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Efficacy of biorational insecticides and entomopathogenic nematodes (EPNs) against brinjal ash weevil, *Myllocerus subfasciatus* Guerin-Meneville (Coleoptera: Curculionidae)

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ABSTRACT: Field experiments were conducted during 2020-21 to evaluate different biorational insecticides and entomopathogenic nematodes against ash weevil, *Myllocerus subfasciatus* Guerin-Meneville. The results revealed that the IPM Module 3 [comprised of soil application of *Metarhizium anisopliae* @ 5 kg/ha followed by soil drenching with EPN *Heterorhabditis indica* @ 20 kg/ha at 30 DAT and foliar spray with *M. anisopliae* @ 5kg/ha at 45 DAT] was found superior with the lowest number of *M. subfasciatus* adults per plant (mean ± SE) (2.06 ± 0.07) and the lowest leaf damage (4.35 ± 0.59 per leaf per plant), followed by the IPM Module 2 [comprised soil application of neem cake followed by application of entomopathogenic nematode *H. indica* @ 20 kg/ha at 30 DAT and foliar application of *B. bassiana* @ 5 kg/ha at 45 DAT] which was also found significant in reducing the number of *M. subfasciatus* and leaf damage by ash weevil (2.30 ± 0.15; 4.85 ± 0.69 respectively), compared to untreated check (2.91 ± 0.13, 5.63 ± 0.34 respectively).

Keywords: Ash weevil, *Myllocerus subfasciatus*, EPNs, *Heterorhabditis indica*

INTRODUCTION

Brinjal or aubergine (*Solanum melongena* L.) is the most widely used vegetable across many countries *viz*., Central, South and Southeast Asia, some parts of Africa and Central America (Harish *et al.*, 2011). It is native to India and grown in almost all parts of India. In India, brinjal is cultivated in 7.6 lakh hectares with a production of 12695 MT (average productivity of 17.5 t/ha). Ash weevil, *Myllocerus subfasciatus* Guerin-Meneville is the most widely distributed across India and attained major pest status in brinjal crop. Adults lay eggs in the soil and grubs are exclusively root feeders resulting in stunting and wilting of the plant. Adults feed on the foliage of brinjal and other hosts by making characteristic notches along the leaf margins. Since, the grubs have subterranean nature of feeding there is difficulty in managing this notorious pest. Nagesh *et al.* (2016) reported that *M. subfasciatus* is considered as quarantine pest as the immature stages can be easily disseminated through the transportation of planting material. Regular surveys and interactions with farmers revealed that the ash weevil is a major pest after the shoot and fruit borer, *Luecinodes orbonalis* Guenee and under favourable conditions, ash weevils have the potential to cause 100% yield loss in brinjal crop (Shanmugam *et al.*, 2018; 2021).

The farmers depend on soil and foliar application of insecticides to manage grubs and adults in brinjal. As the control options for this pest mainly rely on the use of chemical pesticides, concerns about environmental safety, insecticide resistance and effects on non-target organisms are often raised. This alarming situation has increased the pressure to shift from chemical-intensive management to alternative eco-friendly management strategies**.** Entomopathogenic nematodes (EPNs) in the genus *Steinernema* and *Heterorhabditis* and their associated bacteria (*Xenorhabdus* spp.) have been successfully commercialized as potent biological control agents for a variety of curculionid species. These EPNs: can kill hosts rapidly, are easy to apply, and are exempt from federal and local registration requirements in most countries because of their safety for mammals and plants (Georgis *et al.*, 1991). Therefore, certain biorational insecticides (neem cake, NSKE 5%, neem oil 3%, *Metarhizium anisopliae, Beaveria bassiana*) and EPNs are exploited for the management of the ash weevil damage in the brinjal crop.

MATERIALS AND METHODS

This study was conducted for two seasons in *rabi,* 2020 and *kharif,* 2021 at an experimental block of ICAR-Indian Institute of Horticultural Research, Bengaluru, India [13° 8’ 18’’ N and 77° 28’ 40’’ E 890m amsl]. The seeds of brinjal *cv*. Arka Harshitha were sown in portrays and thirty days old seedlings were transplanted in an area of 45m x 1m. The experiment was laid out in

Table 1. Treatment details

IPM Module		Details
M1	Module 1	➢ Soil application of neem cake @250kg/ha before transplanting ➢ Soil drenching of NSKE 5% @25kg/ha at 30 DAT (days after transplanting) ➢ Foliar spray with neem oil 3% at 45 DAT
M2	Module 2	➢ Soil application of neem cake @250kg/ha before transplanting ➢ Application of EPN *Heterorhabdidtis indica* @ 20kg/ha at 30 DAT ➢ Foliar spray with *Beaveria bassiana* 1x10^8 CFU/g/mL @ 5kg/ha at 45 DAT
M3	Module 3	➢ Soil drenching with *Metarhizium anisopliae* 1x10^8 CFU/g/mL @ 5kg/ha ➢ Soil application of EPN *H. indica* @ 20kg/ha at 30 DAT ➢ Foliar spray with *M. anisopliae* 1x108 CFU/g/ml @ 5 kg/ha at 45 DAT
M4	Module 4	➢ Farmers practice Soil application of Carbofuran 3G@15kg/ha ➢ Soil drenching with Chlor pyriphos 20EC @ 2.5 L/ha at 30 DAT ➢ Foliar spray with Fipronil 5SC @ 750 mL/ha at 45 DAT
M5	Module 5	➢ Untreated check

randomized block design (RBD). All the recommended package of practices were followed. For managing shoot and fruit borer, pheromone traps and tricho cards of egg parasitoid, *Trichogramma chilonis* were used. No other plant protection measures were implemented other than the treatments. A total of five integrated pest management (IPM) modules were evaluated against ash weevil, *M. subfasciatus*. Each module was replicated three times. The treatment details are as follows.

Observations were recorded during *rabi,* 2020 and *kharif,* 2021, on the ash weevil feeding damage based on the visual scoring of the leaf damage on 0.00 -10.00 scale, where 0.00 = no damage; 1.00 = 10%; 2.00 = 20% 3.00=30%; 4.00=40%; 5.00=50%; 6.00=60%; 7.00=70%; 8=80%; 9.00=90%; 10.00 = 100% leaf damage. Data was recorded randomly on 10 plants per replication per module and in each plant, leaves were scored randomly (n = 10) for leaf damage by ash weevil. The number of adults present per plant (n=10) were also recorded.

Statistical analysis

The data on the mean leaf damage and mean number of ash weevil present in each module for each season were subjected to one-way ANOVA. The statistical analysis was performed using GraphPad Prism (v 9.3.1) software. The means were compared using the Tukey's multiple comparison test.

RESULTS AND DISCUSSION

Attempts to identify alternate IPM module has been planned to combat the ash weevil menace with selected biorational insecticides and EPNs. Different IPM modules were evaluated and the results on the mean leaf damage and mean number of *M. subfasciatus* analyzed across the seasons *rabi* and *kharif* during 2020-2021 are given below.

***Rabi,* 2020**

Leaf damage: Data revealed that there was a statistically significant difference in the mean leaf damage among the IPM modules evaluated ($F_{4,15} = 40.5, P < 0.0001$). All the modules were significantly superior over untreated check, Module 5 (Leaf damage, mean ± SE; 9.25 ± 0.24). The Module 1 (6.03 ± 0.05) and Module 3 (4.58 ± 0.35) (*P = 0.019*); Module 1 (6.06 ± 0.05) and Module 5 (9.25 ± 0.24) (*P < 0.0001*); Module 2 (5.13 ± 0.41) and Module 5 (9.25 ± 0.24) (*P < 0.0001*); Module 3 (4.58±0.35) and Module 4 (5.69 ± 0.21) (*P<0.0001*) were significantly different with each other with respect to leaf damage by ash weevil.

Leaf damage caused by ash weevil was not significantly different between Module 1 (6.03 ± 0.05) and Module 2 (5.13 ± 0.41) (*P = 0.22*); Module 1 (6.03 ± 0.05) and Module 4 (5.69 ± 0.21) (*P = 0.91*); Module 2 (5.13 ± 0.41) and Module 3 (4.58 ± 0.35) (*P = 0.65*); Module 3 (4.58 ± 0.35) and Module 4 (5.69 ± 0.21) (*P = 0.09*) (Fig.1A).

Ash weevil adults: No significant difference was observed in the mean number of *M. subfasciatus* adults in different modules ($F_{4,15} = 7.929, P = 0.143$). The mean number of *M. subfasciatus* adults were not significantly different between Module 1 (2.11±0.05) and Module 2

(2.29 ± 0.22) (*P = 0.772*); Module 1 (2.11 ± 0.05) and Module 3 (1.93 ± 0.02) (*P = 0.98*); Module 1 (2.11 ± 0.05) and Module 4 (2.36 ± 0.06) (*P = 0.512*); Module 2 (2.29 ± 0.22) and Module 3 (1.93 ± 0.02) (*P = 0.305)*; Module 3 (1.93 ± 0.02) and Module 4 (2.36 ± 0.06) (*P = 0.151*); Module 4 (2.36 ± 0.06) and Module 5 (2.79 ± 0.02) (*P = 0.091)*. Whereas, significant differences in the mean number of ash weevils were observed between the Module 1 (2.11 ± 0.05) and Module 5 (2.79 ± 0.02) (*P = 0.0043*); Module 2 (2.29 ± 0.22) and Module 5 (2.79 ± 0.02) (*P = 0.039*); Module 3 (1.93 ± 0.02) and Module 5 (2.79 ± 0.02) (*P = 0.0008*) (Fig.1B).

***Kharif,* 2021**

Leaf damage: Statistically significant difference was observed in mean leaf damage among the IPM modules ($F_{4,15}$ = *101.4, P < 0.0001*). All the modules were significantly superior over untreated check, Module 5 (9.16 ± 0.29). Statistically significant difference was observed between Module 1 (6.07 ± 0.06) and Module 2 (4.48 ± 0.19) (*P = 0.0004*); Module 2 (4.48 ± 0.19) and Module 4 (5.56 ± 0.14) (*P = 0.*011); Module 2 (4.48 ± 0.19) and Module 5 (9.16 ± 0.29) (*P <0.0001*); Module 3 (4.12 ± 0.21) and Module 4 (5.56 ± 0.14) (*P < 0.*0001). But there was no significant difference between Module 1 (M1) (6.07 ± 0.06) and Module 3 (4.12 ± 0.21) (*P =0.39*); Module 2 (4.48 ± 0.19) and Module 5 (9.16 ± 0.29) (*P = 0.69*) (Fig.1C).

Ash weevil adults: There was no significant difference in the mean number of *M. subfacsiatus* adults in different IPM modules evaluated ($F_{4,15}$ = *2.019, P =0.143*) (Fig. 1D).

Pooled analysis (*Rabi,* 2020 and *Kharif,* 2021)

Leaf damage: Significant differences in the mean leaf damage was observed among the IPM modules evaluated ($F_{4,15}$ = *101.4, P < 0.0001*). All the modules were significantly superior over untreated check, Module 5. All the modules showed significant difference with each other except Module 1 (6.05 ± 0.04) and Module 4 (5.63 ± 0.12) (*P = 0.436*); Module 2 (4.83 ± 0.24) and Module 3 (4.35 ± 0.20) (*P = 0.362*).

However, significant differences were observed between the Module 1 (6.05 ± 0.04) and Module 2 (4.83 ± 0.24); Module 3 (4.35 ± 0.20) and Module 4 (5.63 ± 0.12); Module 1 (6.05±0.04) and Module 3 (4.35±0.20) (*P < 0.0001*); Module 3 (4.35 ± 0.20) and Module 4 (5.63 ± 0.12) (*P < 0.0001*); Module 2 (4.83 ± 0.24) and Module 4 (5.63 ± 0.12) (*P =0.017*) (Fig.1E).

Ash weevil adults: The pooled data analysis revealed significant differences in the mean number of *M. subfasciatus* adults in different modules ($F_{4,15}$ = *5.33, P = 0.0018*). The number of ash weevils per plant (mean ± SE) was significantly different between Module 2 (2.39 ± 0.18) and Module 5 (2.91 ± 0.13) (*P = 0.022*); Module 3 (2.06 ± 0.07) and Module 5 (2.91 ± 0.13) (*P =0.0007*). There was no significant difference observed between Module 1 (2.39±0.18) and Module 2 (2.30 ± 0.15) (*P = 0.99*); Module 1 (2.39 ± 0.18) and Module 3 (2.06 ± 0.07) (*P = 0.43*); Module 1 (2.39 ± 0.18) and Module 4 (2.43 ± 0.09) (*P = 0.99*); Module 1 (2.39 ± 0.18) and Module 5 (2.91 ± 0.13) (*P = 0.43*); Module 2 (2.30 ± 0.15) and Module 3 (2.06 ± 0.07) (*P = 0.71*); Module 2 (2.30 ± 0.15) and Module 4 (2.43 ± 0.09) (*P = 0.96*); Module 3 (2.06± 0.07) and Module 4 (2.43 ± 0.09) (*P = 0.325*) ; Module 4 (2.43 ± 0.09) and Module 5 (2.91 ± 0.13) (*P = 0.102*) (Fig. 1F).

On the whole, the Module 3 that comprised soil application of *M. anisopliae* @ 5kg/ha followed by soil drenching with EPN *H. indica* @ 20kg/ha at 30 DAT and foliar spray with *M. anisopliae* @ 5kg/ha at 45 DAT was found to be superior and recorded lower leaf damage and the lowest number of *M. subfasciatus* adults (4.35±0.59, 2.06 ±0.07 respectively). The Module 2 that comprised soil application of neem cake followed by application of entomopathogenic nematode *H. indica* @ 20kg/ha at 30 DAT and foliar application of *B. bassiana* @ 5kg/ha at 45 DAT was also found on par with Module 3 in reducing the leaf damage and number of *M. subfasciatus* adults (4.83 ± 0.24, 2.30 ±0.15 respectively). Interestingly, the Module 3 and Module 2 were also statistically at par with Module 4, a synthetic insecticide intensive farmers practice that comprised soil application of carbofuran 3G@15 kg/ha followed by soil drenching with chlorpyriphos 20EC @ 2.5 L/ha at 30DAT and foliar spray with fipronil 5SC @ 750 mL/ha at 45 DAT (5.63±0.34, 2.43 ± 0.09 respectively).

In conclusion, among the IPM modules, Module 3 [integrated with soil application of *M. anisopliae* @ 5kg/ha followed by soil drenching with EPN *H. indica* @ 20kg/ha at 30DAT and foliar spray with *M. anisopliae* @ 5kg/ha at 45 DAT] and the Module 2 [comprised of soil application of neem cake followed by application of EPN, *H. indica* @ 20kg/ha at 30 DAT and foliar application of *B. bassiana* @ 5kg/ha at 45 DAT] which are mainly comprised of biorational insecticides and EPNs were found effective in managing *M. subfasciatus*. The results indicate the efficacy of tested biorational insecticides in reducing the incidence of *M. subfasciatus* and leaf damage and also found at par with the chemical insecticide application (Module 4). Hence, the present study affirms that the soil application of EPNs and the use of biorational insecticides are effective in managing

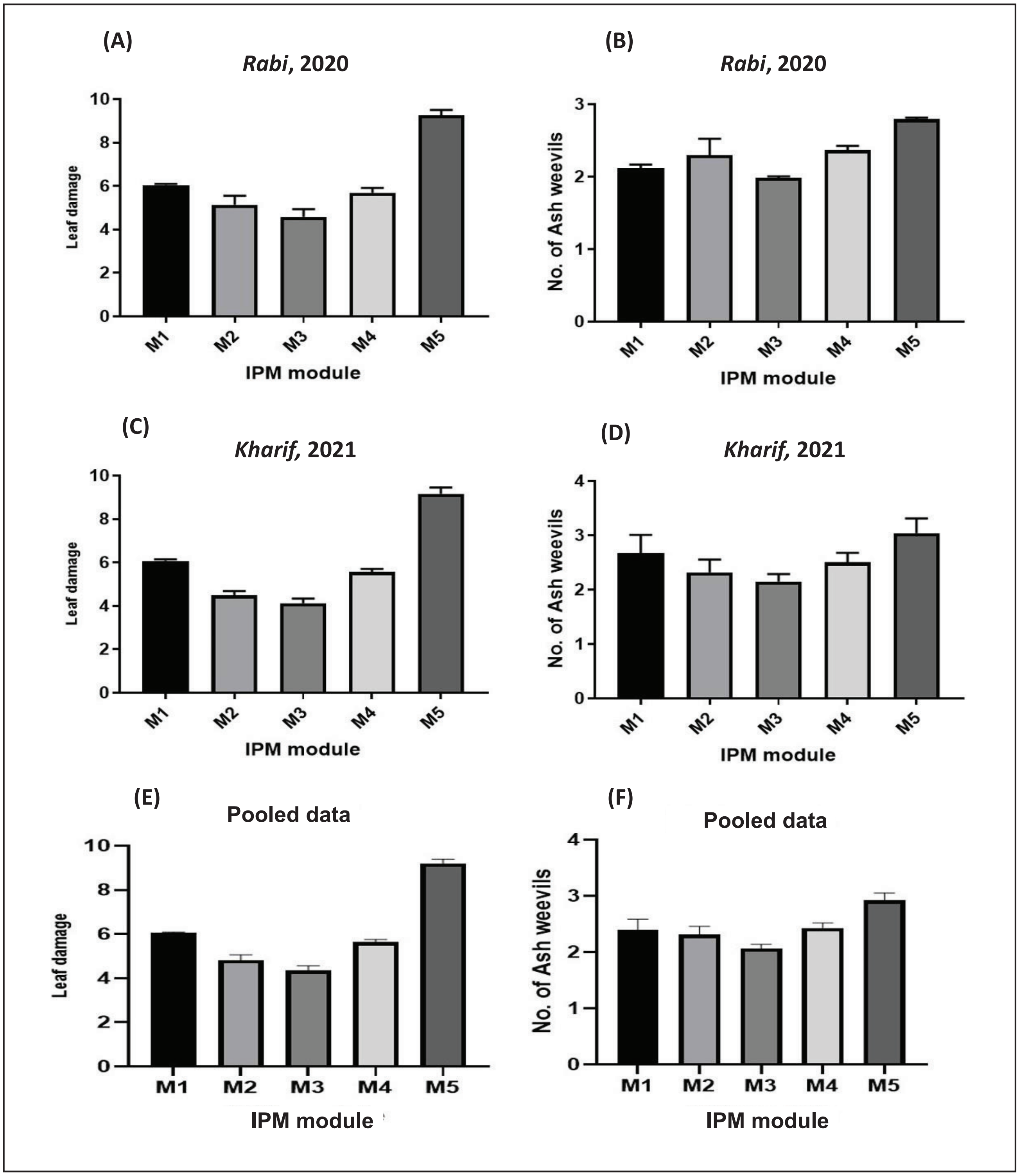

Fig 1. Mean leaf damage and adult ash weevils recorded in different IPM modules during 2020-2021. (M1: Soil application of neem cake @250kg/ha before transplanting-Soil drenching of NSKE 5% @25kg/ha at 30 DAT-Foliar spray with neem oil 3% at 45 DAT; **M2:** Soil application of neem cake @250kg/ha before transplanting -Application of EPN *Heterorhabdidtis indica* @ 20kg/ha at 30 DAT - Foliar spray with *Beaveria bassiana* 1x10^8 CFU/g/mL @ 5kg/ha at 45 DAT; **M3:** Soil drenching with *Metarhizium anisopliae* 1x10^8 CFU/g/mL @ 5kg/ha Soil application of EPN *H. indica* @ 20kg/ha at 30 DAT -Foliar spray with *M. anisopliae* 1x108 CFU/g/ml @ 5 kg/ha at 45 DAT ; **M4:** Farmers practice Soil application of Carbofuran G@15kg/ha - Soil drenching with Chlor pyriphos 20EC @ 2.5 L/ha at 30 DAT - Foliar spray with Fipronil 5SC @ 750 mL/ha at 45 DAT; **M5:** Untreated check).

the subterranean population of ash weevils. These biointensive modules also restrict the usage of synthetic insecticides in managing ash weevils.

Similar findings were reported by Shanmugam *et al.* (2021), where mulching along with EPN, *H. indica* at 2.5 kg/ha recorded zero incidence of *M. subfasciatus* up to 30 DAP (days after planting) and 2.5-7.5 per cent damage up to 150 DAP. The application of EPNs *Steinernema carpocapsae, Steinernema glaseri* and *H. indica*, against *M. subfasciatus* were aslo evaluted by several researchers (Manjunatha *et al.*, 2016; Nisthiskarani *et al.,* 2019), where, Manjunatha *et al.* (2016) reported that *S. carpocapsae* caused greater mortality (20-100 %) than *H. indica* (16-92 %) against pre-pupal stages and *S. carpocapsae* caused 16-92 % mortality in the third instar larvae, while *H. indica* caused 12-80 % mortality. Nisthiskarani *et al.* (2019) found that the application of EPN, *S. glaseri* was effective at the third instar stage of *M. subfasciatus.* Similarly, the studies of Nagesh *et al.* (2016) revealed that seven strains of EPNs, (*Heterorhabdidtis bacteriophora* NBAIIHb105, *H. indica* NBAIIHi101, *H. indica* NBAII Himah, *Steinernema abbasi* NBAIISA01, *Steinernema abbasi* NBAIISA04, *S. carpocapsae* NBAIISc04, *S. glaseri* NBAII Sg01) caused > 80% mortality at 40 IJ/cm^2 in *M. subfasciatus* larvae. Our findings also fall in line with Umamaheshwari *et al.* (2021) who revealed the strength of native strains of EPNs *H. indica, S. carpocapsae, S. glaseri* in combination with *B. bassiana and Bacillus subtilis* against ash weevil, *M. subfacsiatus* under field conditions. Their study revealed that the combination of *H. indica* and *B. subtilis* reduced the leaf damage by ash weevil in brinjal to the tune of 76% .

The present findings affirm that incorporating the EPNs in the IPM modules resulted in greater efficay against *M. subfasciatus*. In general, the use of EPNs for insect pest control has many benefits, including minimal harm to natural enemies, lack of environmental pollution, and end-user safety. Hence, the application of EPNs might play a key role in targetting the ash weevils in brinjal as ensures environmental sustainability through biointensive IPM. Reduced chemical input costs, diminished on and off-farm environmental effects, more efficient and su stainable pest management are some advantages of applying biointensive IPM.

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