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Mass Production of Entomopathogenic Nematodes- A Review

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Abstract*—Utilization of entomopathogenic nematodes (EPNs) is an ecofriendly method of crop protection. EPNs can be easily mass produced. Production approaches are either in vivo or in vitro methods (solid and liquid). Most nematodes intended for commercial application are produced in solid or liquid fermentation technology. However, for laboratory research and small greenhouse or field trials, in vivo production of entomopathogenic nematodes is the common method of propagation. Mass production of EPNs is influenced by the amount of progeny required, time, resources, the costs of production, as well as the level of expertise available. The differences in nematode life cycle and bacterial symbiosis play major role in final nematode yields. This review describes the general biology of EPNs and gives an overview of studies to date on EPNs mass production.*

Keywords*— *Entomopathogenic nematodes, bacterial symbiosis, biocontrol agent, in vivo mass production, in vitro mass production.

I. INTRODUCTION

Entomopathogenic nematodes (EPNs) are widely used as biocontrol agent against economically important insect pests in different farming systems, viz. fruit orchards, vegetable garden, turf grass, nurseries and greenhouses which provide environmentally safe and sustainable crop protection. EPNs can be considered good candidates for commercialization as biological control agents as they can rapidly kill the insect host; have a broad pest host range; have active searching behavior; they can be mass produced; have potential for application in integrated pest management programs; and are considered safe for vertebrates and most non-target invertebrates, therefore minimizing the registration requirements (Lacey and Georgis 2012, Lacey et al. 2015) .The use of EPNs for biocontrol involved a step-by-step scientific and technical development. Mass production of the nematodes played a key role in the commercially development of insect pests control. Steiner (1923) identified the species *Aplectana kraussei* for the first time. Later, Glaser and Fox (1930) identified a nematode infecting grubs of the Japanese beetle (*Popillia japonica*) at the Tavistock Golf Course near Haddonfield, New Jersey, USA. This nematode was described by Steiner as *Neoaplectana* (=*Steinernema*) *glaseri* (Rhabditida: Steinernematidae) from Belgium as a natural pathogen of *Hoplia philanthus* (Coleoptera: Scarabaeidae) (Steiner1929). A new species of entomopathogenic nematode, *Heterorhabditis bacteriophora,* was described by Poinar in 1975, as a new species as well as a member of new genus, and family (Heterorhabditidae) of Rhabditida. Currently, over 118 species of *Steinernema* and 20 species of *Heterorhabditis* have been described from different habitats all over the world (Hunt and Sergei,2016). Besides these, other nematode species, *Oscheius* (=Heterorhabditoides) species have been shown to use pathogenic bacteria to parasitize insect hosts. *O. chongmingensis* , *O. carolinensis*, *O.rugaoensis* and *Caenorhabditis briggsae* have been identified as potential insect pathogens (Nguyen and Hunt 2007,Zhang et al.2008,Ye et al.2010,Dillman et al.2012,Zhang et al. 2012).

II. BIOLOGY OF ENTOMOPATHOGENIC NEMATODES

The life cycle of EPNs is characterized by an egg stage, four juvenile stages, and an adult stage. Only the third juvenile stage is the infective juvenile that is free-living in the soil, non-feeding, encased in a double cuticle with closed mouth and anus and capable of surviving for several weeks in the soil, before infecting a new host individual. Therefore, the only stage used in biological control is the third instar infective juvenile. The infective juveniles actively penetrate through the midgut wall or tracheae into the insect body cavity (hemocoel) containing insect haemolymph. EPNs have a mutualistic partnership with Gram-negative Gamma-Proteobacteria in the family Enterobacteriaceae. *Xenorhabdus* bacteria are associated with steinernematids nematodes while *Photorhabdus* are symbionts of heterorhabditids. *Xenorhabdus* occurs naturally in a special intestinal vesicle of *Steinernema* IJs (Bird and Akhurst1983) while *Photorhabdus* is distributed in the foregut and midgut of *Heterorhabditis* IJs(Boemare et al.1996) .An IJ carries

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between 0 and 2000 cells of its symbiont bacterium in the anterior part of the intestine(Spiridonov et al.1991,Endo and Nickle 1994,Forst and Clarke, 2002). *O. chongmingensis* and *O. carolinensis*, and *Caenorhabditis briggsae* have been found to associate with insect pathogenic bacteria of the genus *Serratia*, while *O. carolinensis* may have additional associates (Torres-Barragan et al. 2011). *O. chongmingensis* and *C. briggsae* require their bacterial partners to cause host death, to grow and reproduce within killed insects, and emerging dauer juveniles are associated with the vectored pathogen (Ye et al.2010) .The nematode provides protected shelter for the symbiotic bacteria and carries the bacteria into the host. Nematode and bacteria overcome the insect immune system and the host insect is killed within 48 hours post infection (Adams and Nguyen,2002) .The bacteria break down the host tissues, and provide food sources for the nematode, which feeds and multiplies on bacterial cells and degrading host tissues. During the process, the bacteria themselves provide a protected niche by producing antibiotics that suppress the competition from other microorganisms(Kondo and Ishibashi, 1986). Due to the different symbiotic bacteria associated with EPN, heterorhabditid nematodes turn the host cadaver red, purple, orange, yellow, brown or sometimes green, whereas steinernematid nematodes turn the insect cadaver tan, ochre, gray or dark gray. J_4 stage nematodes develop into egg laying female or male adults in the insect cadaver and hereby run through four juvenile stages (J_1 - J_4) and the adult stage has up to three generations(Kaya and Gaugler,1993).After reproduction and depletion of all nutrients, a high nematode population density triggers the nematode development into IJs again. In the case of *Steinernema*, IJs become colonized by bacteria *via* one or two founder bacterial cells. The life cycle of Heterorhabditid is similar to that of Steinernematids except for the fact that the IJs always develop into self-reproducing hermaphrodites(Poinar, 1990). Strauch *et al.* (2000) observed that offspring of the first generation hermaphrodites can either develop into amphimictic adults or into automictic hermaphrodite, both can occur simultaneously.The development into amphimictic adults is induced by favourable nutritional conditions, whereas the development of hermaphrodites is induced by low concentrations of nutrient. The lifecycle is completed in a few days and thousands of new IJs emerge, searching for new hosts. The cycle from entry of IJs into a host until emergence of new IJs is dependent on temperature and varies for different species and strains. Generally, life-cycle of EPNs (infective juvenile penetration to infective juvenile emergence) is completed within 12-15 days. The optimum temperature for growth and reproduction of nematodes is between 25^0 C and 30^0 C.

III. MASS PRODUCTION OF ENTOMOPATHOGENIC NEMATODES

The most important requirement for successful and economically reasonable usage of EPNs in crop protection is their production on large scale at competitive cost within a short time (Ehlers,2001). Entomopathogenic nematodes can be easily cultured either *in vivo* or *in vitro* in the laboratory. Mass production of entomopathogenic nematodes has evolved from the first large scale *in vitro* solid media production by Glaser (1940),to the *in vivo* production by Dutky *et al*. (1964) to the three dimensional solid media *in vitro* process by Bedding(1981,1984) and to the *in vitro* liquid fermentation production method by Friedman(1990).

3.1 IN VIVO MASS CULTURE

In vivo production is a simple process of culturing EPNs in live insect hosts (Table.1). *In vivo* nematode production is based on the White trap method; the method involves the natural migration of IJs away from the infected host cadaver into a surrounding water layer, from where it can be harvested. This method was devised, reconstructed and later on modified by several workers(White,1927,Dutky et al.1964, Poinar,1979, Woodring and Kaya, 1988,Abdel-Razek and Abd-elgawad,2007, Lindegren et al.1993). Gaugler *et al*.(2002) developed LOTEK system which does not rely on nematode migration to a reservoir. The system consists of perforated trays to secure insects, harvesters with misting nozzles that rinse IJs through the holding trays into a central bulk storage tank and use of a continuous deflection separator for washing and concentrating IJs. The hosts used *in vivo* methods must be susceptible, have high multiplication potential, and reared easily using cheap materials. The choice of host species and nematode for *in vivo* production should depend on nematode yield per cost of insect and the suitability of the nematode for the pest target (Chen et al. 2004,Blinova andIvanova,1987, Costa et al. 2007).The most common insect host used for *in vivo* production is the last instar of the greater wax moth *Galleria melonella* (L.) (Lepidoptera: Pyralidae). *G. melonella* occurs naturally in bee hives and is reared using artificial diets made of cereals, wax, yeast and glycerol. Production of cocoons and the extreme fragility of nematode infected larvae (*G. melonella*) are some of the drawback. The silkworm (*Bombyx mori*) is a Lepidopteran insect that feeds on mulberry leaves and twigs and is highly susceptible to entomopathogenic nematodes. The yellow mealworm, *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae), is an alternative host for *in vivo* nematode

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production. The structural integrity of nematode infected mealworm cadavers has enabled the development of mechanized methods for packing, thereby reducing labor costs. Nematode yield in general is proportional to host size(Flanders et al.1996, Kaya.and Stock, 1997).Maximum number of IJs per larva (*Steinernema* sp. and *Heterorhabditis* sp.) is found in the large sized *Galleria mellonella* larvae (20-22 mm). However, the production of *Heterorhabditis* sp. per unit body weight is always greater than that of *Steinernema* sp. in *Galleria* larvae (Raj Kumar et al.2003). *In vivo* production yields are also dependent on nematode doses (Boff et al.2000).Inoculation method, nematode concentration and host density also effect *in vivo* production of *S. carpocapsae* and *H. bacteriophora* in *G. mellonella* and *Tenebrio molitor* (Shapiro-Ilan and Gaugler,2002,Shapiro-Ilan et al.2012). *In vivo* can be accomplished by pipetting or spraying nematodes onto a substrate, immersion of insects in a nematode suspension, or applying the nematodes to the insect's food. It was observed that host immersion was about 4 times more efficient than pipetting inoculum on to the hosts (Shapiro-Ilan and Gaugler, 2002).Environmental factors including temperature, aeration, and moisture can affect yield (Shapiro-Ilan et al.2012, Grewal et al.1994,Dolinski et al.2007).In general nematode yield is proportional to insect host size (Ehlers and Shapiro Ilan,2005).Other factors affecting yield are inoculum and temperature. The efficiency of *in vivo* culture production also relies on the quality of media, i.e., insect hosts. For example, in production operations that produce their own insect hosts for nematode culturing, a host diet that is improved for insect production translates into improved efficiency in the overall process (Morales-Ramos et al. 2011). Additionally, in a tri-trophic interaction, the nutritional quality of insect host's diet can also impact the quality and fitness of entomopathogenic nematodes that are reared on those insects. The nematode's role in community dynamics will be affected as host diet effects impact entomopathogenic nematode ecology thereby fitness is impacted by differential nutrition (Shapiro-Ilan,2008). Best yields are achieved with intermediate inoculum dosage because higher doses create lower yield due to EPN competition for nutrients(Shapiro-Ilan et al.2002).Optimum production temperatures lie between 18°C and 28°C for different species(Burman and Pye,1980, Hazir et al,2001,Karagoz et al. 2009,Morton and Gracía-del-Pino, 2009).It is also crucial to maintain adequate aeration and humidity throughout the production process(Shapiro-Ilan and Gaugler 2002). Advances in mechanization and production geared toward application of nematodes through infected host cadavers can improve efficiency and economy of scale(Shapiro-Ilan et al.2016).

Table.1: Nematode mass production in in vivo method.

Nematode species	Host	References
Neoaplectana carpocapsae (DD-136 strain), *Steinernema glaseri,S. carpocapsae,S. feltiae, S. masoodi, S. seemae, S. thermophilum,S.*sp.	*Galleria mellonella*	(White, 1927, Dutky,1964,Poinar, 1979, Blinova and Ivanova, 1987, Woodring and Kaya 1988, Lindegren et al. 1993, Gaugler et al.2002, Chen et al. 2004, Abdel-Razek and Abd-elgawad,2007, Costa et al. 2007).
Heterorhabditis bacteriophora, H. indica, Heterorhabditis sp.	*G. mellonella*	(Poinar, 1979, Woodring and Kaya, 1988, Lindegren et al.1993, Flanders et al.1996, Kaya and Stock, 1997, Boff et al.2000,Raj Kumar et al.2003)
H. bacteriophora	*Corcyra cephalonica*	(Shapiro-Ilan and Gaugler 2002, Raj Kumar et al.2003)
Steinernema sp.,*S.glaseri,S. feltiae, S. thermophilum,S. carpocapsae,S.masoodi, S. seemae*	*C.cephalonica*	(Blinova and Ivanova, 1987, Karunakar et al. 1999, Ganguly and Singh,2000, Singh and Gupta 2006, Khan et al. 2007, Ali et al.2008, Shapiro-Ilan et al. 2012).
N.carpocapsae	*Diatraea saccharalis*	(Folegatti et al.1988)
S. feltiae	*G.mellonella, Achroia grisella*	(Saenz and Luque, 2000)
H. bacteriophora ,S. carpocapsae,S. glaseri , Heterorhabditis sp.	*G.mellonella, Achroia grisella Bombyx mori*	(Saenz and Luque, 2000, Zaki et al. 2000, Prabhuraj et al. 2003)

H.indica,S. *glaseri*	*Chilo sacchariphagus indicas*	(Karunakar et al. 1999)
H. bacteriophora	*Tenebrio molitor*	(Shapiro-Ilan et al.2002)
S. masoodi,S. seemae, S. carpocapsae,S. glaseri S. thermophilum,H. indica	*H. armigera*	(Subramanian,2003 , Ali et al.2008, Rishi and Prasad 2012).
S. carpocapsae	*S. litura*	(Ali et al.2008, Gupta et al.2008)
H. indica	*P. xylostella*	(Rishi and Prasad, 2012)
*S.*sp., *H. bacteriophora*	*Odontotermes obesus*	(Devi et al.2018)
S. carpocapsae, H. bacteriophora	*Capnodis tenebrionis*	(Morton and Gracia-del-Pino, 2009).

3.2 IN VITRO MASS CULTURE

In vitro culturing of EPNs is based on introducing nematodes to a pure culture of their symbiotic bacteria in a nutritive, non-living medium. Such media must use sterile ingredients to avoid unwanted bacterial contamination, retain the nematode's specific symbiotic bacterium and provide all the necessary nutrients. The medium is sterilized, and then inoculated with bacteria, followed by the nematodes. Nematodes are then harvested within 2-5 weeks in water. *In vitro* mass production of *Steinernema glaseri* was attempted for the first time in USA for prevention of *Popillia japonica* (Glaser,1932,McCoy and Glaser,1936).The presence of symbiotic bacteria was discovered from DJ (dauer juvenile) of *Steinernema felitiae* (McCoy and Glaser, 1936). Later on *Xenorhabdus nematophilus*, the symbiotic bacteria was isolated and identified from *S. carpocapsae* (Poinar and Thomas, 1966).House *et al.*(1965) devised a dog food based medium to produce the DD-136 strain of *Neoaplectana carpocapsae* on a commercial scale. Hara *et al.*(1981) who stressed on monoxenicity, produced 125 million nematodes / week from 100 dog food agar Petri dishes at a cost of $ 0.28 per million. Bedding (1976) developed methods for production of *Neoaplectana* spp. Bedding (1981) soaked shredded plastic foam in pig's kidney-beef fat homogenate (animal protein and lipid based medium). Several species of neoaplectanid and heterorhabditid nematodes were reared successfully with this method with an average yield of 6×10^5-10×10^5 infective juvenile (*N. carpocapsae*) per gram of medium, at a cost of less than $ 0.02 per million. As an improvement to the previous method, Bedding [27] coated shredded polyether polyurethane sponge with a homogenate of chicken offal (for steinernematids) or chicken offal and 10 per cent beef fats (for heterorhabditds), sterilizing the medium in large autoclavable bags and adding the appropriate bacterium and nematode and was able to produce about 50,000 million IJs of *N. bibionis* in a week. In Pakistan, *S. pakistanense, S. asiaticum, S. feltiae* and *H. indica* were mass produced using chicken offal media(Tabassum and Shahina, 2004).Entomopathogenic nematodes were reproduced in solid culture method as 47,000 DJ/ml (Buecher and Popiel,1989).Solid culture method is economically feasible up to a production level of approximately 10×10^{12} nematodes/month(Friedman et al.1989,Ramakuwela et al. 2016).Liquid culture for entomopathogenic nematodes was attempted for the first time by Stoll in 1952 .He cultured them in the shaker by using liver extracts yielding approximately 400 DJ/ml at 21^0C-25^0C and pH of 6.0-6.5, and he had an important observation that, reproduction was more in the dark. Buecher and Hansen (1971) examined the effects of quantity of air flow and shear stress on the growth of entomopathogenic nematodes after the air was supplied to the liquid culture media. Pace *et al*. (1986) attached the flat–blade impeller to the 10 L Bioreactor and then inoculated *Xenorhabdus nematophilus*. After incubation for 24 hours, they reinoculated *Steinernema carpocapsae at* 2,000 DJ/ml and incubated for 10 days while oxygen saturation of 20% was maintained at 23 -28˚C, 180 rpm. *S. feltiae* strain 42 was reared in liquid culture along with its bacterial symbiont, *X. nematophilus.* First-stage juveniles developed into reproducing adults in a maintenance salts medium containing resuspended *Xenorhabdus* cells and the yeast *Kluyveromyces marxianus* or cholesterol. Friedman *et al.* (1989) observed that costs of production decrease rapidly up to a capacity of approximately 50×10^{12} infective juveniles/month in liquid fermentation technique. Using this method *S.carpocapsae*, *S.riobrave*, *S.scapterisci*, *S.feltiae*,

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S.kushidai and *S.glaseri* have been produced at 80,000 L scale and *H.bacteriophora*, *H.indica* and *H.megidis* have been produced at 300-2000L level with yield capacity as high as 250,000 IJs /ml (depending on the nematode species). An improved method has been developed by Lunau *et al*.(1993) where axenic nematode eggs are placed on a pure culture of the symbiont. Culture times vary depending on media and species, and may be as long as three weeks though many species can reach maximum IJ production in two weeks or less(Ehlers et al.2000).Large scale production was further advanced through several measures including using bags with gas permeable Tyvac ® strips for ventilation, automated mixing and autoclaving, simultaneous inoculation of nematodes and bacteria, sterile room technology, and automated harvest through centrifugal sifters(Gaugler and Han ,2002,Neves et al. 2001,Wang et al.2007).Once the culture is completed, nematodes can be harvested from media via centrifugation (Surrey and Davies,1996). Media containing materials of plant origin generally were reported to have low productivity than those of animal origin (Abe, 1987, Wouts, 1981, Ehlers, et al.1998,Vyas et al.1999, Shapiro-Ilan and McCoy 2000,Vyas et al.2001,Hussaini et al.2000,2002,2007,Kaya et al. 2006,Prabhu et al.2006, Umamaheswari et al.2008, Somwong and Petcharat,2012, Upadhyay et al. 2013,Sunanda and Siddiqui,2013, Shapiro-Ilan and Xuehong, 2014,Ferreira and Malan,2014, Banu and Meena,2015,Yadav et al.2015).

IV. STRATEGIES FOR MASS CULTURE

Although these nematodes are easily produced *in vivo* or *in vitro* on various complex semisolid organic media, the cost of mass production using these methods is a major constraint on nematode commercialization. A large scale liquid culture system would constitute a more cost-effective approach. EPN production with *in vitro* solid technology gives rise to higher nematode yields per gram of solid media than *in vivo* technologies. However, costs associated with solid media technologies are much higher than *in vivo* technologies. The high production cost is mainly associated with labour, materials and storage area, while large scale commercial farms' nematode needs can be met by the capital investment mass propagation methods using fermentation chambers [101-103]. Although mass production in submerged culture offers cost-efficiency, capital and technical expertise is still required. Understanding the biology of both the nematodes and bacterial partner is important for mass production. Phase shifting of the bacterial symbiont, time and concentration of the nematode inoculums, low percentages of nematode copulation, and fermentation parameters (oxygen concentration, pH, temperature, agitation, etc.) are some of the other factors which create problem in mass production (Ferreira and Malan,2014 ,Kaya et al.2006,Ehlers,2001,Gil et al.2002,Ehlers,1994,Ehlers et al. 1992,Zervos et al. 1991).The quality of infective juveniles depends on method of production and media composition. Recovery can also be affected by nutritional factors, aeration, CO_2, lipid content, and temperature (El-Sadawy, 2011).Diets rich in lipids, glucose and yeast extract content increased juvenile yields in *in vitro* production (Han et al.1992,Kooliyottil et al.2013,Chavarría-Hernández et al. 2010).Nematode virulence is correlated with the percentage of dauer juveniles retaining *Xenorhabdus* and the number of bacteria per dauer juvenile. *Xenorhabdus* subspecies vary in their virulence for a given host. Virulence of *S. glaseri* was restored by culturing these nematodes on *X. nematophilus* subsp, *poinari*. Nematodes with small juveniles were more productive than large nematodes. Nematode yield is inversely proportional to the size of the species. Higher yields of *H. indica* whose juveniles are small in size but *S. yirgalemense* is a large nematode and yet the highest yielding nematode species in *G mellonella* .Maximum average yields reported include 300,000 and 320,000 IJs per ml for *H. bacteriophora* and *S. carpocapsae* respectively, 138,000 per ml for *H. megidis* ,71,470 IJs per ml for *S. feltiae* and 450,000 IJs per ml for *H. indica*. Trait deterioration is a major concern to industrial producers of entomopathogenic nematodes(Bilgrami,2006).Trait changes as a result of continuous subculturing in *S. carpocapsae* and *H. bacteriophora*. These investigators studied trait stability of *P. luminescens* and *X. nematophila* after serial *in vitro* subculturing and demonstrated that phase variation (Phase I to Phase II) in *P. luminescens* and *X. nematophila* strains occurred within ten subculturing cycles. Furthermore, phenotypic variation was controlled in *X. nematophila* strains by selection of primary variants; however, trait change was not detected after prolonged culturing. When phenotypic variation in *P. luminescens* was controlled, changes in the primary variant like cellular morphology and prevalence of inclusion bodies with different sizes were observed((Inman et al.2012,Inman and Holmes,2012).Inman and Holmes (2012) have described the role of trehalose, a non-reducing sugar found in abundance within insect hemolymph that seems to aid in maintenance of Phase I variant of *P. luminescens* over extended periods of time. Minimization of serial passages, introduction of fresh genetic material, improved cryopreservation methods

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of stock cultures (Bai et al. 2004) or creation of homozygous inbred lines are the probable precautions against strain deterioration (Bai et al. 2005, Chaston, 2011).The quality of nematodes produced *in vitro* solid culture is similar to that produced *in vivo* (Dunphy and Webster,1989, Glaser et al.1940,Han et al.1997).High quality of EPNs can be produced using liquid culture provided good media as well suitable environmental conditions in the bioreactor (Johnigk et al.2004,Hirao and Ehlers,2010,Indriyanti and Muharromah,2016).

V. ECONOMIC VIABILITY

Low-cost mass production of entomopathogenic nematodes (EPNs) is an important prerequisite towards their successful commercialization. During the past few years, a distinct cottage industry has emerged that produces entomopathogenic nematodes mostly *in vivo* for the home lawn and garden markets. Small scale farmers will benefit using cheap materials and those from their farms. However, commercial scale production is impracticable due to high production costs, lacks economies of scale and low nematode yields per gram of insect biomass. The advantage of *in vitro* solid media method are that capital costs are low, limited expertise is required and the logistics of production are flexible. This technology has the lowest mass production costs and is the method of choice for larger companies with multiple products in industrialized countries. Nematodes have been commercially developed by several companies in large liquid fermentation tanks which range from 50,000 up to 100,000 L fermenter(de la Torre,2003, Dillon et al.2012) in North America, Europe, Australia and Asia for the control of a vast array of pests, ranging from pests occurring in greenhouses to those occurring on golf-course turfs. In 1982,the first company which commercialized the liquid culture methods for entomopathogenic nematodes was Biosys (Palo Alto, California).They made mass production of *S. carpocapsae* in large scale of 80,000 L and their commercial products 'Biosafe' and 'BioVector' were used against lawn and garden pests. In 1983, Biotechnology Australia, produced nematodes on particles of sponge impregnated with an artificial diet and the product 'Otinem' was utilized against black vine weevils in Australia and Europe. Currently, E-Nema GmbH and Microbio Ltd. are doing mass production in Europe. Becker Underwood (formerly Micro Bio Ltd.) is owned by a USA company but operates out of Little hampton, United Kingdom, e-nema is based in Germany, and Koppert has its home in The Netherlands In addition, there are smaller producers like Andermatt Biocontrol based in Switzerland, bionema in Sweden and Owiplant in Poland, which produce nematodes using an improved solid-state Bedding system. In Korea, The Sesil, a company has started in vivo nematode production using the greater wax moth, *Galleria mellonella* (L.), larvae. The company produces 200 packs of *S. carpocapsae* Pocheon strain and 380 packs of an unidentified Korean isolate of *Heterorhabditis* sp. a day. The nematodes are sold for use against caterpillars on vegetables, fungus gnats on mushrooms and other insect pests of greenhouse plants. In Korea, WooGene B and G is currently producing the mass culture of entomopathogenic nematodes. A Chinese company Guangzhou Greenfine Biotechnology uses a solid culture method to produce several entomopathogenic nematode species both for Chinese and international markets.

VI. FUTURE PROSPECTS

Entomopathogenic nematodes have emerged as important biological control agents against soil-dwelling as well as plant-boring insects. The role of nematodes in controlling insect pests will be enhanced by continued research and improved quality control. Recent advances in mass-production and formulation technology, and the discovery of numerous isolates/strains, together with the desirability of reducing pesticide usage, has resulted in a surge of scientific and commercial interest in these insect-killing nematodes. This has culminated in the commercial availability of many nematode products for use in several medium and high-value markets. Each approach has its advantages and disadvantages relative to production cost, technical know-how required, economy of scale, and product quality(Grewal et al.2005)and each approach can be improved further.

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