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RESEARCH **Open Access**

Compatibility of entomopathogenic fungi and botanicals against sucking pests of okra: an ecofriendly approach

Jaydeep Halder*, Pratap A. Divekar and A. T. Rani

Abstract

Background: Okra, *Abelmoschus esculentus* (Linn.) Moench, is one of the most important traditional vegetables in India. The crop is attacked severely by sucking pests, viz., jassid, *Amrasca biguttula biguttula* Ishida, and whitefly, *Bemisia tabaci* Genn., throughout its growth period. To control these sucking pests, different entomopathogenic fungi (EPF), viz., *Beauveria bassiana*, *Metarhizium anisopliae*, and *Lecanicillium lecanii* were tested alone and their 1:1 combinations with neem oil against these sucking pests and compared to Imidacloprid 17.8% SL as chemical control under field conditions during 2018 and 2019.

Results: Among the EPF tested, the lowest jassid (1.16, 1.27 leaf^{-1}) and whitefly (1.33, 0.84 leaf^{-1}) population was recorded in plots treated with *L. lecanii* during the two consecutive years. Combination of *L. lecanii* and neem oil at half of their recommended concentrations had the lowest jassid and whitefly population among all the treatments and maximum reduction over control and at par with chemical control, i.e., Imidacloprid 17.8 SL at 0.33 ml l^{-1}. Moreover, all these biopesticides were found relatively safe to the polyphagous predators (*Micraspis discolor*, *Menochilus sexmaculatus*, and spiders) and at par with untreated control. In contrast, Imidacloprid 17.8 SL was the most toxic among all the treatments with the lowest numbers of spiders and ladybird beetles.

Conclusion: Combination of the EPF like *B. bassiana*, *M. anisopliae*, and *L. lecanii* with neem oil at half of their recommended concentrations could be a viable ecofriendly option in the management of the sucking pests of okra, along with the conservation of natural enemies.

Keywords: Entomopathogenic fungi, Neem oil, Okra, Jassids, Whitefly, Predators, Efficiency

Background

Okra, *Abelmoschus esculentus* (Linn.) Moench, also known as lady's finger (family: Malvaceae), is one of the most important traditional vegetables in India. It is a rich source of fiber, antioxidants, ascorbic acid, and folate. Okra is also a wonderful source of calcium, phosphorus, and potassium. The crop losses owing to insect pests are a major constraint in agricultural production and productivity. Among the different insect pests, sucking ones are gaining importance as besides sucking the sap and thereby devitalizing the plants, some serve as a vector in transmitting viral diseases (Rai et al. 2014). Okra jassid, *Amrasca biguttula biguttula* Ishida (Hemiptera: Cicadellidae), and whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), are important ones causing damage from early seedling to fruit maturity. Biological control of insect pests using different entomopathogenic microorganisms is gaining importance due to their target specificity, self-perpetuity, and obvious safety to the environment. The pest control prospects chiefly of entomopathogenic fungi (EPF), viz., *Beauveria bassiana*, *Metarhizium anisopliae*, and *Lecanicillium* (=*Verticillium*) *lecanii*, have been proved beyond doubt over the decades. Another important fact to be considered in favor of these EPF is that, to date, there has no report of

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developing resistance. Among the botanicals, neem, *Azadirachta indica* A. Juss, is gaining importance due to its diverse mode of action against a wide range of insect pests across the agricultural and horticultural crops, easy accessibility, and low cost of production (Halder et al. 2012). Literatures pertaining to the compatibility of different entomopathogens and neem oil against major vegetable sucking pests and their effect on the beneficial fauna are very scanty. Therefore, an attempt was made to find out the compatibility of different entomopathogens and neem oil alone against the major sucking pests of okra and their 1:1 combinations with neem oil to explore the possible role of compatibility, if any.

The present study aimed to evaluate the most potential bioagents, especially the EPF against the major sucking pests of okra and their compatibility with neem oil.

Methods

The field experiments were carried out on an experimental farm of ICAR-Indian Institute Vegetable Research, Varanasi (82° 52′ E longitude and 25° 12′ N latitude), Uttar Pradesh, India, during the rainy seasons (August to November) of 2018 and 2019. The experimental site comes under the alluvial zone of Indo-Gangetic plains having soils silt loam in texture and low inorganic carbon (0.43%) and available nitrogen (185 kg ha^{-1}). The experiment was laid out in randomized complete block design with three replications for each treatment. Seeds of okra (cv. Kashi Pragati) were sown in the plot size of 5 × 4-m area with plant to plant spacing of 45 cm and row to row distance of 60 cm during the first week of August. The recommended concentrations of fertilizers (N:P:K = 120:60:60) were applied as basal. Hand weeding and irrigations were provided as and when required, and usual crop husbandry measures were undertaken except plant protection measures.

Talc-based formulation of 3 entomopathogenic fungus (EPF), viz., *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) both commercial formulation (1×10^8 cfu g^{-1}) and NBAIR strain (1×10^{10} cfu g^{-1}), *Metarhizium anisopliae* (Metchnioff) Sorokin (Hypocreales: Clavicipitaceae) NBAIR strain (1×10^{10} cfu g^{-1}) and *Lecanicillium lecanii* R. Zare & W. Gams (Hypocreales: Clavicipitaceae) NBAIR strain (2×10^9 cfu g^{-1}) were considered for the experiments. Neem oil (0.5%) (Himedia Laboratories Pvt. Ltd., CAS No. 8002-65-1) was prepared by dissolving in emulsifying water containing Triton-X-100 (Himedia Laboratories Pvt. Ltd., CAS No. 9002-93-1) as an emulsifier. All the microbial insecticides at their recommended concentrations and neem oil (0.5%) alone and their 1:1 combinations with neem oil were tested for their efficacy against both sucking pests of okra. Both the nymphs and adults of okra jassids and adults of whiteflies were counted. In addition to these biopesticides, Imidacloprid 17.8% SL was taken as chemical control. The treatment details along with their concentrations were as follows: T1 = *B. bassiana* at 5 g l^{-1}; T2 = *M. anisopliae* @ 5 g l^{-1}, T3 = *L.* (=*Verticillium*) *lecanii* @ 5 g l^{-1}, T4 = *B. bassiana* (2.5 g l^{-1}) + neem oil (2.5 ml l^{-1}), T5 = *M. anisopliae* (2.5 g l^{-1}) + neem oil (2.5 ml l^{-1}), T6 = *L. lecanii* (2.5 g l^{-1}) + neem oil (2.5 ml l^{-1}), T7 = Imidacloprid 17.8% SL @ 0.33 ml l^{-1}, T8 = untreated control. A total of three rounds of sprayings were done at 15 days interval starting from seedling stage when whitefly and jassid infestation started. The spray solutions were prepared just before the application and the spraying was carried out with the help of pneumatic knapsack power sprayer during the evening hour using spray fluid @ 500 l ha^{-1}. The data were recorded 1 day before spray and 1, 3, 5, 7, 10, and 15 days after spray (DAS) from 3 leaves (top, middle and bottom) per plant, and 5 plants per plot were selected. In addition, the adult population of polyphagous predators and spiders, identified at ICAR-Indian Institute Vegetable Research, Varanasi, by the authors, were counted after each spray and expressed as a number of predators/plant. According to the IOBC (International Organization for Biological Control) classes of toxicity, the pesticides/biopesticides tested under the field conditions were classified as N: harmless or slightly harmful (0-50% reduction); M: moderately harmful (51-75% reduction), and T: harmful (75% reduction) (Boller et al. 2005). The critical difference (CD) at 5% level of significance was worked out with the SAS program (version 9.2) from the data of mean population before the spraying and subsequent various days' intervals after spraying.

Results

Bio-efficacy of the EPF and neem oil alone and their combinations on okra jassids and whiteflies

The effect of different EPF and neem oil alone and their 1:1 combinations against jassids and whiteflies are depicted in Tables 1 and 2. Significant differences were observed among different treatments against the jassid population (F = 14.79; df = 7; P < 0.05) in year 2018. Among the EPF tested, the lowest jassid population per leaf (1.16) was recorded in plots treated with white halo fungus, *L. lecanii*, at its recommended concentration and thereby registered the highest percent reduction over control (PROC) of 62.22 followed by *B. bassiana* (1.19 and 61.24, respectively) in 2018. When *L. lecanii* and neem oil were blended at half of their recommended concentrations and sprayed, the combination had the lowest jassids population (1.07 $leaf^{-1}$) among all the treatments and maximum (65.15) PROC. In case of the whitefly, *L. lecanii* alone and its combination with neem oil were the best in terms of high PROC and low whitefly population in 2018.

Table 1 Effect of different entomopathogenic fungi and neem oil alone and their 1:1 combination against major sucking pests of okra during 2018

Treatment	Jassids leaf^{-1a}						Whitefly leaf^{-1a}					
	Before spray	1st spray	2nd spray	3rd spray	Mean	PROC[b]	Before spray	1st spray	2nd spray	3rd spray	Mean	PROC
T1	3.18	1.28	1.42	0.87	1.19	61.24	2.56	1.05	1.09	2.03	1.39	48.52
T2	3.74	1.13	1.13	1.37	1.21	60.59	2.67	1.27	0.97	2.2	1.48	45.19
T3	3.89	1.19	1.28	1.01	1.16	62.22	2.49	1.17	0.95	1.87	1.33	50.74
T4	3.58	1.07	1.13	1.25	1.15	62.54	3.15	0.95	1.13	2.06	1.38	48.89
T5	3.44	1.23	1.23	1.05	1.17	61.89	3.57	0.88	0.99	2.45	1.44	46.67
T6	3.15	0.95	1.08	1.18	1.07	65.15	3.04	1.53	1.14	1.2	1.29	52.22
T7	3.59	1.33	1.35	0.98	1.22	60.26	2.98	1.84	1.15	1.39	1.46	45.93
T8	3.49	3.02	3.90	2.29	3.07	–	3.19	1.70	1.97	4.43	2.70	–
SEM (±)	–	–	–	–	0.31	–	–	–	–	–	0.19	–
LSD (5%)	–	–	–	–	0.67	–	–	–	–	–	0.47	–

[a]Mean of 15 observations over three sprays of different treatments at 15 days interval; [b]percent reduction over control (PROC) = ((pest population in control − pest population in treatment/pest population in control) × 100)

Next year, i.e., 2019, the experiment was replicated and significant differences were noted within the treatments against jassids (F = 26.31; df = 7; P< 0.05) and whiteflies (F = 36.26; df = 7; P< 0.05). The lowest jassid and whitefly population (1.27 and 0.84 leaf^{-1}, respectively) and the highest PROC (56.95 and 53.07, respectively) were recorded from the treatment T3, i.e., spraying of *L. lecanii* at its recommended concentration. Among all the treatments, *L. lecanii* (2.5 g l^{-1}) + neem oil (2.5 ml l^{-1}) was found superior in terms of management of whiteflies and jassids during both years. Combination of the EPF with neem oil was found compatible even at half of their recommended concentrations and could be a viable ecofriendly option in the management of these sucking pests. In paradox, spraying of Imidacloprid 17.8% SL at 0.33 ml l^{-1} was at par with these EPF alone and their combinations with neem oil.

Bio-efficacy of the EPF and neem oil alone and their combinations against predators

The predators collected from okra ecosystem were identified as predatory ladybird beetles, i.e., *Micraspis discolor* (Fabricius) and *Menochilus sexmaculatus* (Fabricius) (Syn: Cheilomenes sexmaculata (Fabricius)) (Coleoptera: Coccinellidae), and true spiders, viz., lynx (*Oxyopes lineatipes* (Koch)) (Araneae: Oxyopidae) and jumping spiders (*Marpissa* spp.) (Araneae: Salticidae).

The impact of the EPF alone and their combination with neem oil were studied against predatory ladybird

Table 2 Effect of different entomopathogenic fungi and neem oil alone and their 1:1 combination against major sucking pests of okra during 2019

Treatment	Jassids leaf^{-1}						Whitefly leaf^{-1}					
	Before spray	1st spray	2nd spray	3rd spray	Mean	PROC	Before spray	1st spray	2nd spray	3rd spray	Mean	PROC
T1	3.43	1.46	1.24	1.29	1.33	54.92	2.51	1.16	1.06	0.81	0.96	46.37
T2	3.22	1.51	1.30	1.36	1.39	52.88	2.47	1.11	1.03	0.82	0.93	48.04
T3	3.09	1.36	1.24	1.21	1.27	56.95	2.63	0.98	0.91	0.78	0.84	53.07
T4	3.36	1.31	1.16	1.16	1.21	59.32	3.13	0.93	0.81	0.72	0.77	56.98
T5	3.16	1.39	1.18	1.18	1.25	57.63	3.19	0.96	0.82	0.74	0.79	55.87
T6	3.47	1.26	1.05	1.08	1.13	62.76	3.16	0.89	0.79	0.66	0.73	59.22
T7	3.65	1.31	1.35	1.12	1.26	57.29	2.64	0.95	0.86	0.72	0.79	55.87
T8	3.70	3.54	3.03	2.28	2.95	–	3.16	2.24	1.82	1.46	1.79	–
SEM (±)	–	–	–	–	0.29	–	–	–	–	–	0.18	–
LSD (5%)	–	–	–	–	0.65	–	–	–	–	–	0.48	–

[a]Mean of 15 observations over three sprays of different treatments at 15 days interval; [b]percent reduction over control (PROC) = ((pest population in control − pest population in treatment/pest population in control) × 100)

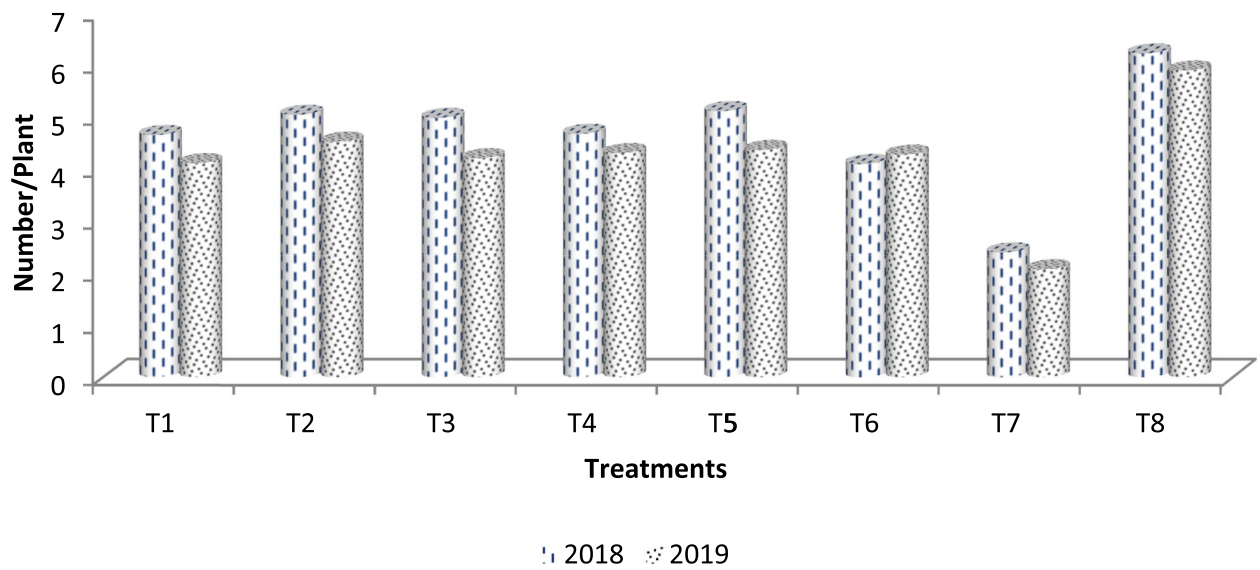

Fig. 1 Effect of different entomopathogenic fungi and neem oil alone and their combination (1:1) against *Menochilus sexmaculatus*

beetles and spiders (Figs. 1, 2, and 3). All these tested biopesticides alone or their combinations with neem oil were classified as N (harmless or slightly harmful) according to the IOBC categories for natural enemies, viz., lady bird beetles and spiders prevalent okra ecosystem as their reductions were < 50%. All the stages of these predators were abundant in the biopesticides treated and untreated control plots. In contrast, Imidacloprid 17.8 SL was the most toxic among the treatments. Imidacloprid-treated plots had the lowest numbers of spiders (1.43 and 1.07 plant^{-1} in 2018 and 2019, respectively), lady bird beetles, viz., *M. discolor* (0.56 and 0.49 plant^{-1}) and *M. sexmaculatus* (2.39 and 2.07 plant^{-1}), as compared to untreated control (4.28, 3.91, 2.87, 2.63, 6.22, and 5.89 plant^{-1}, respectively). Imidacloprid 17.8% SL was classified as M (moderately harmful) against *M. sexmaculatus* and spiders, and T (harmful) against *M. discolor* of IOBC categories for natural enemies.

Discussion

Bio-efficacy of the EPF and neem oil alone and their combinations on okra jassids and whiteflies

Three EPF alone and their combinations with neem oil were found effective against the nefarious sucking pests

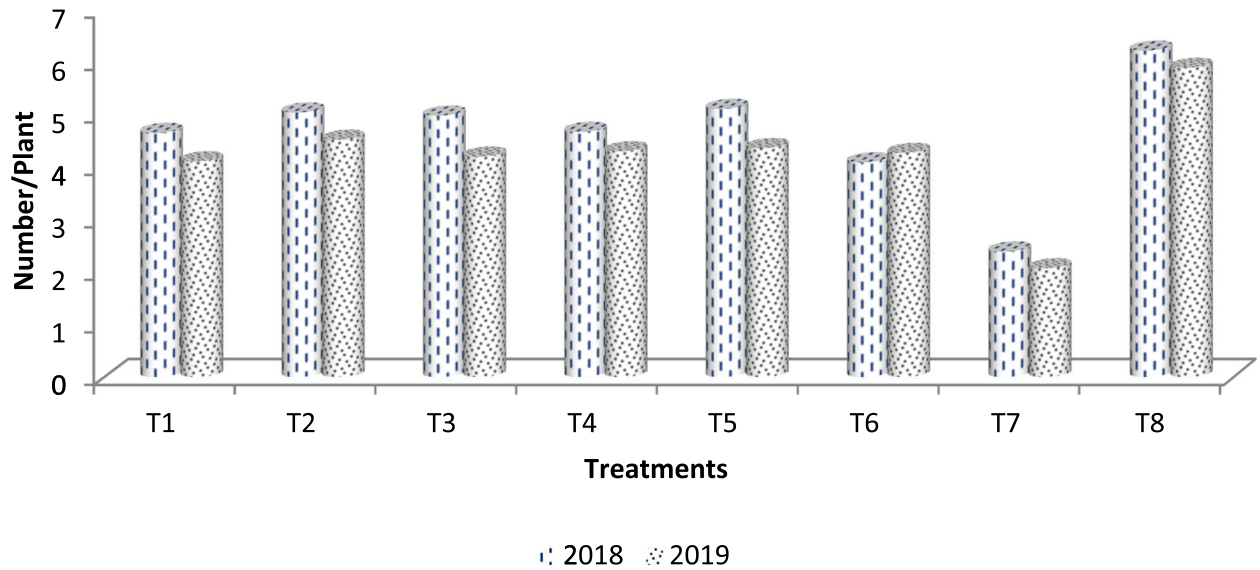

Fig. 2 Effect of different entomopathogenic fungi and neem oil alone and their combination (1:1) against *Micraspis discolor*

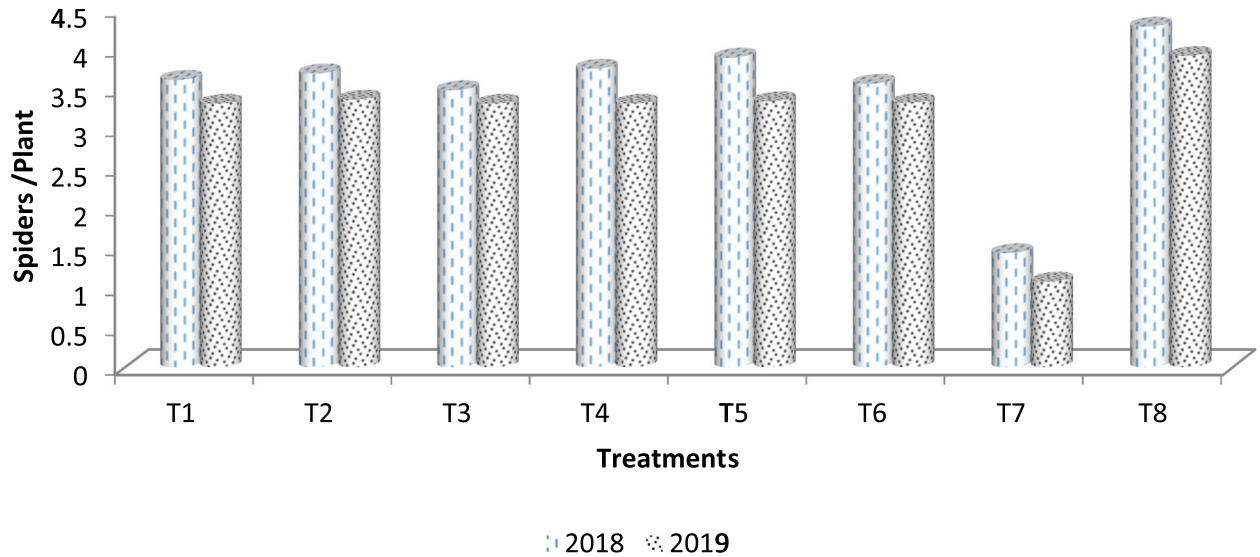

Fig. 3 Effect of different entomopathogenic fungi and neem oil alone and their combination (1:1) against spiders

of okra. *L. lecanii* was the most promising among the tested EPF under field conditions. Combination of *L. lecanii* with neem oil at half of their individual concentrations was the best treatment in reducing jassids and whiteflies infesting okra in 2018 and 2019. Raheem and Al-Keridis (2017) observed that *L. lecanii*, *B. bassiana*, and *M. anisopliae* isolates were promising as a fungal biocontrol agent (or pathogens) for whitefly control in the field. They also concluded that among the 3 EPF, *L. lecanii* was more virulence than others against *B. tabaci* infesting tomato. In another study, Scorsetti et al. (2008) documented that *L. lecanii* was highly virulent against *B. tabaci* and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) in organic and conventional horticultural crops in greenhouses and open fields in Argentina. White halo fungus at 7 g l^{-1} gave significantly a high mortality of okra jassid which was in conformity with the present findings (Baladaniya et al. 2010). Maketon et al. (2008) revealed that *M. anisopliae* (strain CKM-048) at the concentration of 1.25×10^{13} conidia ha^{-1} showed good controlling efficacy with the 73.33 ± 10% mortality rate of jassids, *A. biguttula biguttula* in aubergine, *Solanum aculeatissimum*.

Co-application of fungi like *B. bassiana*, *M. anisopliae*, and *L. lecanii* at suitable sub-lethal concentration of neem oil as two-in-one tank mix successfully employed against various insect pests to reduce the selection pressure in target pests. Compatibility of neem product and *Beauveria* against *B. tabaci* was studied (Islam et al. 2010) and highlighted that the highest adult deterrence index (80.15) and oviposition deterrence index (88.25) recorded when neem was combined with of *B. bassiana* (10^8 conidia ml^{-1}). Combinations of the EPF and neem oil (1,1) had lower LT_{50} values than each of their individual indicating the compatibility among them against *Epilachna dodecastigmata* and *Bagrada hilaris* under laboratory conditions (Halder et al. 2017). Neem-based formulation nimbecidine has been reported compatible with *B. bassiana* and *L. lecanii* (Subbulakshmi et al. 2012). In another in vitro study, Depieri et al. (2005) recorded the compatibility of emulsifiable neem oil, aqueous neem seed extracts, and leaves (0.15 and 1.5%) with *B. bassiana* and concluded that all the formulations had no effect on the fungus vegetative growth and on conidia production and viability. Many botanical insecticides including azadirachtin is having a diverse mode of action. The apparent enhancement in activity of neem oil and EPF mixtures was attributed to the possible additive, synergistic, and/or stabilizing effect of neem oil (Halder et al. 2012).

Interestingly, bio-efficacy of Imidacloprid 17.8% SL was at par with the different biopesticides, botanicals, and their combinations. This first generation neonicotinoid, Imidacloprid are being used in the region over a decade. Local farmers frequently applied this insecticide more than their recommended concentration. Due to long-term regular use of this neonicotinoid in agri-horticultural ecosystem of the region caused development of resistance among sucking pests. The green peach aphid, *Myzus persicae* (Sulzer), has developed 5.90-folds resistance against Imidacloprid 17.8% SL during 2010–2018 in Varanasi region (Halder and Rai 2018). Many local farmers also viewed the lower efficacy of Imidacloprid 17.8% SL in recent years. In paradox, microbial insecticides like EPF seldom used for pest management in the area. The reason could be non-

availability of suitable biocontrol agents in the local market and lack of knowledge about their usage (Roy et al. 2017). So, using relatively newer control method in the region, i.e., spraying of EPF alone and combinations with neem oil against the sucking pests of okra, might be the reason for superior result.

Bio-efficacy of the EPF and neem oil alone and their combinations against predators

Biopesticides are promising alternatives to chemical pesticides, and they have opened up new avenue in insect pest management to aid in the promotion of safe, eco-friendly pest management (Prithiva et al. 2018). They are relatively host-specific and do not interfere with other living organisms. In the present experiment, all the 3 EPF alone and in combination with neem oil was found relatively safe to the polyphagous predators under field conditions. The present findings were in accordance with Thungrabeab and Tongma (2007), who reported that *B. bassiana* (Bb.5335) and *M. anisopliae* (Ma.7965) were relatively safe on non-target natural enemies, viz., *Coccinella septempunctata* L., *Chrysoperla carnea* (Stephens) and *Dicyphus tamaninii* Wagner, and beneficial soil insect *Heteromurus nitidus* Templeton. Oil formulation of *B. bassiana* (Bb 112) was found safe to predatory *Cryptolaemus montrouzieri* adults with the highest adult survival of 92.62% at the highest concentration (10^8 spores ml^{-1}) and 100% survival at the lowest concentration tested (10^4 spores ml^{-1}) (Prithiva et al. 2018). In another study, Brown and Khan (2009) reported that *M. anisopliae* isolate was safe against *C. montrouzieri*. Toxicity of Imidacloprid to polyphagous predator, *C. carnea* was studied by Preetha et al. 2009. They recorded that Imidacloprid at the recommended concentration of 0.28 ml/l caused 15.38% egg mortality, 26.67 and 33.33% larval mortality by ingestion and contact, respectively, and 50.00% adult mortality. Imidacloprid was also reported to affect the longevity of *C. carnea* adults (Mathirajan and Regupathy 2002). Toxicity of Imidacloprid to these predators could be the reason for their low population in the respective treatment.

Conclusion

Combination of the entomopathogenic fungi like *B. bassiana*, *M. anisopliae*, and *L. lecanii* with neem oil at half of their recommended concentrations could be a viable ecofriendly option in the management of the sucking pests of okra along with conservation of natural enemies.

Abbreviations

EPF: Entomopathogenic fungi; N: Nitrogen; P: Phosphorus; K: Potassium; cfu: Colony-forming unit; PROC: Percent reduction over control

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Authors' contributions

JH and ATR designed the research. JH and PAD conducted the experiments under field conditions and took the data. JH and PAD statistically analyzed the data. JH, ATR, and PAD wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All experimental works were approved by ICAR-Indian Institute of Vegetable Research, Varanasi, India. Committee's reference number: not applicable

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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