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Integrating entomopathogenic nematodes and microbes for eco-friendly management of brinjal ash weevils, *Myllocerus subfasciatus* Guerin (Coleoptera: Curculionidae)

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ABSTRACT: Ash weevils (*Myllocerus subfasciatus*, *M. viridanus, M. discolor*) are economically important pests of brinjal in India. Entomopathogenic nematodes (EPN) from the families Steinernematidae and Heterorhabditidae have emerged as promising alternatives to toxic chemical pesticides and effective against a spectrum of economically important pests. Hence, studies were conducted to evaluate native strains of EPN *viz., Heterorhabditis indica, Steinernema carpocapsae* and *S. glaseri* against ash weevils, *Myllocerus subfasciatus* in brinjal under field conditions. EPN were tested individually and in combination with microbial biopesticides *viz., Bacillus subtilis* and *Beauveria bassiana.* Among the EPN species, *H. indica* recorded the maximum mortality of 65.13% followed by *S. glaseri* (56.1%) and *S. carpocapsae* (42.3%). When integrated with microbial bioagents, *H. indica* in combination with *B. subtilis* recorded maximum mortality of 69.47% grub mortality and 76% reduction in leaf damage due to ash weevils. Thus the study proves the antagonistic potential of EPN and its compatibility with other microbial biopesticides widening its scope as an excellent component in IPM of brinjal.

Keywords: Ash weevil, Brinjal, EPN, *Myllocerus*, management

INTRODUCTION

Brinjal (*Solanum melongena* L.) is a staple vegetable in India and other countries mostly in South and Southeast Asia. It is often also described as a poor man's vegetable because it is popular amongst small-scale farmers and low income consumers. In India, brinjal, is cultivated in an area of 7.30 lakh ha with a production of 12801 MT and productivity of 17.5 MT per ha (Anonymous, 2018). Brinjal is highly prone to attack from a wide variety of insect pests that reduce the yields by 26 to 92%. Hence farmers tend to spray chemical insecticides 25 to 80 times per season to manage the pests and avoid the yield loss (Kumar, 2012).

Ash weevils (*Myllocerus subfasciatus*, *M. viridanus, M. discolor*) are among the major insect pests of brinjal and other solanaceous crops (Ayyar, 1920) and reported as economically important (Kalyanam, 1967). Shanmugam *et al.* (2018) reported upto 60% yield loss and many time complete crop failure due to ash weevils in brinjal. Larvae are exclusively root feeders resulting in plant stunting and, finally, wilting of the plant. Adults feed on the foliage of brinjal and other hosts by making characteristic U-shaped cuts along the leaf margin leading to a sickly appearance of the plant (Tewari and Kumar, 1983). To manage the weevil menace, soil application of chemical insecticides is scientifically not recommended especially in short duration vegetable crops because of the persistence of pesticide residues in soil and freshly consumable produce. In addition, the grub stages remain in the soil feeding on the roots which make it more difficult for the microbial bioagents or botanical insecticides to reach and establish in the deeper rhizospheric niche of the grubs and cause mortality. Hence, farmers resort to the use of chemical pesticides to tackle the weevil problem. However, inadvertent use of chemical pesticides raises concerns about public safety, soil and water pollution, insecticide resistance and effects on non-target organisms. This alarming situation has increased the pressure to shift from chemical intensive management to alternative eco-friendly control strategies. Entomopathogenic nematodes (EPN) from the families Steinernematidae and Heterorhabditidae are excellent candidates that possess a unique combination of attributes that make them a promising alternative for chemical pesticides against a variety of economically important pests (Grewal *et al.,* 2005). They are non-toxic to humans, relatively specific to their target pests and can be applied with standard pesticide equipment. EPN have been exempted from registration by U.S. Environment Protection Agency (EPA).

Hence, studies were conducted to evaluate native strains of EPN *viz., Heterorhabditis indica, Steinernema carpocapsae* and *S. glaseri* individually and in combination with microbial biopesticides *viz., Bacillus subtilis* and *Beauveria bassiana.* against brinjal ash weevils, *M. subfasciatus* under field conditions.

MATERIALS AND METHODS

Maintenance of EPN Culture

Cultures of EPN *Heterorhabditis indica* and *Steinernema carpocapsae* used in this study were isolated from brinjal fields and maintained in Nematology Laboratory, ICAR – Indian Institute of Horticultural Research, Bengaluru. *S. glaseri* was obtained from Department of Nematology, Tamil Nadu Agricultural University, Coimbatore and used in this study. All the three test EPN strains were cultured on final instar larvae of greater wax moth, *Galleria mellonella* as per Woodring and Kaya (1998). Using white traps, the emerging infective juveniles (IJ) were harvested within 3 days of first emergence and viable EPN were used for field studies.

Field trial

For field evaluation, trials were conducted in brinjal fields (cv. Lalita F1) naturally infested with ash weevils at Block VI, ICAR – Indian Institute of Horticultural Research (IIHR), Bengaluru. Pre application sampling was done to estimate the grub population by digging soil beneath the brinjal plants (30 cm deep) and also counting the number of weevils feeding the leaves per plant. They were tested individually and in combination with entomopathogenic microbes, *Beauveria bassiana* and *Bacillus subtilis* which were maintained at Biocontrol Laboratory, Division of Crop Protection, ICAR-IIHR, Bengaluru. The treatments were T1 – *Steinernema glaseri* (Sg) @ 2.5 x 10^9 Ijs/ha; T2 – *S. carpocapsae* (Sc) @ 2.5 x 10^9 Ijs/ha; T3 – *Heterorhabditis indica* (Hi) @ 2.5 x 10^9 Ijs/ha; T4 – Sg + *Beauveria bassiana* (Bb- 1 x 10^6 cfu/ml); T5 – Sc +Bb; T6 – Hi +Bb; T7 – Sg + *Bacillus subtilis* (Bs- 1 x 10^6 cfu/ml); T8 – Sc +Bs; T9 – Hi +Bs; T10 – Chemical –Chlorpyriphos 2 ml lit-1 and T11 – Untreated control. Each treatment was replicated thrice in a randomised block design.

EPN were applied as soil application in the evening hours and the entomopathogenic microbes were applied as soil drenching and spraying @ 5 ml lit^{-1} of water. The chemical insecticide, Chlorpyriphos @ 2 ml lit^{-1} was applied as positive control. Observations were recorded on % grub mortality (cumulative and corrected) after 2-3 weeks, % leaf area damaged by adults and brinjal yield (t ha^{-1}). Dead larvae were again observed in the laboratory to confirm if the mortality was due to EPN and or microbe.

RESULTS

Initial weevil population was 3.2 ± 0.84 adults per plant and 4.6 ± 1.23 grubs per plant. Field trials revealed that among the three EPN species tested against ash weevils (*Myllocerus subfasciatus*) of brinjal, *H. indica* caused 74.8 per cent grub mortality and 85.9 per cent reduction in leaf damage. When integrated with bacterial bioagent *B. subtilis*, it recorded the highest grub mortality (78.9%) and reduction in leaf area damaged (88.5%) due to adult weevils coupled with the maximum brinjal yield (38.4 t ha^{-1}). It was followed by integration of *S. glaseri* with *B. subtilis* which recorded 69.6 per cent grub mortality and 14 per cent leaf area damaged due to ash weevils and contributed to 15.9 per cent increase in yield compared to untreated control. When *S. glaseri* was applied alone, it recorded 65.3 per cent grub mortality, 17.8 per cent leaf area damage and 14.6 per cent increase in yield compared to control. In combination with *B. bassiana*, *H, indica* recorded 61.3 per cent grub mortality and 12.4 per cent increase in yield while *S. glaseri* recorded 56.2 per cent grub mortality and 7.1 per cent increase in yield, in comparison with untreated control (Table 1; Fig. 1).

Among the three EPN strains, *S. carpocapsae* recorded significantly lower grub mortality as 54.2 per cent when applied alone, 60.6 per cent when combined with *B. subtilis* and 42.6 per cent when combined with *B. bassiana.* It recorded 5.2 to 7.8 per cent increase in yield compared to control, when applied singly and in combination with bioagents. Chlorpyriphos recorded 62.8 per cent grub mortality and 75 per cent reduction in leaf damage coupled with 13.7 per cent increase in yield. The lowest yield (31.2 t ha^{-1}) was recorded in control plants without any treatments which also recorded the highest leaf area damaged due to adult weevils (68.4%) (Table 1; Fig. 1).

DISCUSSION

Our findings fall in line with the earlier findings of Gowda *et al.* (2016) who revealed the biocontrol potential of *H. indica* and *S. carpocapsaae* against brinjal ash weevils under laboratory conditions. Also Nithiskarani *et al.* (2019) observed that *S. glaseri* was more effective than *H. indica* against third instar larvae of *M. subfasciatus* and caused 95 and 87.5 per cent grub mortality in soil test assays. Field evaluation of *S. glaseri* and *Heterorhabditis* sp. against brinjal root weevil, *Myllocerus discolour* evidenced the highest reduction in grub population (93.8%) at 10 days after *Heterorhabditis* application followed by *S. glaseri* (92.9%) (Prabhuraj, 2000). *B. subtilis* 26D was observed to disarrange the microbiome development and cause high mortality of *Leptinotarsa decemlineata* in potato (Sorokan *et al.,* 2016). *B. bassiana* is a promising entomopathogenic fungus that has a huge biocontrol potential against a broad spectrum of insect pests (Umamaheswari *et al.* 2020). Devi (2007) observed maximum larval mortality of *G.*

Table 1. Effect of EPN singly and in combination with microbial entomopathogens on leaf damage due to ash weevil in brinjal (cv. Lalita F1) under field conditions

Treatment	Corrected grub mortality (%)	Leaf area damaged (%)	Reduction in leaf damage (%)	Yield (t/ha)	Increase in yield (%)
T1 - Sg	65.3[d]	17.8[de]	73.9	35.8[cd]	14.6
T2 - Sc	54.2[f]	27.0[f]	60.6	32.8[gh]	5.2
T3 - Hi	74.8[b]	9.7[ab]	85.9	37.5[b]	20.1
T4 - Sg + Bb	56.2[f]	18.1[de]	73.5	33.4[fg]	7.1
T5 - Sc + Bb	42.6[g]	30.7[f]	55.1	32.3[h]	3.6
T6 - Hi + Bb	61.3[e]	12.6[bc]	81.6	35.1[e]	12.4
T7 - Sg + Bs	69.6[c]	14.0[cd]	79.5	36.2[c]	15.9
T8 - Sc + Bs	60.6[e]	22.0[e]	67.8	33.6[f]	7.8
T9 - Hi + Bs	78.9[a]	7.9[a]	88.5	38.4[a]	23.1
T10- Chlorpyriphos @ 2 ml lit^{-1}	62.8[de]	17.1[d]	75.0	35.5[de]	13.7
T11-Control	-	68.4[g]		31.2[i]	
CD (0.05)	2.82	4.29		0.89	

Hi – *H. indica*; Sg – *S. glaseri*; Sc – *S. carpocapsae*; Bb – *Beauveria bassiana*; Bs – *Bacillus subtilis*

mellonella upto 80% when EPN, *S. carpocapsae* and *H. indica* were applied in combination with *Beauveria bassiana*, *Nomuraea rileyi* and *Lecanicillium* sp. than when applied singly. Bhagat *et al.* (2008) evaluated different combinations of EPN *viz.* S. *carpocapsae, H. indica* and biopesticides *viz., B. bassiana* and *M. anisopliae* against *Agrotis ipsilon* in maize and found that H. *indica* + *M. anisopliae* treated plots recorded significantly higher yield compared to other biopesticides. Hence, similar results were observed in the present study revealing higher insect mortality with combination of EPN and biopesticides.

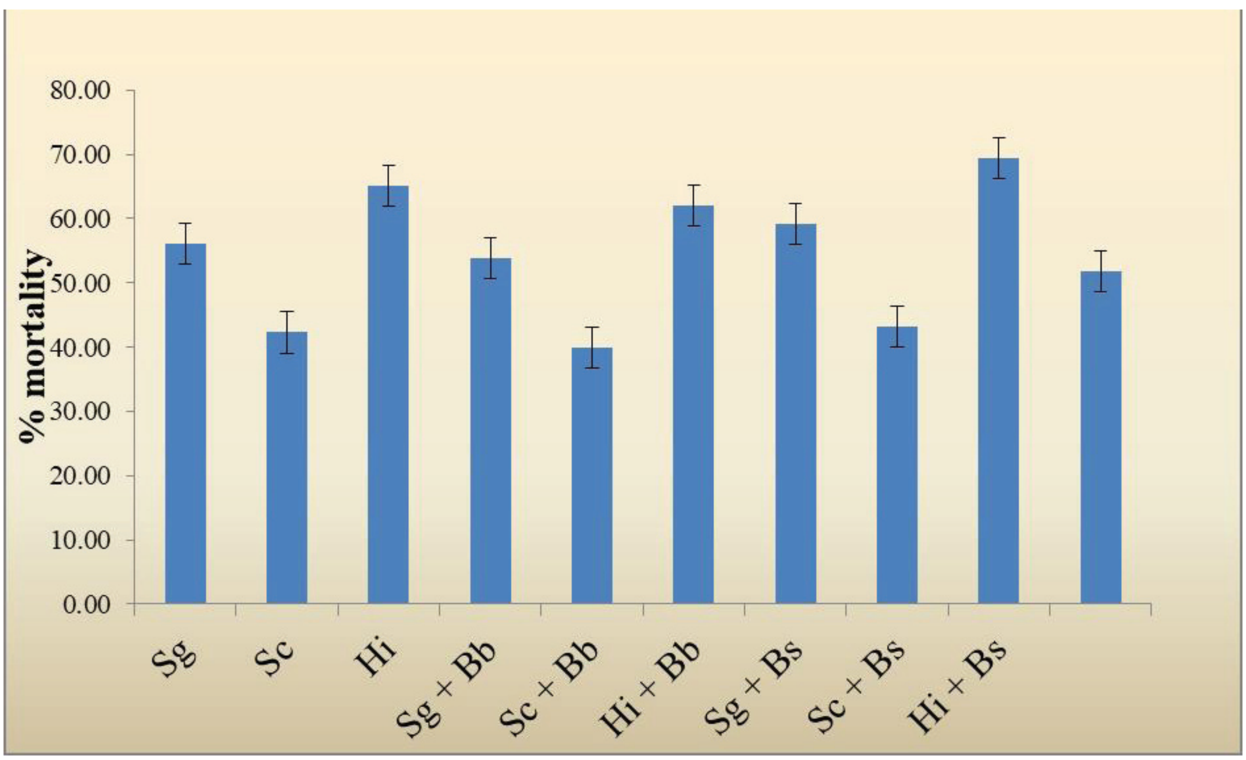

Fig . 1. Effect of EPN singly and in combination with microbial entomopathogens on mortality of ash weevils in brinjal (cv. Lalita F1)

CONCLUSION

Thus our study proves the biocontrol potential of EPN and its compatibility with other microbial biopesticides widening its scope as an excellent component in IPM of brinjal. Future line of work needs focus on development of effective formulations with longer shelf life and higher field efficacy.

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