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Entomopathogenic nematodes and entomopathogenic bacteria, *Bacillus thuringiensis* interaction effect on insect population

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Abstract

Entomopathogenic nematodes and entomopathogenic bacteria, Bacillus thuringiensis are extensively used biocontrol agent against a wide range of insect pest. The two biocontrol agents are used in combination so that there increase in their efficacy and reduction in cost of production. This review attempts to highlight the work done in different insect pest management programme.

Keywords: Entomopathogenic nematodes (EPNs), entomopathogenic bacteria, *Bacillus thuringiensis*, interaction on insect mortality, insect pests

Introduction

Microbial entomopathogens like entomopathogenic nematodes and entomopathogenic bacteria are widely used as potential biocontrol agent against a wide range of insect pests. The intervention of more than one biocontrol agent can enhance the efficacy of the other partners; many studies have been conducted in this regard. The goal of combining other control agents with EPNs is effective pest control with reduced use of hazardous synthetic insecticides, increased consistency and control levels, and lower costs through reduced rates of EPNs and /or chemicals. The combination of two controlling agents could have three different effects: synergistic, antagonistic or additive. Beline (2018) [4] reported that entomopathogens and other biological control agents can be synergistic, additive, or antagonistic depending on the specific biological control agents as well as their rate, timing of application, and the host species. As demonstrated by Ferguson and Stiling (1996) [6], synergistic interactions result in a higher mortality than the combined individual mortalities of the pest population. Additive interactions occur if the natural enemies do not interact, and thus, the total level of mortality is equivalent to the combined individual mortalities caused by each agent. The antagonistic interactions occur if the total mortality is less than when either natural enemy acts alone. Roy and Pell (2000) [22] reported that synergistic interactions between pathogens and insect predators or parasitoids can enhance control efficacy, whereas antagonistic interactions reduce total control efficacy. Interactions between biopesticides vary in nature: they might become more effective (synergistic); they might have no interaction (additive, or complementary); or they might be less effective than when they are used separately (antagonistic) (Koppenhofer and Grewal 2005) [11]. Two control agents applied together might act independently of one another against a given pest, and their effects would be additive. This type of response will be observed if the action sites of the two components differ, i.e. if each one has a completely different mode of action and these modes of action are totally independent. They also might interact synergistically or antagonistically, thus rendering the combination more or less effective in control than in the case of an additive effect.

Entomopathogenic Nematodes

Entomopathogenic nematodes in the families steinernematidae and heterorhabditidae are soil inhabiting insect pathogens that possess potential as biological control agents. Nematodes, working with their symbiotic bacteria, kill insects in 24-48 hr. The non-feeding infective Juvenile seeks out insect hosts; when a host has been located, the nematode penetrates into the insect body, usually through natural body openings (mouth, anus, spiracles) or areas of thin cuticle. Once in the body cavity, a symbiotic bacterium (*Xenorhabdus* for steinernematidae and *Photorhabdus* for heterorhabditis) is released from the nematode, multiplies rapidly and causes rapid insect death.

The nematodes feed upon the bacteria and liquefying insect; and mature into adults. Thus, entomopathogenic nematodes are a nematode-bacterium complex.

Selection of an EPN for control of a particular pest insect is based on several factors that include the nematode's host range, host finding or foraging strategy, tolerance of environmental factors and their effects on survival and efficacy (temperature, moisture, soil type, exposure to ultraviolet light, salinity and organic content of soil, means of application, agrochemicals, and others). The four most critical factors are moisture, temperature, pathogenicity for the targeted insect, and foraging strategy. Besides these, interactions with many other soil organisms may affect EPN performance. Interactions between EPNs and other biological control agents can be synergistic, additive or antagonistic, depending on the specific biocontrol agents as well as their rates and timing and host species, depending on the specific EPN target pest combination as well as the application strategy(mixture/sequential, dose rate etc). The use of combined biocontrol agents could be a potential strategy to reduce pest resistance caused by intensive use of chemical insecticides and to manage restrictions of current insecticides. The opportunities for using entomopathogenic nematodes against insect pests in the soil and cryptic habitats in agricultural pest are excellent. Entomopathogenic nematodes appear to be compatible with many herbicides, fungicides, acaricides, insecticides, nematicides, *Bacillus thuringiensis* (Kaya *et al*., 1995) [9]. The combination of EPNs and other control agents has proved to be synergistic and produces higher mortality than either agent alone.

Entomopathogenic bacteria

Bacillus thuringiensis

The invertebrate pathogen, *Bacillus thuringiensis* is also one of the most commonly used biological control agent proving its efficacy against many insect species with no adverse effect on beneficial species. Crystal (Cry) toxins produced by the *Bacillus thuringiensis* (Bt) are a large family of pore-forming toxins (PFTs) that target the intestinal cells of insects (Schnepf *et al*., 1998; Rosasgarcia, 2009) [24, 21]. In nature, *B. thuringiensis* vegetative cells, are taken up by the larva by ingestion or more accidentally by wounding through the cuticle. *Bacillus thuringiensis* subsp. *aizawai*, *B. thuringiensis* subsp. *kurstaki*, *B. thuringiensis* subsp. *israelensis*, *B. thuringiensis* subsp. *sphaericus*, and *B. thuringiensis* subsp. *tenebrionis* are effectively used for controlling different groups of target insects. For example, *Bacillus thuringiensis* subsp. *aizawai*, and *B. thuringiensis* subsp. *kurstaki* are effective against caterpillars, *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *sphaericus* target mosquito larvae, and *B. thuringiensis* subsp. *tenebrionis* is effective against some coleopterans. *Cyclocephala hirta* is not very susceptible, *C. pasadenae* has intermediate susceptibility, *Anomala orientalis* is highly susceptible to *B. thuringiensis* subsp. *japonensis*. Early instars of *Plutella xylostella* are susceptible to commercial *B. thuringiensis* subsp. *kurstaki* the high and specific toxicity makes *B. thuringiensis* a leading biocontrol agent. The primary reason for the utilization of *B. thuringiensis* is fast acting, easy to produce at low cost, easy to formulate, and has a long shelf life. It also can be applied using conventional application equipment and systemics (i.e. in transgenic plants). *B. thuringiensis* toxins are selective and negative environmental impact is very limited. There are currently no less than 73 families of crystal (CRY) toxins comprising a total of 732 toxins, 3 families of cytotoxic (Cyt) proteins including 38 different toxins and 125 Vegetative Insecticidal Proteins (VIPs) belonging to 4 different families (Crickmore *et al*., 2014) [5]. When Bt is ingested, alkaline conditions in the insect gut (pH 8-11) activate the toxic protein (delta-endotoxin) that attaches to the receptors sites in the midgut and creates pore in midgut cells. This leads to the loss of osmoregulation, midgut paralysis, and cell lysis. Contents of the gut leak into insect's body cavity (hemocoel) and the hemolymph leaks into the gut disrupting the pH balance. Bacteria that enter body cavity cause septicemia and eventual death of the host insect. Insects show different kinds of responses to Bt toxins depending on the crystal proteins (delta-endotoxin), receptor sites, production of other toxins (exotoxins), and requirement of spore.

Interaction between Entomopathogenic nematodes and *Bacillus thuringiensis*

The combined effects of two pathogens on overall insect mortality have been well documented. The combination of EPNs and other control agents has proved to be synergistic and produces higher mortality than either agent alone (Kaya and Koppenhofer, 1996; Koppenhofer, 2003; Koppenhofer and Grewal, 2005; Koppenhofer and Wu, 2017) [10, 13, 11, 12].

The idea of contaminating nematode infective juveniles with *Bacillus thuringiensis* may help to provide another pathway for *Bacillus thuringiensis* spores to reach the insect haemocoel avoiding any other obstacle preventing this process when ingested. Natural infection involving *Photorhabdus / Xenorhabdus* mainly starts from the body cavity, since they are released at that site from the nematode hosts. *B. thuringiensis* causes feeding inhibition due to midgut damage in the treated larvae. Two pathogens inside the insect haemocoel may result better control results. Pesticides based on *Bt* and entomopathogenic nematodes are often used simultaneously, and most researchers consider these two plant protection agents to be fully compatible, with their synergistic effect having been described (Koppenhofer, 2003) [13].

The results from a combined application of a low dose of the commercial preparation IMC 10,001.1, containing β-exotoxin of *Bacillus thuringiensis* var. *thuringiensis* with a low inoculum of *Neoaplectana carpocapsae* DD-136 suggested a possible synergistic action of the two substances and resulted in an increased percentage of mortality of third and fourth instar larvae of *Tipula paludosa* under laboratory condition (Lam and Webster, 1972) [16]. Combination application of the nematode *Neoaplectana carpocapsae* and *B. thuringiensis* var. *kurstaki* did not result in significantly greater control than that achieved by the nematode used alone against the artichoke plume moth under field condition (Bari & Kaya, 1984) [2]. The interaction between the *Steinernema feltiae* and *Bacillus thuringiensis* subsp. *kurstaki* on *Spodoptera exigua* was investigated. *S. feltiae* did not produce progeny in *B. thuringiensis*-infected hosts (neonate larvae of *Spodoptera exigua*). Those hosts which had a dual infection had *Bacillus thuringiensis* infection in the anterior part and *S. feltiae* infection in the posterior part of the body. In general, *B. thuringiensis* killed insects were not satisfactory hosts for *S. feltiae* (Kaya & Burlando, 1989) [8]. When the insect host was exposed to *Bt* and nematode simultaneously, dual infections occurred. The developing nematodes in *Bt*-infected insects were smaller and more hyaline, and had less food reserves stored in their intestinal cells than those of the controls

Nematode development *N. carpocapsae*, *H. heliothidis* in larvae of the elm-leaf beetle and wax-moth larvae that were simultaneously infected with *Bacillus thuringiensis* var San Diego, *israelensis* and *kurstaki* was reduced considerably. Such reductions were dependent on the timing of the initial infections of the two organisms. When nematodes were allowed to enter wax-moth larvae 24 h before B.t. kurstaki was introduced, nematode development was almost normal (Poinar *et al*., 1990) [20]. After combining and immediately applying both the nematode (*S. carpocapsae*, *H. bacteriophora*) and *Bacillus thuringiensis* subsp. *kurstaki*, positive results were obtained against insects in the soil and on foliage (*Cyclocephala hirta*, *Otiorhynchus sulcatus*, *Trichoplusia ni*) (Kaya *et al*., 1995) [9]. Koppenhofer and Kaya (1997) [15] have demonstrated an additive or synergistic interaction between *B. thuringiensis* subsp. *japonensis* (Btj) and *H. bacteriophora* or *Steinernema glaseri* (Steiner) on white grubs, *Cyclocephala hirta* and *Cyclocephala pasadenae.* Koppenhofer and Kaya (1997) [15] showed additive and synergistic interaction between EPNs (*H. bacteriophora*, *S. glaseri* or *S. kushidai*) and *B. thuringiensis* subsp. *japonensis* Buibui strain for scarab grub (*Cyclocephala hirta* and *C. pasadenae*) control. To achieve additive or synergistic effects, larvae had to be exposed to Btj for at least 7 days before the addition of nematodes. This interaction was observed between Btj and *H. bacteriophora* or *S. glaseri*, but not with the most pathogenic nematode, *S. kushidai*. Combination application of both the nematode *H. bacteriophora* HP88 and the bacteria *B. thuringiensis* var. *kurstaki* did not result in significantly greater control of black cutworm than that achieved by the nematodes used alone under laboratory condition (Shamseldean and Ismail, 1997) [26]. Combined treatment (*Bacillus thuringiensis* subsp. *kurstaki* and nematodes *Steinernema carpocapsae* All both at half rate) resulted in 58% control of *Plutella xylostella in* field trials conducted on watercress (*Rorippa nasturtium aquaticum*) (Baur *et al*., 1998) [3]. *Bacillus thuringiensis* sub sp. *japonensis* (Btj) combined with EPNs (*H. bacteriophora* and *S. glaseri*) overall resulted in weak synergistic effects against third instars of different white grub species *Cyclocephala hirta*, *C. pasadenae*, *Anomala orientalis*. The combination should be more effective or equally effective at lower rates when applied against grubs, i.e., young third instars or second instars. Combinations of nematodes and Btj at economic application rates provided acceptable control levels whether applied simultaneously or with a 4-day delay between Btj and nematode application (Koppenhofer *et al*., 1999) [14]. Simultaneous application of *S. carpocapsae* and *Bacillus thuringiensis* subsp *israelensis* against early instars of *Tipula paludosa* under field condition were found to be successful and economically feasible (Oestergaard *et al*., 2000) [19]. Schroer *et al*. (2005) [25] observed promising results against *Plutella xylostella* on cabbage either using a weekly rotation of EPN and Bt or both biological agents together. Yi and Ehlers (2006) [28] observed an additive effect when *S. carpocapsae* and *B. thuringiensis* were simultaneously applied against early 3rd instar of *P. xylostella.* Salem *et al*. (2007) [23] found that the combination of *S. carpocapsae* All and *B. thuringiensis* subsp. *aizawai* against 2nd and 5th instar larvae of *Spodoptera littoralis* exhibit an additive interaction in the laboratory. When both *Xenorhabdus nematophila* K1 of *Steinernema carpocapsae* and *Bacillus thuringiensis* subsp. *kurstaki* were fed to late instars of *Plutella xylostella*, they showed significantly enhanced mortality, in which *X. nematophila* cells were recovered from the hemocoel of the treated *P. xylostella*. This study suggests that *X. nematophila* can be applied to control *P. xylostella* in a mixture with Bt in the field without its nematode host (Jung and Kim, 2007) [7]. An additive interaction between *S. carpocapsae* and *B. thuringiensis aizawai* aiming to control noctuid moths (*S. exigua* and *A. gamma*) in the open field of spinach (Lanzoni *et al*., 2014) [17]. Results of Btk and EPN *H. bacteriophora* and *S. feltiae* combinations showed additive and synergistic effects in the different time intervals. *P. brassicae* are better controlled if they are first exposed to Btk. The best mortality effect, when the EPNs were used with Btk at 12 h and 24 h time intervals. It seems that Btk as stressor cause a synergistic effect and make the larvae more susceptible (Arman *et al*., 2017) [1]. Synergistic interactions were observed for the combination of *H. beicherriana* LF (1X10^3 IJs / plant) and *B. thuringiensis* (HBF-18) (1.14X10^{10} CFU / plant) against *Holotrichia parallela* third instar larvae, resulting in a sizable white grub reduction up to 83.9% (Li *et al*.,2021).Integration of entomopathogenic nematode (*H. bacteriophora*) and *B. thuringiensis* var. kurstaki can be effectively used against sixth instar larvae and adults of *Rhynchophorus ferrugineus* with 100% larval mortality and 94.24% adult mortality (Yasin *et al*., 2021) [27].

Conclusion

Integrated pest management (IPM) applies multiple methods to suppress pest populations, thereby reducing dependence on conventional insecticides, which can have unintended harmful consequences for the environment and human health. Biocontrol agents like viruses, bacteria, fungi, protozoa, and nematodes have an important role in the IPM, and investigations on their combined effects could be very helpful in controlling pests. The toxicity of a given component of the combination should not affected by the other components. A good knowledge of biological parameters of insect and, the interaction among entomopathogens could play a key role to expand IPM programs. This calls for the isolation and identification of more virulent strains of entomopathogens. Soil biotic communities should be considered in EPN research and application. Moreover, the field evaluation of these substances in combined manners can provide substantial information and help in developing new strategies for IPM based crop production systems.

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