15	Pune	Sept., 2012	Potato- leaf eating caterpillar	*N. rileyi*	150
16	Nandurbar	Sept., 2012	Cotton- sap sucking pests	*V. lecanii*	40
17	Kolhapur	Oct.-Nov., 2012	Soybean, Sugarcane - White grub	*M. anisopliae*	30
18	Solapur	Nov., 2012	Rabi Sorghum - sucking pests	*M. anisopliae*	12
19	Solapur	Nov., 2012	Safflower - aphids	*M. anisopliae*	24
20	Ahmednagar	Nov., 2012	Chickpea - gram pod borer	*Ha*NPV	60
21	Sangali	Nov., 2012	Rabi Sorghum- white grub	*M. anisopliae*	10
22	Ahmednagar	Dec., 2012	Chickpea - gram pod borer	*Ha*NPV	50
23	Dhule	Dec., 2012	Chickpea - gram pod borer	*Ha*NPV	50
24	Nashik	Dec., 2012	Wheat - aphids	*V. lecanii*	50
25	Pune	Dec., 2012	Mango - hoppers	*M. anisopliae*	24
26	Pune	Dec., 2012	Mango- aphids, white flies	*V. lecanii*, *B. bassiana*	50
27	Satara	Dec., 2012	Sugarcane- white grub	*M. anisopliae*	25
28	Ahmednagar	Jan., 2013	Tomato - sap sucking pests	*M. anisopliae*, *B. bassiana*	15
29	Nashik	Jan., 2013	Lucerne- aphids	*V. lecanii*	25
30	Nashik	Jan., 2013	Onion - thrips, leaf blotch	*M. anisopliae*, *Pseudomonas fluorescence*	25
31	Ahmednagar	March, 2013	Lucerne - Leaf eating caterpillars	*Ha*NPV, *Sl*NPV *M. anisopliae*	10
32	Pune	April-May, 2013	Potato - white grubs	*M. anisopliae*	30
33	Ahmednagar	July, 2013	Brinjal - shoot & fruit borer, jassids, white flies, aphids	*M. anisopliae*, *V. lecanii*, *B. bassiana*	10
			Total Area (ha)		**1130**

Table 4. Pathogenicity of mycoinsecticides and NPV against target pests of crops

Sr. No.	Biopesticide	Crop	Target pest	Infectitious conditions (%)*
1	*M. anisopliae*	Mango	Hopper	60-74
		Sugarcane	White grub	50-78
		Soybean	White grub	48-70
		Cotton	Sap sucking pests	50-68
		Sorghum	Sap sucking pests White grub	52-60 54-78
		Safflower	Aphids	58- 75
		Tomato	Sap sucking pests	50-62
		Onion	Thrips	60-68
		Potato	White grub	51-77
		Brinjal	Pest complex	50-65
		Polyhouse crops	Thrips, Mites	54-62 60 50-
2	*B. bassiana*	Mango	Aphids White flies	47-60 54-70
		Tomato	Sap sucking pests	52-65
		Brinjal	Pests complex	48-70
3	*V. lecanii*	Cotton	Sap sucking pests	56-80
		Wheat	Aphids	60-75
		Mango	Aphids White flies	68-80 52-72
		Tomato	Sap sucking pests	60-76
		Lucerne	Aphids	64-78
		Brinjal	Pests complex	56-75
4	*N. rileyi*	Soybean Potato	Leaf eating caterpillar	52-80 65-80
5	Trichoderma+ *P. lilacinus*	Pomegranate	Root-knot nematode	47-65 (decline in population)
6	*Ha* NPV	Chickpea	Pod borer	80-92
7	*Sl*NPN	Lucerne Soybean	Leaf eating caterpillar	85-90 80-92