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Entomopathogenic Nematode Application in Egypt and Russia: Challenges and Opportunities

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Abstract

Biocontrol is a safe and environmentally sound alternative to chemical insecticides. Current status of entomopathogenic nematode (EPN) research and application as bio-pesticide in Egypt and Russia is outlined. Adoption of organic agriculture has induced fundamental changes in organic farmer management practices. Therefore, new challenges for organic agriculture to include EPN against insect pests in such practices in both countries are discussed. Major impediments and solutions for EPN commercialization in Egypt and Russia are presented. More financial, informative, regulatory, and technical support is needed in order to exploit the potential for increased pest management through EPN applications in both countries. We pointed out perceived reasons to develop techniques that exploit the potential for effective pest management through different EPN application tactics. Local EPN production should be encouraged to eliminate or reduce transport, packaging, formulation and storage costs. Furthermore, local EPNs may be more adaptive and less expensive without any risk to Egyptian and Russian fauna and flora than imported strains. We addressed also guidelines necessary to increase the number of people interested in the activities of EPN application against pests hoping to reap clean and safe agro-products for man and the environment.

Key words: Entomopathogenic nematode, Egypt, Russia.

Introduction

The field of entomopathogenic nematology has faced periods of spreading and clipping wings in the last five decades. Its most important periods of exponential growth were in 1980s and early 1990s. At the beginnings of this period, fueled by lavish venture capital money and unrestrained enthusiasm for biotechnology, a flush of biopesticide companies arose to exploit the extraordinary potential biologicals offered as environmentally benign alternatives to chemicals **(Gaugler, 1997).** Although the widely predicted environmental driven surge in demand for biologicals was never fully materialized, research developments and application levels concerning entomopathogenic nematodes (EPNs) differed among countries. In advanced countries like USA, the stable knowledge that entomopathogenic nematodes can provide excellent arthropod pest control in some situations remains frequently unexploited by growers for several reasons. Some of these reasons are well documented in details **(Shapiro-Ilan *et al*., 2002; Georgis *et al*., 2006).** For example, the potentially widespread use of EPNs in turf **(Frank and Walker, 2006)** and cotton **(Shapiro-Ilan *et al*., 2002)** were supplanted by less expensive chemical pesticides and insect resistant germplasm, respectively. Admittedly, inferior performance of EPNs compared to chemical insecticides in many situations and inconsistent performance of EPNs, due to product quality control failures or environmental variation, are the greatest impediments to the reliability/adoption of EPNs in pest management programs **(Lewis *et al*., 1998; Dolinski *et al*., 2012).**

On the other hand, issues of pesticide resistance, safety, health and environmental concerns leading to banning of some effective fumigant and granular pesticides, and development of pest biotypes that break host plant resistance have provided real opportunities to integrate the use of EPNs in pest management programs **(Dolinski *et al*., 2012).** In addition, technical advances in EPN formulation, shelf-life, production and application have provided further scope to integrate the use of EPNs in pest management programs. Unfortunately, useful EPN applications are more frequently unexploited in many countries than in USA. The present study reviewed the current obstacles and possible solutions of managing arthropod pests using entomopathogenic nematodes in two countries with quite different history of agricultural economics: Egypt and Russia. The two countries cover a broad range of system and climate types within different contexts. Russia is now experiencing the permanent increase of areas under organic agricultural management, whilst Egypt has an established organic sector. Such a sector can benefit from, and in return help in the development and spread of EPN utilization. This is especially timely since organic agriculture is growing continuously in Egypt to meet the demands of European Union (EU), USA, Australia, and Japan markets; the EU is the major importer of the Egyptian produce. Consumer market in Russia is demonstrating the increase in inner demand for eco-friendly products, including organic food. Therefore, this report will also address the development of organic farming in Egypt and Russia as a primary candidate where EPN application may have real opportunities. We will throw light on different aspects of their organic agriculture to clarify possible inclusion of EPN utilization. Then, we will briefly address research programs in Egypt and Russia that are being pursued to contribute to development of efficacious EPNs against insect pests. More importantly, we will focus on the relevant techniques and informative approaches required in order to thrive the commercial use of EPN in both countries. Therefore, key points conveying perceived reasons to develop cultural practices that exploit the potential for effective pest management through different EPN application tactics in Egypt and Russia are reviewed.

History of EPN researches in Egypt and Russia:

The real start on EPN research in Egypt began in the 1970s when Dr. El-Kifl worked on the biological control potential of *Neoaplectana* (= *Steinernema*) *carpocapsae* against the cotton leafworm, *Spodoptera littoralis*; one of the most economically important insect pests in Egypt. This work was part of her Master and Ph.D. research programs **(El-Kifl, 1980 and 1984)** and was followed by that of **Abdel-Kawy (1985).** On the other hand, the fundamental Egyptian survey to isolate indigenous EPNs was published by **Shamseldean and Abd-Elgawad (1994).** Since then, researchers in Egypt have been rearing EPNs mostly on *Galleria mellonella* and their biocontrol potential was tested against main insect pests such as the *black cutworm*, *Agrotis ipsilon*, and the hairy rose beetle, *Tropinota squalida* **(El-Kifl and Sammour, 1989; Hussein, 2004).** Since *Heterorhabditis* spp. were apparently found to be more common than *Steinernema* spp. in Egyptian fauna, additional research to control other insect species was done as well **(Haukeland *et al*., 2014).** For some of these insects, especially those that do not live in soil (e.g., the red palm weevil), it is unclear whether control with EPNs would ever be practical or economically feasible **(Abd-Elgawad, 1996; El-Bishry *et al*., 2000).** Egyptian researchers are trying to solve such problems and to be acquainted with the new advances in entomopathogenic nematology through joint scientific research projects with developed countries.

Ivan N. Filipjev – the founder of modern nematology in Russia - was also the first Russian scientist who described entomopathogenic nematode. In 1934 he published description of the nematode *Steinernema feltiae* **(Filipjev, 1934).** The material for description was sent to him from Udmurt Republic of Russian Federation (North-East of the European part), where local scientists discovered intensive infestation of armyworms (Lepidoptera) by these nematodes. Up to now ubiquitous *S. feltiae* is valid species, thanks to complete and precise description by I.N. Filipjev. In Russia it was Galina V. Veremchuk who initiated applied research in entomopathogenic nematodes in the All-Union Plant Protection Institute, St.-Petersburg-Pushkin. On this stage she was mainly active in taxonomy of Steinernematidae, describing new forms of steinernematids, though species she described are considered now as junior synonyms of other steinernematid species **(Nguyen and Hunt, 2007).** She also adapted the techniques for the rearing of entomopthogenic nematodes on laboratory insects and artificial media. Her pupil – Dr. L.G. Danilov was the first specialist in Russia who proposed the commercial products based on the entomopathogenic nematodes

Organic farming in Egypt and Russia:

The general principles in the International Federation of Organic Movements (IFOAM) basic standards state that the management of pests, diseases and weeds should occur through the application of biological and cultural means. Cropping practices (rotations, intercropping and diversity) and soil fertility practices are central to such a management **(Altieri, 1999).** The organic standards permit the use of inputs such as biologicals should preventative measures not be sufficient. In Egypt, the organic cropping systems closely resemble traditional, market-oriented cropping systems where buyers usually require that the crops be grown without the application of pesticides. Typical rotations might be a wheat–clover–maize–clover rotation or herbs and spices (often perennial), such as coriander or chamomile are grown alternately on other plots. Organic agriculture in other Egyptian farms ranges from field crops such as corn and barley to vegetables, fruits, and aromatic and medicinal plants **(http://www.sis.gov.eg/En/Templates/Articles/tmpArticles.aspx?ArtID=835#.U52zJ3Zaut8).** Yet, some growers have stopped growing corn as it cannot be sold organically, whilst others reported that tomato is too difficult to produce organically **(Oelofse *et al.*, 2011).** This has led to a more shortage of cultivated areas where EPNs could be used to combat pests of corn and tomato. Main EPN-susceptible corn and tomato pests proved to be good EPN targets include the cotton leafworm, *Spodoptera littoralis* **(Abbas and Saleh, 1998; Abdel-Razek and Abd-Elgawad, 2007),** the black cutworm, *Agrotis ipsilon* (Shamseldean *et al*., 1994; Hussein, 2004), the green worm, *Spodoptera exigua* **(Abd-Elgawad and Aboul-Eid, 2001),** and the sugarcane stem borers, *Sesamia cretica* **(Saleh *et al*., 1999).** Other insect pests presented in table (1) may be prime candidates for control by EPN in Egypt. Yet, relevant EPN techniques should be sought out to reduce the efficacy gap between laboratory and field results. As for the above-mentioned reluctance for cultivation of a few crops by some growers, it is not necessarily problematic as it most likely reflects what systems fit best in the local context. Also, contracting companies play a central role in the selection and certification of certain farms and farmer typologies **(Oelofse *et al*., 2011).** Farms only become certified organic when a certain target crop is present—this is the reason for the general lack of drastic change towards EPN applications on the organic farms since pests of these crops might not be a good target for EPNs. Also, some organic farmers manage pests based on a ‘wait-and-see’ strategy and did not consider insect pests a major problem unless they abundantly exceed the economic threshold level. Pest management strategy in Egyptian organic farms is based upon the use of a variety of control types, including natural sprays (such as garlic extracts), copper sulphate and various types of pheromone traps as well as biocontrol agents such as *Bacillus thuringiensis*, *Trichogramma* spp., *Beauveria bassiana*, predators and EPNs. Unfortunately, almost all references about organic agriculture in Egypt do not mention EPNs due to their limited application, lack of information, or both.

Admittedly, the lack of system redesign on most organic farms is evident in the curative nature of applied pest controls **(Oelofse *et al*., 2011).** However, prophylactic control measures might well be implicit to some of the farming systems in Egypt and Russia based upon farm history as well as farmers specific knowledge.

Table (1). Mortality of insects exposed to Egyptian heterorhabditid nematode isolate EGB1 and numbers of emerging nematode-infective juveniles (IJs) **(Laboratory tests adapted from Shamseldean *et al*., 1994).**

Insect host		Mortality (%)	Mean number of IJs/insect cadaver
Common name	**Scientific name**		
Greater wax moth	*Galleria mellonella*	100	>250,000
Egyptian cotton leafworm	*Spodoptera littoralis*	100	>100,000
Common mole cricket	*Gryllotalpa gryllotalpa*	100	>50,000
Greasy or black cutworm	*Agrotis ipsilon*	100	>100,000
Spin bollworm	*Earias insulana*	100	>50,000
Pink bollworm	*Pectionophora gossypiella*	100	>250,000
Pink borer of sugar cane	*Sesamia cretica*	100	>75,000
Grape fruit worm	*Polychrosis botrana*	100	>10,000
Honey dew moth	*Cryptoplapes gnidiella*	100	>10,000
Apple stem borer	*Zeuzera pyrina*	100	>250,000
Fruit fly (larvae)	*Ceratitis capitata*	100	No emerge
Fruit fly (pupae)	*Ceratitis capitata*	100	No emerge
Looper caterpillars	*Chlorissa* spp.	100	>10,000

For example, in Fayoum governorate (Egypt), organic farmers' crop selection and rotations resemble traditional farming systems, which have been adapted to the area, thus organic farms might have implicitly adopted preventative strategies. Therefore, scoring for organic and conventional farms was similar in Fayoum, whilst organic farms scored substantially higher than conventional farms in four other cases of the same study in China and Brazil **(Oelofse *et al*., 2011).** Such a score was based on diversity crop index, pest, disease, and weed control, and soil fertility management. In such a case where organic and conventional farms are similar, managing pests is a major challenge for organic farmers, particularly where knowledge access is limited and/or pest pressure is decisive for whether farmers can operate organically. Therefore, organic farmers in the contexts of both countries require both EPNs-substantial knowledge support and a pragmatic set of related rules which can take into consideration the local context in both countries.

Guthman (2004) stressed that due to economic pressure, organic farmers contracted to certain crops are more inclined to rely upon input substitution, rather than system redesign. Hence, EPN application could sometimes replace some insecticides within the same system. Needless to remind that there are still limited number of organic farms especially in Egypt. **Hammad *et al*. (2011)** recorded 460 organic farms (= 17,000 hectare) in Egypt. Thus, organic farms, though growing quickly, are still like small islands in a sea of conventionally managed land.

The situation in Russia is different in the terms of the market history and contemporary legislation. Organic agriculture was practically absent during Soviet period, despite serious concern of USSR population about the quality of agricultural products. The shortages of consumer goods and food during Soviet period decreased significantly the interest of population toward ecologically safe food.

Despite open discussions on this topic during so called ‘glasnost’ period (last years of USSR existence) Soviet agriculture was unable to answer the needs of population. In the last decade growing premium sector of in-Russia consumer market is acting as positive stimulating factor onto development of eco-friendly food production. Still, legislative changes in the rural life in Russia are very slow and up to now no regulations or regulatory document related to the organic agriculture are accepted on the Federation level. On the other side there is permanent interest from population in such eco-friendly production. Only in 2013 the law about the status of organic farming was presented in ‘Gosudarstvennaya Duma’ (lower chamber of Russian Parlament), but this document still has to pass several hearings and will be approved, as expected, only in 2015. According to the estimates of the ‘Euromonitor international’ Russian market of organic food in 2012 constituted $148 mln. US dollars and demonstrated 7.8 % growth in comparison with the year 2011. Some experts predict 30% growth in 2010 – 2015. Small producers are preparing for such predicted market changes and soon established the Union of Organic Agriculture **(Mentyukova, 2013).** Absence of proper legal basis results in the inability of Russian producers to obtain the certification of their products as organic food. In some areas of Russia (e.g. in Kaliningrad area – Russian enclave between Poland and Lithuania) farmers are asking for the organic certification from corresponding structures of European Union. Both the cost of such certification and everyday burden of paper work to keep at active are behind the acceptable level for the majority of small producers.

The inherited problem with organic agriculture is that it cannot produce large quantities per unit area to meet the growing demand for this type of food, leading to higher prices and make it limited only to high-income earners. If production was little compared to the cultivated area, farmers will offset the price of the product. Such a scheme may not be profitable for the most part of Egyptian population whose income is low. Nevertheless, organic ‘niche’ market crops with a high-value can influence organic farmer’ management decisions and consequently may justify rather costly EPN application. Fulfillment of agro-ecological ideals, based upon system redesign to create bio-diversified cropping patterns and enhance pest management including tactful EPN application, is perhaps an end goal for organic farmers in Egypt and Russia.

The use of EPNs in Egypt and Russia:

Throughout the different periods of this science, research has been done on different branches of its related disciplines in Egypt and Russia. Relevant research programs have not negated the common and main interest in the use of entomopathogenic nematodes (EPNs) as biological control agents with a clear aim at the avoidance of hazardous chemical insecticides. These programs encompass a variety of approaches such as studies on EPN applications, enhancement of their effectiveness, their interactions with other soil micro-organisms/insecticides, and integration of biocontrol with other management techniques **(e.g. Saleh and Sammour, 1995; Shamseldean *et al*., 1999; Salama and Abd-Elgawad, 2002; Shamseldean, 2010).** Basic information on the criteria and techniques of the entomopathogenic nematode's production and utilization has been afforded in Arabic by **Abd-Elgawad, (1998)** through the Arab League in an effort to stimulate EPN research and introduce beneficial biocontrol methods in the Arab countries. Then, processing EPNs for commercial production has been tried in both countries **(e.g. Abd-Elgawad *et al*., 2003; Hussein and Abdel-Aty, 2012).**

Two products: 'Nemabact' and 'EntonemF', which are really a suspensions of *Steinernema carpocapsae* and *S. feltiae* juveniles, correspondingly, are available in Russia now. These products are produced and distributed by "Biodan' company in Sankt-Petersburg and 'Biometodika' company (Moscow region). 'Nemabact' (*Steinernema carpocapsae*) is proposed for the control of different pests including trips, grubs, cabbage flies and even bark beetles. Researchers in different areas of vaste Russian territory are testing this product against new pests, as e.g. against currant shoot borer (*Incurvaria capitella* Cl.) in Siberia **(Ovchinnikova *et a*l., 2013).**

EPN diversity in Egypt includes both indigenous and imported species. The latter yielded poor or inconsistent results in many cases of field trials, probably because they were poorly adapted to local agro-climatic conditions. Indigenous EPN might be more suitable for inundative release against local insect pests because they are adapted to the local environment. Searches for effective indigenous species and strains resulted in a number of new nematode species/isolates from different parts of Egypt **(e.g. Shamseldean and Abd-Elgawad, 1995a; Salama and Abd-Elgawad, 2001; Hussein and Abou El-Souud, 2006; Abd-Elgawad, 2008a and 2014).** These include *Steinernema abbasi*, *S. carpocapsae*, *S. kushidai*, *S. glaseri*, *Heterorhabditis bacteriophora*, *H. indica*, *H. taysearae*, and *H. egyptii*. The latter two species have been described from Egypt by Shamseldean, **Abou El-Sooud, Abd-Elgawad and Saleh (1996) and Abd-Elgawad and Ameen (2005),** respectively. Many other heterorhabditids and some steinernematids have been isolated from Egyptian soils but have yet to be identified **(Abd-Elgawad *et al*., 2013).**

The overall goal of such biocontrol agents is the identification and deployment of highly effective EPN strain(s) against insect pests before their development into ready-for-sale plant protection products. Admittedly, indigenous EPN strains proved very effective in lab tests (e.g. Table 1). Commercial EPN products are available worldwide and characteristics of some commercialized species have been documented **(http://www.biocontrol.entomology.cornell.edu/ pathogens/ nematodes.html).** Yet, local EPNs may be more adaptive and less expensive without any risk to fauna and flora than imported strains. So, the relatively high efficacy demonstrated by local EPNs may nominate them for further experimentations and development. In fact, very few of them are being commercialized locally; e. g. NOBUG© in Egypt but on a very limited scale. The active ingredient, *Steinernema carpocapsae*, of this product is placed on moist polyether-polyurethane sponge to control mainly caterpillar and pupal stages of lepidopterous insect pests. Usually, a 5-litre package is diluted by 300 litre of water per one Feddan (Feddan = 4200 m^2) for arthropod pest control in such fields as tomato, pepper, lettuce, and cabbage. Moreover, increase in their efficacy appears possible when such biocontrol agents are integrated with other management methods **(e.g. Dimetry *et al*., 1996; Salama and Abd-Elgawad, 2002; Dreves, 2014).**

Laboratory and field studies demonstrated that target insect pests for EPNs in Egypt include mainly the cabbage white butterfly [*Artogia (pieris) rapae*], cabbage webworm (*Hellula undalis*), strawberry scarab (*Temnorhynchus baal*), corn stem borer or greater sugarcane borer (*Sesamia cretica*), cabbage moth (*Plutella xylostella*), red palm weevil (*Rhynchophorus ferrugineus*), cotton leafworm (*Spodoptera littoralis*), cutworm (*Agrotis ipsilon*), hairy rose beetle (*Tropinota squalida*), greater wax moth (*Galleria mellonella*), and leopard moth (*Zeuzera pyrina*). For example, laboratory percentage mortality of some insect species exposed to Egyptian EPN isolates and numbers of emerging nematode-infective juveniles are presented (Table 1). Despite the general high efficacy shown in Table 1, nematodes have very limited usage for such pests on a large scale in both countries. That is because the definitive test of host suitability is efficacy under field conditions where a minimum of three solid field trials to establish host suitability is required **(Shapiro-Ilan *et al*., 2002).** An analysis of nematode host suitability (based on field efficacy) for some of the most extensively studied insect pests is illustrated in table (2). The table only includes host-nematode combinations with at least three refereed publications on field efficacy to confirm reliability. Reliable outcome should come from multi-site and multi-season tests. The best matches tend to be for nematodes that have high virulence toward hosts in a protected environment or cryptic habitat, e.g. leafminer, black cutworm, borers, etc. Nematode applications to environments exposed to temperature extremes, ultraviolet radiation or desiccation, e.g. some *Spodoptera* spp., are prone to failure. On the other hand, **Abd-Elgawad and Nguyen (2007)** found differences ($P \leq 0.05$) in heat and desiccation tolerance among four Egyptian heterorhabditid isolates extracted from distant regions which could stimulate additional surveys for EPNs in areas characterized by extreme environmental conditions. Also, understanding the fundamental basis of molecular and physiological architecture of survival in EPNs will enable the improvement in nematode ability to withstand environmental extremes and thus to persist longer **(Glazer, 2002).**

Nernatode end-users in Russia and Egypt need to know more about characteristics of nematode species and the proper use of each one to reach a sounder pest management. **Abd-Elgawad (2001)** discussed matching nematode and insect species to achieve optimal field performance in the light of nematode-host selection process and characteristics of commercially available nematode species. For best matching, the nematode and host together must produce the highest infective juvenile yield per cost unit per gram of host. It may ever be possible to determine from EPN characteristics the species that is most likely to control certain insect species. EPN foraging strategy should also be considered **(e.g. Dreves, 2014).**

Table (2). An analysis of host suitability for entomopathogenic nematodes against various insect pests (adapted from **Shapiro-Ilan *et al.*, 2002**).*

Pest	Nematode species	Host suitability[a] (%suppression)	Number of references in analysis
White grub (*Tropinota squalida*)	*H. bacteriophora*	Good (68)	3
Leafminer (*Liromyza trifolii*)	*S. carpocapsae*	Good (68)[b]	3
Black cutworm (*Agrotis ipsilon*)	*S. carpocapsae*	Excellent (86)	7
Diamondback moth (*Plutella xylostella*)	*S. carpocapsae*	Fair (56)	5
Corn earworm (*Helicoverpa zea*)	*S. riobrave*	Excellent (90)[c]	4
Borers (*Synanthedon* spp.)	*S. feltiae*	Excellent (86)	4
Spodoptera spp.	*S. carpocapsae*	Poor (27)[d]	3
Tomato leafminer (*Tuta absoluta*)	*Steinernema feltiae*	Fair (45)[e]	3

*Only pests (by genus or species) with at least three refereed publications on field efficacy for each nematode species were included, and only if the rate of nematode application was not excessive (i.e not > 125 nematodes cm^{-2}).

[a] Host suitability ratings: Excellent, Good, Fair, and Poor are based on suppression levels of 80-100, 60-79,40-59, and < 40%, respectively. The suppression levels were calculated by averaging results from field trials in the associated references.

[b] indoor applications in high humidity.

[c] Soil applications.

[d] Above ground applications.

[e] Foliar application (Jacobson and Martin, 2011).

Some *Steinernema* spp. are ambushers using sit-and-wait tactics. So, ambusher EPN are preferably used against insect hosts who spend most of their time at or near the soil surface; e.g. *S. feltiae* is the best choice against fungus gnat larvae. Other EPNs are cruiser. They move through the soil profile searching for potential hosts; e.g. *Heterorhabditis bacteriophora* is the best choice against the black vine weevil. **Abd-Elgawad (2001)** reported inter- and intra-specific variation in virulence and reproductive potential of newly isolated Egyptian nematodes. Admittedly, more native species with diversity of target traits should be sought after to overcome behavioral and environmental barriers and optimize field performance. **Abd-Elgawad (2001)** stated minute and tentative definitions of terms for EPN classification and Egyptian key insect pests for differential host test so that they can offer us an indispensable clue for careful analysis of nematode-host matching.

On the other hand, if economic factors are not favourable even a strategy involving the most suitable match of nematode to target pest is doomed to failure. Economic factors include the grower's perceived need to control the pest, the relative cost of nematodes compared with other management options, the value of the commodity (e.g. per ha), and the overall importance of the commodity in the agricultural market **(Shapiro-Ilan *et al.*, 2002).** Exemption of EPNs from the Environmental Protection Agency pesticide registration has clearly benefited commercialization efforts. Niche markets tend to be amenable to EPN use not only because the crop value is high, but also because the commodity occupies a small enough segment of the agricultural market for would-be competitors to shy away from registration costs and seek alternatives. On the contrary, major row crops, such as maize, cotton, soybeans and wheat are often unreachable for EPN marketing because the crop value is low, and the market segment is huge. But for a two-fold goal; i.e. controlling plantparasitic nematodes and insect pests simultaneously by EPN **(Abd-Elgawad and Aboul-Eid, 2001 and 2002; Keila *et al.*, 2008)** has shown a promise in Egypt, EPN use in such crops is likely to always be unattainable. Yet, to achieve the first goal necessitates optimal application tactics of EPN, e.g. delivery of the dauer stage juveniles near the plant roots for effective phytonematode control **(Abd-Elgawad *et al.*, 2013).** Another important point is to enlighten the end-user by the indirect benefits gained by using such biocontrol agents for the safety of human being and environment. For example, in Egypt, EPN product is more expensive by only about 20% when its price is compared to the absolute price of chemical insecticide to control the black cutworm. A solid media mass production for the production of *S. carpocapsae* at the standard application rate of 1 x 10^9 nematodes $Feddan^{-1}$ costs L.E. 120. On the other hand, grower cost for Hostathion 40 EC (Emulsifiable Concentrate), a chemical insecticide, is L.E. 100. So, Hostathion is about 20% less expensive than EPNs. Yet, if we take into account the insecticide damage/pollution to the environment generated from soil, plants, animals, and water, it is apparent that such adverse effects and their impact on the health and social services exceed many times the original price of bio-pesticide. Therefore, EPNs are still commercialized in countries such as USA **(http://cru.cahe.wsu.edu/CEPublications/PNW544/PNW544.pdf)** although such a bio-insecticide costs the grower US\$ 52.33 ha^{-1} compared to US\$ 10-20 ha^{-1} for chemical insecticide **(Georgis, 2002).** A new citriculture system in Florida unintentionally suppresses native and augmented entomopathogenic nematodes **(Campos-Herrera *et al.*, 2013).** On the other hand, modifying some orchard planting sites conserves entomopathogenic nematodes, reduces weevil herbivory and increases citrus tree growth, survival and fruit yield **(Duncan *et al.*, 2013).** Yet, a plausible comparison with standard insecticides should be based on cost, ease-of-use, and efficacy.

The bacterial symbiont, *Photorhabdus* sp., which was isolated from Egyptian *Heterorhabditis* sp. induced the high rate of mortality for larvae of the cotton leaf worm with an LC_{50} value of 60 bacterial cells/larva compared to 72 and 85 cells per larva for other two bacterial symbionts, *P. luminescens subsp. akhurstii* and *P. luminescens*, respectively. These effects were thought to be attributed to different bacterial enzymes that damage the host hemolymph **(Abd-Elgawad and Abdel-Razek, 2005).** However, correlation coefficients between the relative activities of lipase, esterase, catalase and protease for six Egyptian heterorhabditid isolates versus the nematode-induced mortality of *G. mellonella* and *R. ferruginous* for these EPN strains were low, which indicates that the activities of these enzymes might not reflect nematode virulence **(Abd-Elgawad *et al*., 2003).** When the symbionts extracted from five Egyptian isolates of heterorhabditid nematodes were grown in yeast salt broth, in the absence of the nematodes, the bacteria produced a toxin protein that was lethal when injected into the hemolymph of the insect pest **(Abd El-Zaher *et al*., 2008).**

Poor storage and post-application survival are major obstacles to the expand use of EPNs. In general, no worldwide EPN formulation meets the 2-year shelf-life requirement of standard chemical pesticides **(Grewal, 2002).** In addition, lack of capital and expertise often hinders developing an EPN *in-vitro* production industry, especially one based on costly and stainless steel fermenters and centrifuges, which require highly skilled workers in Egypt and Russia. Therefore, the default strategy in Egypt is to scale-up EPN *in-vivo* production. This technique could be achieved through American-Egyptian collaboration through LOTEK system **(Gaugler *et al*., 2002)** and the nematodes could be reared on hosts such as *G. mellonella* and *Tenebrio molitor*. Success in the mass production of these nematodes, especially native isolates, would constitute an important step towards their implementation in Egypt. An automated process such as the LOTEK system could provide for local custom production biopesticides. Local production would eliminate or reduce transport, packaging, formulation and storage costs. Furthermore, only "fresh" biological agents would be applied, providing improved efficacy. Moreover, this technology even offers the promise of producing locally adapted strains, rather than "one size fits all" approach used in the U.S. and Europe.

Nonetheless, research on *in vitro* solid culture of EPNs is being conducted with varying degrees of success in various laboratories in Egypt and Russia where sponge-, alginate-, and water-based formulations of EPNs are used on a small scale **(Shamseldean and Atwa, 2006; Abd-Elgawad, 2008a, Tarasova and Lugovaya, 2014).** For example, in a field experiment, three locally-made formulations were sprayed as suspensions of indigenous *H. bacteriophora* EG6-IJs produced in *Galleria* larvae onto loamy soil planted with banana. The three formulations involved 0.1% formalin solution, gelatinous pellets and sponge foam, but otherwise were the same. In this experiment, sponge foam gave better nematode persistence in soil than the other two treatments. Differences in nematode viability among the three treatments just before field applications (i.e., while in aqueous suspension), were not significant and ranged between 84.5 for gelatinous pellets and 93% for sponge foam. However, EPN applied in the form of nematode-infected *G. mellonella* cadavers, a fourth treatment, had superior persistence in soil compared to any of the above- formulations that applied the nematode-IJs **(Abd-Elgawad *et al*., 2003).** EPNs that emerged from the cadavers into soil were still able to kill insects 8 weeks after application. Also, in a tomato field with sandy loam soil, treatments involving a liquid culture of the bacterium *Serratia marcescens* and a nematode suspension containing four Egyptian isolates of *H. bacteriophora* were applied separately through drip irrigation in addition to a third treatment of 24% liquid oxamyl sprayed simultaneously on the shoots of tomato. The treatments increased the tomato yield by 21.2%, 60.2% and 63.7%, respectively, compared to the untreated check **(Abd-Elgawad and Aboul-Eid, 2001).** The results suggested that the yield increase in *H. bacteriophora*-treated plots was due to both insect (primarily) and plant-parasitic nematode (secondarily) control. The field was infected with the Egyptian cotton leafworm, *Spodoptera littoralis*, and the green worm, *S. exigua*. The percentage of cotton leafworm-infected tomato fruits were 7, 3, 8.5, and 9% in treatments involving oxamyl, *H. bacteriophora*, *S. marcescens*, and the untreated control, respectively.

Although the overall goal of EPN research in Egypt and Russia is the identification and use of highly effective strains against economically important insect pests, no companies yet exist for the development and sale of these plant protection products. Nonetheless, small special units associated with some research institutes and universities are producing and marketing EPNs in Egypt on a limited scale (Haukeland *et al*., 2014). This production is used primarily for scarab pests of perennial ornamentals such as roses, as well as lepidopterous insects in annual crops as mentioned above.

Major impediments and solutions for EPN commercialization in Egypt and Russia:

The insufficiency of EPN usage against insect pests in Egypt and Russia on commercial scale may be attributed to various sources. EPN cost and reliability are reasons in common with many other regions. The influence of these two factors will automatically impose themselves in more detail when talking about EPN use. Moreover, one can venture to add the general heavy burden of economy in case of Egypt. Many Egyptian farmers and small scale agricultural companies are not keen in adopting policies more suited to the improvement of life quality. They are rather interested in meeting the real cost of living through traditional agricultural practices because of their low standard of living. On the other hand, Egyptian researchers were retrograde at the beginning of EPN discipline to the degree that these nematodes were primarily imported to be used on small scale especially for post-graduate studies **(e.g. El-Kifl, 1980 and 1984; Abdel-Kawy, 1985**). No sooner did Egyptian researchers initiate some collaborative research projects with USA then they succeeded in EPN extraction, identification, and application against economically important insect pests of Egypt **(e.g. Shamseldean and Abd-Elgawad, 1994; Salama and Abd-Elgawad, 2001; Abd-Elgawad and Nguyen, 2007; Abd-Elgawad, 2008a; Shamseldean, 2010; Abu-Shady *et al.*, 2011; Abd-Elgawad, 2014).** Yet, a number of difficulties still remain for getting more and sound ground for EPN as commercial biocontrol agents. These include issues related to their formulation, packaging, shelf-life/storage, mass-culture, variable field performance, and potential negative effects on non-target/beneficial organisms. Limited market is due to EPN cost, efficacy and persistence in the field as well as ease-of-use, product stability, and lack of information on their usage **(Georgis, 2002).** In addition, Egyptian and Russian farmers' expectations of broad-spectrum activity and rapid performance based on experience with long-used chemical insecticides are making growers insist on their use, even though most of such synthetic chemicals are banned in developed countries **(Abd-Elgawad, 2008b).** Moreover, some pests for which EPNs provided acceptable control do not exist in both countries; e.g. the Diaprepes root weevil (*Diaprepes abbreviates*) in citrus **(Duncan *et al.*, 1996),** and the black vine weevil (*Otiorhynchus sulcatus*) on cranberries **(Georgis *et al.*, 1991).** Also, other pests which are candidate for successful control by EPN may infest very limited planted areas. These include pests of turf, golf courses and the like with minimal opportunities especially in Egypt. Therefore, such pests-damaged areas may be amended by ready-made kits provided by landscape grass suppliers on the basis of planted metric landscape selling. Therefore, exploitation of EPNs as biocontrol agents of insect pests has been less sophisticated in Egypt and Russia than in other countries; e.g. Korea, Brazil, and USA **(Dolinski *et al.*, 2012).** Other drawbacks which hinder the advance of applied entomopathogenic nematology in Egypt and Russia include insufficient awareness of modern insect pests-control strategies especially by small scale farmers, limited financial resources available through governmental bodies for advancing this discipline, unsatisfactory response from the private sector to contribute to the development of EPN applications, and imperfect activity of relevant agricultural extension. Also, there should be closer collaboration and coordination between the nematologists and other researchers of relevant disciplines in every aspect of the production and application of the EPN product. Multidisciplinary research for developing relevant commercial EPN products may include nematologists, entomologists, molecular biologists, plant pathologists, formulation chemists, agricultural engineers, and the likes. No sophisticated molecular approach has contributed to EPN applications compared to current trends which make *Bacillus thuringiensis*-transgenic cotton, for example, the most popular option to control its insect pests. Eventually, more financial, media, regulatory, and technical support would need to be forthcoming in order to exploit the potential for increased pest management through EPN applications in both countries.

EPN application in Russia is facing numerous obstacles, but probably the most important impediment for this technology in Russia is over-complicated bureaucratic system of new products registration in pest control. As it is indicated by **Anishchenko *et al*., (2010)** the state registration of microbial pesticides is overseen by such federal institution as Rossel'khoznadzor (Russian Agricultural Control). It is known Rossel'khoznadzor is responsible for registration, use, production and transportation, sale, (including import and export of pesticides). The task to register new biocontrol agent, including EPN-based one is obviously behind the reach for any small producer.

Key points and perceived reasons for success in using EPNs in both countries:

1. Relevant researchers should act quickly to compensate for the lack of experience and the acquisition of new skills and techniques. Technical progress in EPN production and utilization will create further chances to integrate the use of EPNs in pest management strategies in both countries. Current methods of detecting the nematode in the field, requiring trapping and monitoring insects, are tiring and costly for routine monitoring. Future research will utilize a newly developed molecular primer/probe set that can quantify EPNs present in soil samples **(Campos-Herrera *et al*., 2011).** Use of this tool will facilitate efforts to understand the relative contributions of EPN species in the control program. The molecular probe can also help growers identify fields in which nematode applications are likely to be profitable **(Dolinski *et al*., 2012).**

2. A cottage industry and/or small enterprises should be initiated as a first and urgent step in order to economize for EPN production and distribution. Capital outlays should be kept as modest as possible. For example, we should consider that Biosys (a former EPN company) entered into a cost-effective long-term contract agreement with a contract manufacturer to provide fermentation facilities rather than build its own fermentation manufacturing facility **(Georgis, 2002).** Also, by tapping into the infrastructures of other agrochemical companies through alliances, Biosys was able to launch many products without spending the time and money to create its own sales and distribution networks.

3. Relevant EPN techniques should be considered: Soil temperature where nematodes are to be applied should be above 55°F and less than 90°F. Nematodes are also affected by suboptimal soil type, thatch depth, and irrigation frequency. EPNs require a moist, not saturated, soil so they can move around and locate their host. Protect nematodes from excessive exposure to ultra violet (UV) rays which can inactivate and kill them. Select proper day time (sunset) for application of EPNs and proper period to target the susceptible

stage of the pest. Select the proper nematode species to match the most susceptible pest stage. Take into account that storage of formulated nematode species varies: steinernematids at 39-46°F; heterohabditids at 50-60°F. Do not leave in a hot vehicle. Select the application rate and method to maximize contact between EPNs and the target pest **(Dreves, 2014).** In all cases, refer to the manufacturer's label for recommendations. Modern pest management tactics should be disseminated to stakeholders. The relatively high cost of bio-insecticide nematodes should encourage growers to consider ways to target specific zones within fields which have the insect pests for EPNs application to minimize wasted money and unprofitable expense. So, instead of random EPN application, nematodes should be deliberately sprayed on insect aggregates **(Abd-Elgawad, 2014).** Biocontrol potential can be enhanced by using EPNs that can best tolerate extremes in heat, cold, or desiccating conditions or other stress factors. Hence, characterization of environmental tolerance among EPN strains and species can aid in selecting which nematode to use in a particular target system **(Shapiro *et al.*, 2014).** Continued discovery of novel EPNs, or novel uses for them, is certain to lead to new and improved pest control. Use of nematodes may also be expanded by increasing host suitability through genetic improvement or better formulation and packaging. Improvements in production technology, distribution, and application will be a key to reducing nematode costs and insuring quality. In this vein, **Gaugler (1997)** proposed local level cooperatives to produce nematodes cheaply and effectively for on-site use. This strategy may be ideal in Egypt and Russia. Application of nematodes in infected hosts instead of aqueous suspension may also be a potential approach, which could reduce costs of in vivo production because several labor-intensive steps would be avoided **(Shapiro-Ilan *et al.*, 2002).** When applying agrichemicals in the area where EPNs are to be used, be sure that there is enough separation time between applications of toxic compounds and EPNs **(Dreves, 2014).** Some chemicals have been found to affect nematode efficacy when nematodes are exposed to them. These should be applied with care when used in conjunction with nematodes. On the other, when the nematodes coexisted with cytoplasmic polyhedrosis virus of 100 x 10^6 polyhedra inclusion bodies per ml, which reproduced within and killed *R. ferruginous* larvae, nematode survival was adversely and progressively affected by the increase in the viral suspension. Among tested concentrations, a viral suspension equal to one-fifth the size of the nematode and virus suspension is the best for nematode activity in integrated control against the red palm weevil **(Salama and Abd-Elgawad, 2002). Likewise, Abd El-Zaher and Abd-Elgawad (2012)** assessed the antagonistic potential for the bacterial symbionts of EPNs against common micro-organisms of Egyptian soil. The EPNs symbionts, *Photorhabdus* spp., could be used as a biopesticides **(e.g. Abd El-Zaher *et al.*, 2008 and 2012).**

4. More resources from governments and industry should be devoted to support

different facets of EPN research with common interest in their usage as biocontrol agents.

5. Raising awareness of farmers and extension personnel concerning EPN as bio-insecticides. Information days (day-long teaching events), targeting farmers and extension specialists, should be organized to transfer knowledge, technologies and methodologies in terms of nematology-related issues. These events should be held at sites where demonstration trials may be done to inform (and educate) farmers and extension officers about the importance, mode-of-action, application and impact of EPN on insect pests, crops and soil health.

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الملخص العربي

تطبيق النيماتودا الممرضة للحشرات في مصر وروسيا: التحديات والفرص

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تعتبر المكافحة البيولوجية بديل آمن للمبيدات الحشرية الكيميائية فهي سليمة بيئيًا، ولذلك أوضحنا في الوضع الحالي للبحث العلمي والتطبيق للنيماتودا الممرضة للحشرات بوصفها مبيدات حيوية في مصر وروسيا الاتحادية. لقد أدى تبني الزراعة العضوية إلى تغييرات جوهرية في ممارسات إدارة المزارع العضوية لتشمل التحديات الجديدة للزراعة العضوية استعمال النيماتودا الممرضة للحشرات ضد الآفات، ولذا ناقشنا مثل هذه الممارسات في كلا البلدين وعرضنا المعوقات والحلول لاستخدام وتسويق هذه النيماتودا في البلدين. هناك حاجة إلى المزيد من الدعم المالي، والمعلوماتي، والتنظيمي، والتقني من أجل استغلال الإمكانات المتاحة لزيادة مكافحة الآفات الحشرية من خلال استعمال النيماتودا الممرضة للحشرات في كلا البلدين. أوضحنا الأسباب المتصورة لتطوير التقنيات التي تستغل إمكانات الإدارة الفعالة للآفات من خلال تطبيق النيماتودا الممرضة للحشرات بطرق متنوعة. ينبغي تشجيع الإنتاج المحلي لهذه النيماتودا للقضاء على أو الحد من نقل وتعبئة وتغليف وتكاليف تخزين النيماتودا. وعلاوة على ذلك، قد تكون النيماتودا المحلية من سلالات مصرية وروسية أكثر تكيفًا وأقل تكلفة – دون أي خطر على الحيوانات والنباتات في بلدها – من النيماتودا المستوردة. عرضنا أيضًا الإرشادات اللازمة لزيادة عدد الأشخاص المهتمين باستعمال النيماتودا الممرضة للحشرات ضد الآفات أملاً في جني منتجات زراعية نظيفة وآمنة للإنسان والبيئة.