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Interaction between entomopathogenic nematodes and entomopathogenic fungi in biocontrol mechanism

Gitanjali Devi**Abstract**

Entomopathogenic nematodes are the most important biocontrol agents for the management of insect pest population which can be utilized as a tool in integrated pest management programme. Therefore interaction with other biocontrol agent specially entomopathogenic fungi reflects the success or failure of biocontrol mechanism. This review focuses on different types of interaction of entomopathogenic nematodes with entomopathogenic fungi.

Keywords: Entomopathogenic nematodes (EPNs), entomopathogenic fungi (EPF), insect pest management, interaction, biological control

Introduction

Entomopathogenic nematodes (EPNs) are able to either kill, or hamper the completion of life-cycle of insect. The genera *Steinernema* and *Heterorhabditis* in the family Steinernematidae and Heterorhabditidae of the order Rhabditida are obligate parasites of insect pests. They are distributed all over the world in natural and agricultural soils. They have huge potential as biocontrol agent against a many insect pests of agricultural crops due to their high reproductive capacity, ease of mass culture and their harmlessness to environment. The only free-living stage in the soil is the third stage juvenile which is the infective stage. They are having closed mouth and anus and encased in a double cuticle and are capable of surviving for several weeks in the soil, before infecting a new host. The infective juveniles actively enter through the mid gut wall or tracheae into the insect body cavity to get insect haemolymph. EPNs have a mutualistic association with gram-negative gamma-proteobacteria in the family Enterobacteriaceae and are carrying in their intestines ^[1]. *Xenorhabdus* bacteria are associated with steinernematid nematodes while *Photorhabdus* are associated with heterorhabditids and thereby release the bacteria in the haemolymph. The host insect is killed within 24-48 hours by overcoming the insect immune system ^[2]. The bacteria cause septicemia, and provide food sources for the nematode. During the process, the bacteria produce antibiotics and provide a protected place and suppress the competition from other microorganisms ^[3]. After exhaustion of all nutrients; infective juveniles develop into high population and coming outside the cadaver. The life cycle of heterorhabditids is that IJs always develop into self-reproducing hermaphrodites whereas Steinernematids are amphimictic ^[4]. Strauch *et al.* ^[5] observed that progeny of the first generation hermaphrodites of heterorhabditids can either develop into amphimictic adults or into automictic hermaphrodite both. The pathogenic ability of different species of EPNs varies towards a range of insect order, microclimatic condition, as well as in terms of their strength as commercial products ^[6].

Entomopathogenic fungi (EPF) are important microbial agents against many insect pests ^[7]. There are about 90 genera and 700 species of EPF ^[8]. *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin, *Lecanicillium*, *Isaria fumosorosea* Wize and *Metarhizium anisopliae* (Metschnikoff) Sorokin, are being exploited against insect pests ^[9]. The conidia attaches and penetrates the insect integuments that is achieved by formation of an appressorium ^[10]. Though there is host defense mechanism inside insect host, EPF can cause mortality in the target pest by overcoming the mechanisms ^[11]. Several species of Isoptera ^[12, 13], Lepidoptera ^[14-16], Coleoptera ^[17], Hemiptera ^[18], and Diptera ^[14, 19] are susceptible to various EPF species. Efficacy of biocontrol mechanism increases with combined application of several biocontrol agents instead of one ^[20]. In this regard, entomopathogenic nematodes in combination with

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other biocontrol agents increase their efficacy against insect pests. Entomopathogenic nematodes have been evaluated in combination with insecticides [21-23], biocontrol agents [24, 25], and parasitoids [26, 27]. Stiling [28] observed that combination of different entomopathogenic species and other biological control agents increased the mortality of target insect pests. This promising approach is also a 'dual attack' approach which reduces the application rate of bioagents and the host killing time.

Advantages of biocontrol agents applied in combination

- Different modes of action of different biocontrol agent against the target insect pest.
- More than one stage of the life cycle of the target pest may be effected by different biocontrol agent
- Activity and performance of bioagents are different during different the growing season and soil condition.
- Biocontrol agents are having the ability for persistence in soil even in combination.

Type of interaction (Table.1)

Additive interaction

Koppenhofer and Grewal [29] stated that additive effects are more of independent and magnitude are added up with no increment when there is a combination of two.

Synergistic interaction

When two biocontrol agents are applied together to create an overall effect which is greater than the sum of their individual effects.

Antagonistic interaction

Antagonistic interaction is when the net effect of both organisms is zero. There are two types of antagonistic interaction. Direct antagonism is the infection or predation of entomopathogenic nematodes by another organism, where as indirect antagonism occurs during competition (either interference or exploitation) for resources and spaces [30]. Progeny production in nematodes and fungi are less in infected hosts [31] because the nematodes symbiotic bacteria excluded the other bioagent, the agent infecting first, often excluding the other. The antibiotics produced by *Xenorhabdus nematophilus* or *Photorhabdus luminescens* inhibited the growth of *B. bassiana*. If the fungus was applied before the nematode, the antagonistic interactions between *B. bassiana* and *S. carpocapsae* or *H. bacteriophora* were dependant on temperature [32]. On the other hand, fungal antagonism on the nematodes may be antibiosis (the production of mycotoxins) [33]. *B. bassiana* infected hosts in soil are better avoided by entomopathogenic nematodes. *Photorhabdus luminiscens* is able to inhibit the growth and reproduction of *Beauveria bassiana*, *B. brongniarti* and *Paecilomyces fumosoroseus*, whereas *Xenorhabdus poinari* does not [31].

In some cases, the combination of two different pathogens does not provide an additive or synergistic effect. In a soil test, *S. carpocapsae* and *B. bassiana* combination produced about the same level of mortality as *S. carpocapsae* alone [31].

Mode of action during interaction

It has been observed that in combine application of antagonists, one antagonist may alter feeding behaviour or movement of the target insect, and thereby more susceptible to the other antagonist. Steinhaus [34] was the first to observe that stressed insects are more susceptible to antagonists. After

infection, EPF reduces locomotion, feeding and increasing irritability of target insect [35]. *M. anisopliae* infected insects are less mobile; thereby EPNs can penetrate the host more easily [36]. Scarab reduction by both EPNs and EPF is that the scarab larvae may have been stressed [37-40]. The body length of insects is another factor that are infected already by EPF, are more debilitated, respire more and thus susceptible to the EPNs. In combine application conidia production of EPF reduce by the activities *Photorhabdus luminescens* [41]. Shapiro *et al.*, [42] observed that the antagonistic interactions between two entomopathogens may be a result of toxins. Similarly, Hu and Webster [43] showed that during the first 24 h of infection by the nematode *H. megidis*, *Photorhabdus luminescens* (strain C9) activity, producing antibiotic compounds and minimizing competition from other microorganisms. Isaacson and Webster [44] also showed that *Xenorhabdus* associated with *Steinernema riobrave* produced antibiotic and anti-fungal compounds. A virulent strain of nematode can colonize host more rapidly and suppress the development of the fungus. But the combination of two highly virulent antagonists does not result in the rapid host mortality. Only the combination of a highly virulent nematode with a moderately virulent fungus or vice versa can cause rapid host death. Therefore careful selection of bioagent strains is essential before field testing is made. The EPN infective juvenile and fungal conidia released from infected cadavers can recycle populations, and thus provide longer term protection to crops.

Factors effecting interaction

Interaction between EPNs and EPF depends on the EPN species/strains, EPF species, target insect host, application parameters, and environmental conditions [17, 36, 40, 42].

Insect defense mechanism

The interaction might be due to overcoming the mechanism of larval cellular and humoral defense system. Phagocytosis, nodule formation, cellular encapsulation, melanotic encapsulation, and the production of antimicrobial peptides are some of the defense mechanism. Against nematodes encapsulation and against bacteria phagocytosis is the immediate response [45]. Besides these, injury and microbe infection leads to production of antimicrobial peptides in insect. However, defense responses vary with the species of insect and antagonists involved and their physiological states [46, 47].

Time of application

In some studies, the simultaneous application of nematodes with EPF [32] was found to be successful. Additivity in *Curculio caryae* mortality was achieved following simultaneous application of pathogens [42]. However, the combination of EPNs and EPF in simultaneous applications was unsuccessful in woolly apple aphid (WAA) control [48]. Additivity or slight synergy was achieved between EPNs and *M. anisopliae* against *Holotrichia consanguinea* in simultaneous application [49].

When *M. anisopliae* and *B. bassiana* were applied one week after applying *S. glaseri* and *H. megidis* additive effects were observed and when fungi were applied four weeks after the nematodes synergism were observed [36]. Thus sequential application increased additive or synergistic effects [40]. It was observed that *M. anisopliae* was the most effective fungus against *Eriosoma lanigerum*. Combining *S.*

yirgalamense with *B. bassiana* and *M. anisopliae* was unsuccessful for controlling *Eriosoma lanigerum* [50]. Hence, *M. anisopliae* could be used alone for the management of population of WAA, rather in combination with nematodes.

Local isolate of EPN and EPF

The local isolates, with higher pathogenicity should be utilized than that of the exotic commercial isolates for specific insect control.

Insect host

In non-soil tests, dual infection with *N. carpocapsae* and *Paecilomyces farinosus* and with *N. carpocapsae* and with *B. bassiana* produces accelerated and higher mortality of a susceptible insect species *G. mellonella*. This intensification effect is not observed in *Tribolium castaneum* with *N. carpocapsae* and *B. bassiana* or in *T. castaneum* or *Trogoderma granarium* with *N. carpocapsae* and *P. farinosus* indicating that the insect host has an effect on this interaction between nematodes and fungus [51, 52].

Table 1: Interaction of Entomopathogenic nematode with Entomopathogenic fungi

Entomopathogenic Nematode	Entomopathogenic Fungi	Insect pest	Type of Interaction	References
<i>Heterorhabditis megidis</i> <i>Steinernema glaseri</i>	<i>Metarhizium anisopliae</i> CLO53	<i>Hoplia philanthus</i>	Additive or Synergistic	[17]
<i>H. bacteriophora</i>	<i>Beauveria bassiana</i>	<i>Spodoptera exigua</i>	Additive	[31]
<i>S. feltiae</i> <i>H. heliothidis</i>	<i>B. bassiana</i>	<i>Galleria mellonella</i>	Antagonistic	[32]
<i>H. Megidis</i> <i>S. glaseri</i>	<i>M. anisopliae</i> <i>B. bassiana</i>	<i>H. philanthus</i>	Synergistic and/or additive	[36]
<i>S. yirgalamense</i>	<i>B. bassiana</i>	<i>Eriosoma lanigerum</i>	Antagonistic	[40, 54]
<i>S. yirgalamense</i>	<i>M. anisopliae</i>			
<i>H. indica</i> <i>S. carpocapsae</i>	<i>Paecilomyces fumosoroseus</i>	<i>Curculio caryae</i>	Antagonism	[42]
<i>H. indica</i>	<i>M. anisopliae</i>	<i>Curculio caryae</i>	Additive	
<i>H. indica</i> <i>S. carpocapsae</i>	<i>B. bassiana</i>		Antagonistic	
<i>H. bacteriophora</i>	<i>M. anisopliae</i>	<i>Holotrichia consanguinea</i>	Additivity or slight synergy	[49]
<i>Neoalectana carpocapsae</i>	<i>P. farinosus</i>	<i>Galleria mellonella</i>	Synergistic	[51]
<i>N. carpocapsae</i>	<i>P. farinosus</i>	<i>Tribolium castaneum</i> <i>Trogoderma granarium</i>	Antagonistic	
<i>N. carpocapsae</i>	<i>B. bassiana</i>	<i>Tribolium castaneum</i>	Antagonistic	[52]
<i>H. bacteriophora</i> JPM4	<i>M. anisopliae</i>	<i>Diatraea saccharalis</i>	Synergistic	[55]
<i>H. bacteriophora</i> <i>S. yirgalamense</i>	<i>M. anisopliae</i> <i>B. bassiana</i>	<i>Coptognathus curtippennis</i>	Synergistic	[56]
<i>H. bacteriophora</i> <i>S. feltiae</i> <i>S. kraussei</i>	<i>M. anisopliae</i> V275	<i>Otiorhynchus sulcatus</i>	Synergistic Additive	[57]
<i>S. kraussei</i>	<i>M. anisopliae</i>	<i>Otiorhynchus sulcatus</i>	Synergistic	[58]
<i>Steinernema</i> sp.	<i>B. bassiana</i>	<i>Galleria mellonella</i>	Additive	[59]
<i>S. carpocapsae</i>	<i>B. brongniartii</i>	<i>Ectinohoplia rufipes</i> <i>Exomala orientalis</i>	Synergistic	[60]
<i>S. thermophilum</i>	<i>B. bassiana</i> <i>M. anisopliae</i>	Solid growth media	Antagonistic	[61]
<i>H. zealandica</i>	<i>B. bassiana</i>	<i>Galleria mellonella</i>	Synergistic	[62]
<i>Heterorhabditis</i> sp.	<i>M. anisopliae</i>	<i>Oryctes rhinoceros</i>	Synergistic	[63]
<i>H. bacteriophora</i> <i>S. carpocapsae</i> <i>S. feltiae</i> <i>S. sp.</i>	<i>M. anisopliae</i>	<i>Curculio nucum</i>	No Antagonistic or Synergistic	[64]
<i>H. bacteriophora</i>	<i>B. bassiana</i> simultaneous application Delayed application	<i>Rhynchophorus ferrugineus</i>	Additive Synergistic Additive Synergistic	[65]
<i>H. bacteriophora</i>	<i>M. anisopliae</i> simultaneous application Delayed application			
<i>H. bacteriophora</i>	<i>M. anisopliae</i> <i>B. bassiana</i>	<i>Otiorhynchus sulcatus</i>	Synergistic	[66]
<i>H. bacteriophora</i> <i>S. carpocapsae</i>	<i>M. anisopliae</i> <i>B. bassiana</i>	<i>Curculio elephas</i>	Synergistic	[67]
<i>H. bacteriophora</i> HNI0100	<i>M. anisopliae</i> Ma 9236 <i>B. bassiana</i> Bb 9205	<i>Plutella xylostella</i>	Synergistic	[68, 69]
<i>H. bacteriophora</i>	<i>Nomuraea rileyi</i>	<i>Spodoptera frugiperda</i>	Antagonistic	[70]
<i>S. ichnusae</i>	<i>B. bassiana</i>	<i>G. mellonella</i>	Antagonistic	[71]
<i>N. dutkyi</i>	<i>M. anisopliae</i> <i>B. bassiana</i>	<i>Curculio caryae</i>	Synergistic	[72]
<i>H. bacteriophora</i>	<i>B. bassiana</i>	<i>Rhynchophorus ferrugineus</i>	Synergistic	[73]

Conclusion

Whether the combination of entomopathogenic nematodes and entomopathogenic fungi will be used in integrated management plan will depend on the interaction of individual agent, efficacy of their combination, its cost of production in comparison with chemical insecticides. The combination should also be cheaper than either single bioagent alone at the same efficacy level. Before application, one should know whether releasing one natural enemy against a pest is likely to be more effective than the release of many, where competition between enemies might reduce their overall effectiveness^[53]. We should try to reduce both fungus and nematode application rate to about one fourth of recommended rate and still get more than 80% insect mortality. The interactions with these other microbiological agents in non soil and soil conditions must be understood before their use.

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