ENTOMOPATHOGENIC NEMATODES AS BICONTROL AGENTS

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Abstract

Entomopathogenic nematodes (EPN) are used for management of insect pest. Parasitic relationship is based on symbiotic acting of the nematodes from genera *Steinernema* and *Heterorhabditis* and bacteria *Xenorhabdus* and *Photorhabdus*. They occur naturally in soil environment, but their natural populations mostly are not able to control pests at the level required by farmers. Mass production in liquid fer mentors can provide production for large scale application at competitive price compared to synthetic insecticides. Application is done by common application equipment for pesticides considering some specificity of bicontrol agents and by irrigation equipment. The major challenge for EPN efficacy is moisture. Provided enough moisture during searching and parasiting hosts can provide successful control Adjusting application to specificity of the EPN and the host is often necesary. They are he most efficent against soil stages of the insects. They found place in come rial application in fruit and vegetable production, ornamental, mushrooms. They are among the safest biological control agents what makes the desirable biocontrol agents. EPN's are often exempted from registration when it is scientificaly confirmed presence of natural populations within the country.

Key words: ***insect mangement, Heterorhabditis, Steinernema***

INTRODUCTION

Demand for biological control agents has raised in the last three decades due to the ban of many pesticides. One of biological control agents that are nowadays present are entomopathogenic nematodes. Utilisaing EPN as a biocontrol agents has raised exponential. That growth is consequence of development of mass production and numerous studies of scientist all around the world how to use EPN in agricultural production. First species of EPN was described a century ago by Steiner as *Aplactana kraussei* (syn. Steinernema kraussei).

Entomopathogenic nematodes are the member of the phylum Nematode. The group of animals that are characterized by high diversity and ability to inhabitate all places on the Earth where are other kinds of life present. Entomopathogenic nematodes from the families Steinernematidae and Heterorhabitide have ability to parasite the insects. This ability is based on close relationship with their symbiotic bacteria from the genera *Xenorhabdus* and *Photorhabdus*. EPN from two families evolved convergently and do not share the common ancestor among bacteriovor nematodes (Poinar, 1993

EPN are obligate parasite of insects. Only stage that can be found outside the insect cadaver is 0.5-1.0 mm long third stage juvenile that is infective stage and also called dauer. Once the infective stage locate its potential insect host it tray to find the way how to enter insects body. It can be through

natural openings like mouth, anus and traheae or directly through intersegmental membranes (Georgis & Grewal, 1993). Once the infective stage enters the potential host it releases the coctail of enzimes that tend to supres imune system of the insect and symbiotic bacteria. If the action of symbiotic bacteria and nematodes is sucsesful the insect will day within 24-48 hours. Once the host is death the bacteria start to proliferate in monoxenic culture resulting in degradation of the cadavers intestine what becomes the found source for the nematodes together with bacteria. Sometimes it is necesary just several nematodes to kill an insect and after two to three life cycles within the cadaver several hundreds of thousands of the nematodes can be produced. When the food is depleted infective stage exit the cadaver and search for the next host.

One of the advantages of EPN is ability to actively search for the host. The strategy of searching for the potential host can be from a cruiser to nictating ambusher (Campbell et al., 2000). Cruisers are more successful in finding stationary insects while ambushers are successful in infection of mobile insects. Whatever the searching strategy is the nematodes react actively on the presence of metabolic products of the insects. Actually the searching of the EPN for the potential host is based on chemotaxy since the nematodes does not have eyes. One of the most important metabolites that the EPN utilized for searching for potential host is CO_2 which is metabolic product of all living creatures. They use carbon-dioxide to find the place of metabolic activities and some other insect specific metabolites to find the insect. However, different species of the nematodes do not react in the same way on the certain metabolic product of insects. The nematodes that have narrow life cycle, react only on the metabolic products of their hosts. That is the example of *S. scapterisci* that react only on metabolic products of its host insects the mole crickets. EPN can even utilize the metabolic products of the plants that are injured by herbivore insects. This volatiles attract the nematodes to the place where insects feed on the plants and gave them an opportunity to find the host. However, incests have their imun system and EPN must overcome it (Halem et al., 2011).

MATERIAL AND METHODS

Environmental factors

EPNs have evolved some adaptations to harsh conditions during soil stage where they are exposed to environmental and biological factors. They evolve double cuticle of infective stage where infective juveniles of *H. bacterophora* have also cuticle of previous, second stage juvenile. Mouth opening is closed since it does not feed. Closed mouth prevents energy lose but also decrease pathogen entrance. EPN are found in different range of climatic condition from very cold to even dry and warm desert soil. Ability to warm conditions is desired characteristic of EPN. Temperature above 32°C can have negative effect on life cycle and adaptation is based on presence of specific heat shock proteins (Schlesinger, 1990). Gens responsible for production of these proteins are found in nematode model species *Caenorhabditis elegant* named hsp70, and found also in EPN (Glazer et al., 1991). It is found that this ability is heritable and can be passed to next generation via hybridization (Shapiro-Ilan et al., 1995). Soil moisture is essential for nematode survival (Lui & Glazer, 2000). The nematode are aquatic animals and need to have presence of water around soil particles to move around. EPN in the soil are exposed to different biotical there that can have negative and possitive effect on parasitic life of the nematodes. They have to compete for a pray between other organisms in the soil like bacteria, entomopathogenic fungy etc (Pathak et al., 2014). After penetration of an insect and establishing life cycle they have to repeal also scavengers from preying insect cadaver. They release

also some repellent products of metabolism to prevent to be consumed by some scavengers (Griffin, 2012).

Mass production

EPN are naturally multiply within the insects and it is in vivo production. In vivo production is tidily applied on grate wax moth (*Galleria mellonela*) last instar larva and larvae of mealworm (*Tenebrio militor*). *In vivo* method is usually performed for small scale production for some trials and small scale farm application. It is necessary to establish insect production and also production of nematodes on the insects (Shapiro Ilan & Gaugler, 2002). It is labor intensive and need more space comparing to the methods. It is necessary to adapt production conditions and elements to specific nematode and insect host. That elements are temperature, inoculation dosage, aeration (Polanski et al., 2007). Yield of nematodes is generally dependent of nematode species size. Higher yield is with smaller nematodes and lower with longer nematodes. In case of *S. carpocapsae* it can be 285,000 invective juveniles per grate wax moth, and 114.000 nematodes for *S. feltiae* 114.000 (Đekanović & Nježic, 2022). *In vitro* production of EPN can be in solid and liquid media. The main challenge at the beginning of research of production of EPN in solid media is how to initiate reproductive phase in and artificial media and it is found that presence of bacteria initiate EPN reproduction (Han & Ehlers, 2001). Mass production in liquid media has many technological phalanges but the main advantage is huge amount of EPN are produced in cheapest way. *In vitro* production in liquid media require high technological knowledge of establishing and maintain life cycle. Initially bacteria are inoculated with their food, folowed by nematode inoculation. Maintenance of parameters like temperature, aeration bacteria concentration are essential. For *Heterorhabditis* nematodes even more challenge is to reproduce in liquid media since they cannot perform copulation between males and females in liquid environment. Food source would determinate whether the life cycle will go to amphimictic phase where copulation of males and females hapends, or to hermaphrodite females that can reproduce in liquid media (Ehlers et al., 1997). Formulation of final product is challenging in nematode production since they are live organism and must maintain fitness to parasites pests. Usually nematodes are packed in clay formulation that has shelf life of several weeks to several months depending on the species and temperature (Grewal, 2002).

Application

EPN can be applied with standard equipment for pesticide application, with irrigation equipment and release of infested insects. Even application with air-blast and boom sprayer is effective some specificity must be considered (Unruch & Lacey, 2001). Temperature of the nematode suspension should not exceed 30 °C, due to activity of the pump or sunlight. All sieves smaller than 300 µm must be removed. According to proposals in Europe the pressure in the sprayer should not exceed 5 bars, while in the USA it is 20 bars (Fife et al., 2004). It is not just the pressure that kills the nematodes but rather the flow of the suspension through geometry of nosles. Longer nematodes might be more damaged. Amount of water spent per hectar for application is higher than for pesticides since the nematodes are aquatic animals and require high humidity. It is requirement to put the the biggest nozzles since the nematodes are present in the biggest drops (Boselli et al., 1997). Challenge is application of EPN against above ground pests since rapid evaporation of water cause high mortality of EPN. Application in the evening, when humidity is increased, adding adjuvants can decrease mortality and enhance efficacy. Application of EPN via irrigation system has big advantage since the nematodes are applied with high amount of water. The

biggest challenge is how to provide uniform dosage on all length of the sistem since the nematodes tend to settle down and lower number is distributed at the furteh parts of the system (Kramer & Grunder, 1998). For transplants that are affected with soil pests submerging in suspension with EPN decrease dosage. There is also application in gels or granules like in the case of control of corn root borer *Diabrotica virgifera* (Topper et al., 2008).

Safety and registration

According to experts opinion EPN are among the safer bio control agents (Ehlers, 2005). They are not harmful for farmers, final users of agricultural products and other vertebrates. They could have a short therm negative effect on population of some beneficial organisms, but never cause extinction of another species (Ehlers & Hokanen, 1996). Augmentative application of commercial strain can cause depletion of natural strains of EPN but there are o data that this kind of application cause extinction of another EPN species/population. In many countries EPN are exempted from registration since they are considered macroorganisms. In case when bacteria are considered registration might be more complex. Many countries require that there is scientific evidence of the presence of the species within the country hat application of commercial strains is allowed.

The European and Mediterranean Plant Protection Organization (EPPO) published a document (List of biological control agents widely used in the EPPO region - PM 6/3) in where it is sugested to national plant health organisations the it should be registred only microbial species and dispense them from authorization, or simplify the notification procedures as proposed in EPPO Standards PM 6/1 (First import of exotic biological control agents for research under contained conditions) and PM 6/2 (Import and release of non-indigenous biological control agents) (EPPO, 2016). The document contains a list of seven species of nematodes: *H. bacteriophora, H. megidis, S. carpocapsae, S. feltiae, S. glaseri, S. kraussei* and *P. hermaprodita*.

RESULTS AND DISCUSSION

Field application of EPN against insect pests has been extensively presented in literature. EPN showen high level of control when they tested against numerous insect pests. Most of the pests are soil dwelling organisms and present potentially good target since EPN are from soil environment as well. Good efficacy was demonstrated against soil dwelling pests such as the Large Pine Weevil, *Hylobius abietis* L. (Williams *et al.,* 2013), Oriental Fruit Moth, *Grapholita molesta*, Small Hive Beetle, *Aethina tumida,* Western Corn Rootworm, *Diabrotica virgifera virgifera* (Toepfer *et al.,* 2008). Some successful examples against pests that are in cryptic habitats, like tree borers are the Mediterranean Flat-headed Root Borer *Capnodis tenebrionis* and the Peachtree Borer *Synanthedon exitiosa*. In greenhouses, significant progress has been made against different pests including control of the Sweet Potato Whitefly *Bemisia tabaci*, the Diamond Back Moth *Plutella xylostella,* Tomato Leaf Miner *Tuta absoluta*, Western Flower Trips *Frankliniela occidentalis* and different fungus gnats (Diptera: Sciaridae). Above ground pests in cryptic habitats are also targeted by EPN, like the Red Palm Weevil, *Rhynchophorus ferrugineus* or the Codling Moth, *Cydia pomonella*. Examples of control of adults insects are Plum sawflies – *Hoplocampa flava* and *H. minuta* (Nježić & Ehlers, 2020), where nematodes are applied just before anticipated adult emergens. Control of pests in criptic habitat as mediterania flat had borer – *Capnodis tenebrionisis* is highly demanding and EPN *S. carpocapsae* showed potential for field application (Morton & Garcia-del-Pino, 2008). Application against Curcullio nucum must be considered against bought larva and adults to have sufficient results. Successful application against *F. occidentalis* was when combined

application against soil and leaf stage of the pest. The main challenge for EPN commercial application is to extend good results from the laboratory condition to thefield conditions like in control sap beatle Stelidota geminata (Grujić et al., 2020). Environmental factors are the major obstacle for EPN effective application in the field conditions. Application formulations, breeding of nematodes more tolerant to harsh conditions, improving application technologies and manipulation with environmental factors are the main drivers of improvement of EPN efficacy.

CONCLUSIONS

EPNs are effective against some many insect pests with different success rate. Development of mass production brought this biological control agents to be competitive with synthetic insecticides. The main obstacle in widening their application are limiting results in different environmental conditions. Different approach might bring results but providing activity of the nematodes enough long to penetrate the host is the main target of researchers.

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