Steinernematids and Heterorhabditids as Biological Control Agents for Red Palm Weevil (*Rhynchophorus Ferrugineus Oliv.*)

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استخدام الستينرنيماتيد والهيترورهابدتيد (Steinernematids and Hetrophabditids) في المكافحة الحيوية ضد سوسة النخيل الحمراء (*Rhynchophorus ferrugineus* Olive)

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الخلاصة: لقد اعتبرت المبيدات الكيميائية ومنذ زمن بعيد الطريقة الرئيسية لمكافحة الحشرات، ولكن استخدامها أدى إلى انحسار أنواع من الحشرات لم تكن مستهدفة وظهور فصائل مقاومة للمبيدات ، بالإضافة إلى تلوث المحاصيل الغذائية والماء. لقد أدت هذه الاعتبارات إلى استصدار قوانين حكومية صارمة تحد من استخدام مثل هذه المبيدات، وتشجيع البحوث الهادفة لايجاد طرق المكافحة الحيوية البديلة ، وقد حازت عائلتي (الستينرنيماتيد ، والهيترورهابدتيدا) التي تصيب الحشرات بالأمراض الاهتمام الأكبر. من صفات هذه النيماتودا الطبيعية أنها تبحث وتقتل أنواعاً مختلفة من الحشرات التي تشمل سوسة النخيل الحمراء. وهي لاتضر بالنبات ولا الحيوان ، كما أنها معفاة من الالتزام بالتسجيل، ويسهل نشرها بالرشاشات أو بواسطة الري بالتنقيط. ويمكن أن تتعايش مع العديد من الكيماويات. أدى استخدام بعض من هذه النيماتودا المحسنة الجينات إلى موت ٩٥-١٠٠٪ من يرقات سوسة النخيل الحمراء داخل المختبر، أما في الحقل فقد ماتت ٥٠٪ من اليرقات. بناءً عليه فإن هنالك حاجة ماسة إلى تكثيف العمل نحو تقوية نشاط هذه النيماتودا، ولايجاد التقنيات اللازمة لانتاجها بكثرة، ودراسة إمكانية مقاومتها للظروف الصعبة ، وتحديد مواصفات تنشئتها ، ووضع الاستراتيجيات اللازمة لتوزيعها ، وذلك قبل نشرها تجارياً لمكافحة سوسة النخيل الحمراء والآفات المتخفية التي تصيب الأجزاء العلوية من النباتات المزروعة في المنطقة.

ABSTRACT: Chemical insecticides have long been considered as the primary method of insect control, but their use has been associated with suppression of non-target species, emergence of resistant strains, contamination of food crops and water. These concerns enhanced the enforcement of strict governmental regulations which limited the use of such chemicals and stimulated the search for alternative biological control methods. Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae received a considerable attention. These nematodes seek and rapidly kill a wide range of insects, including the red palm weevil. They are safe to plants and mammals, exempt from registration requirements, easy to apply by sprayers or drip irrigation systems and compatible with many chemicals. Application of genetically enhanced strains of Steinernematids and Heterorhabditids to larvae of red palm weevils resulted in 95 - 100% mortalities in the laboratory and 50% in the field. Additional work is needed to improve virulence, mass production technologies, tolerance to adverse conditions, formulations, and release strategies of entomopathogenic nematodes before they can be commercialized as biological control agents for red palm weevils and other cryptic insects attacking aerial plant parts in the region.

Sustainability of date palm (*Phoenix dactylifera L.*) and its tolerance to adverse ecological conditions, have given this tree a unique status as an important source of food and timber, for thousands of years in the Arabian Gulf Region. There is an estimated 22 million trees in the region with a total production of about 800 thousand tons of dates annually (AOAD, 1995). Although date palm is attacked by a wide range of pests, it is widely accepted that *R. ferrugineus Oliv.* is one of the most devastating insects which, in recent years, destroyed thousands of trees and forced hundreds of farmers to abandon their plantations. The first appearance of red palm weevil in the region in 1985, and its spread to more than 10 thousand farms in 1995, have decreased average yields from about 10 to only 0.7 tons/Ha in infested areas (Hanounik, 1996b).

Previous efforts to control this insect focused mainly on the use of traditional chemical control measures and only recently on pheromone traps. Unfortunately, these attempts were only partially effective. Although chemical insecticides have long been considered as the primary method of control their use has frequently been associated with suppression of non-target species, emergence of chemical-resistant strains and contamination of food crops and waters. These concerns prodded governments to restrict the use of chemicals and even ban several toxicants. This situation stimulated the search for alternative biological control methods. Increasing interest in biological control was associated with a corresponding worldwide

decrease in annual insecticide sales from $7.4 billion in 1978 (Woodburn and Cook, 1979) to $5.4 billion in 1990 (Engel *et al*, 1990).

A number of new, environmentally friendly, bio-control agents, including entomopathogenic nematodes, fungi, bacteria and viruses have been commercialized, in the 1980s, in USA and Europe. However, attempts to introduce these agents, particularly nematodes, to the region, did not occur until early in the 1990s (Hanounik, 1996b).

This paper addresses Steinernematids and Heterorhabditids as biological agents for the control of red palm weevils in the Arabian Gulf Region.

What are Steinernematids and Heterorhabditids?

Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae, with their associated bacteria *Xenorhabdus spp.*, have received a considerable attention as biological control agents. Although these nematodes can seek and rapidly kill, within 48 - 72 hrs, a wide range of soil insects and cryptic insects attacking aerial plant parts (Begley, 1990 and Klein 1990), they do not infect plants or mammals (Gaugler, 1988). These nematodes are compatible with a number of pesticides (Hara and Kaya, 1983) and can be applied with standard chemical sprayers and drip irrigation system (Reed *et al*, 1986) or injected in insect tunnels in tree trunks (Bedding, 1990). Steinernematids and Heterorhabditids with their symbiotic bacteria are exempt from registration requirements in various countries including USA, Canada, Europe, Australia and others (Gaugler, 1988). They have been reported from several countries around the world (Poinar, 1990). Their mass production technologies have developed (Bedding, 1984, 1988b; Friedman *et al*, 1989; Friedman, 1989 and Hanounik, 1996a) and commercialization has already taken place (Georgis, 1990). Unfortunately, the majority of information regarding entomopathogenic nematode technologies are currently protected by patents or hidden in confidential documents by commercial producers and are not available to the public. Taxonomically, members of both families; Steinernematidae and Heterorhabditidae, belong to the order Rhabditida, class Secernentea of the Phylum Nemata (Fig. 1). Although members of both families are bacterial feeders, like other Rhabditids, both can be differentiated from other Rhabditids by their characteristic symbiotic association with the bacterium *Xenorhabdus sp.* Members of the family Heterorhabditidae can be separated from those in steinermatidae by their tooth which is used to penetrate host intersegmental soft tissue (Bedding and Molyneux, 1982) and also by their hermaphroditic life cycle (Wouts, 1984). The family Steinernematidae consists of one genus; *Steinernema (=Neoaplectana)* which contains about nine species. The family Heterorhabditidae consists of one genus; *Heterorhabditis* which contains about seven species. Exact number of species in both genera is subject to discovery of new species. For more details on the taxonomy of entomopathogenic nematodes, the reader is referred to Poinar (1979, 1984), Poinar *et al.* (1987), Wouts *et al.* (1982) and Wouts (1984).

What is *Xenorhabdus Sp.*?

Xenorhabdus species represent a group of bacteria which are symbiotically associated with the entomopathogenic nematodes Steinernematids and Heterorhabditids, and have never been isolated from the soil (Akhurst 1983, 1986a). The genus *Xenorhabdus* consists of two species; *X. nematophilus* and *X. luminescens* which are associated with Steinernematids and Heterorhabditids, respectively (Akhurst, 1986a and b).

Xenorhabdus sp. may occur in primary or secondary form (Akhurst, 1980). Under natural conditions, only the primary form exists. The primary form transforms into the weak secondary form in old cultures when stored at room temperature in the laboratory. The secondary form does not transform back into the primary form and is not efficient for nematode mass production. The primary form is much more important for in-vitro commercial production. The primary form can be isolated from third stage infective juveniles and produces various antibiotics which inhibit other bacteria that might contaminate in-vitro nematode production culture.

Symbiotic Association With *Xenorhabdus Sp.*

Third stage infective juveniles of Steinernematids and Heterorhabditids harbor bacteria *Xenorhabdus* in their gut. The relationship between the bacterium and the nematode is a mutualistic one as neither can survive without the other (Akhurst, 1983).

Although the bacterium *Xenorhabdus* is the principal active bio-control agent, the bacterium can not infect on its own; it needs the nematode as a vector (Poinar and Thomas, 1966; Milstead, 1979). The nematode produces a factor that inhibits the bacterial anti enzyme system of insects (Gotz *et al*, 1981) and this creates suitable conditions for the multiplication of the bacteria in the body of the insect. The gut of the nematode serves as a suitable habitat for the survival of the primary form. On the other hand, *Xenorhabdus sp.* play an important role in the survival, growth and development of the nematode. The bacterium serves as an important food source for the nematode which

selectively feeds on the primary form despite the presence of the secondary form or other bacteria (Akhurst, 1980). In addition to rendering itself as a food source, *Xenorhabdus*, once inside the body of the host insect, causes septicemic death of the insect, produces antibiotics which inhibit foreign bacteria, multiplies and acts on the tissue of the insect rendering it more suitable for the growth of the nematode and development of its reproductive system (Poinar and Thomas, 1966).

Mechanisms of Host - Pathogen Interactions

Because successful host-pathogen interactions may occur only under favorable conditions, good performance in the field can take place only if a virulent nematode and a susceptible host species interact under favorable ecological conditions. In general, nematodes (the vector) actively seek their target insects (the host), achieve physical contact followed by penetration, and delivery of their symbiotic bacterium (the pathogen) into their host. Once the bacterium is delivered, a series of symbio-pathogenic interactions occur favoring bacterial multiplication, host death and nematode reproduction.

The ability of third stage infective juveniles to seek out insects is apparently due to various host attractants including CO_2 (Gaugler *et al*, 1980), heat (Byers and Poinar 1982, Burman and Pye, 1980), insect washing and fecal remains (Schmidt and All, 1979) and tunnels of trunk-boring insect larvae (Bedding and Miller, 1981). Third stage infective juveniles are the only stage in nematode's life cycle which occurs in the soil outside the host. Infective juveniles do not feed; instead they consume their own energy reserves and require oxygen (Burman and Pye, 1980) and moisture (Kondo and Ishibashi, 1985) for survival. Third stage infective juveniles are of a great importance in the commercialization of Steinernematids and Heterorhabditids as bio-control agents. The role of the bacterium as a pathogen and that of the nematode as a vector have recently been demonstrated. The injection of only 100 cells of *axenic Xenorhabdus sp.* (*Xenorhabdus* without nematode) into the susceptible insect host *Galleria mellonella* was sufficient to kill 90 - 100% of the insect (Akhurst, 1986a). Different *Xenorhabdus* species may be different in their pathogenicity. A total of 10,000 cells of *X. nematophilus sub sp. poinarii* of *Steinernema glaseri* were required to kill 50% of injected *G. Mellonella* (Akhurst 1986a). Although the inoculation of insects with axenic nematodes (nematode without *Xenorhabdus*) generally killed those insects and reached the adult stage, the reproduction of such nematodes was rare and poor when occurred (Poinar and Thomas, 1966). *Xenorhabdus spp.* are essential for nematodes survival and efficiency.

Ecology

Steinernematids and Heterorhabditids are soil-borne; they are apparently adapted to gradual desiccation patterns, which occurs naturally in the soil following irrigation or rainfall. Moore (1965) and Womersley (1990) showed that gradual desiccation favors nematode survival and Kondo and Ishibashi (1985) reported that *S. feltaiae* was most effective at soil moisture of 25 - 40%. This is probably why nematodes have been reported to be more effective in the soil and stem galleries where desiccation is much slower than exposed aerial plant parts. Nematode tolerance to gradual dehydration has led the industry to exploit this unique characteristic in the development of stable nematode-based formulations by immobilizing them on clay (Bedding, 1988a).

Minimum and maximum survival temperatures vary with nematode species and strains (Kaya 1977; Molyneux, 1985, 1986). *S. feltaiae* survives -10C° to 35C° (Schmiege, 1963), remains infective between 9 and 33C° (Dukty *et al*, 1964 and Molyneux, 1986) and reproduces efficiently between 25 and 35C° (Kaya, 1977 and Molyneux, 1986). Optimal temperature ranges of different nematodes seem to correlate with their geographical origin. Although Steinernematids are generally known to tolerate a lower range of temperature, and have frequently been reported from cooler geographical regions, compared to Heterorhabditids (Molyneux, 1984, 1985, 1986 and Akhurst and Bedding, 1986), recent recovery of *S. riobravis* from Texas (Raulston *et al*, 1992) and *S. Abbasi* from the Sultanate of Oman (Elawad *et al*, 1996), with a thermal niche of 10 - 39C° and 19 - 37C°, respectively could provide unprecedented opportunities for the use of entomopathogenic nematodes as biological control agents in the Arabian Gulf Region.

Biotic agents in the soil including predators and pathogens (Mankau, 1980) may affect nematode performance as indicated by decreased nematode survival in non-sterilized compared to sterilized soils (Ishibashi and Kondo, 1986).

Performance

Despite of the impressive attributes of Steinernematids and Heterorhabditids as biological control agents, their intolerance to adverse ecological conditions, such as rapid desiccation, temperature extremes and ultra-violet light (Gaugler and Boush, 1978 and 1979) has limited their use mainly to soil-

inhabiting insects and insects with cryptic feeding habits, including stem and fruit borers (Bedding, 1990, Lindegren and Barnett, 1982, Cossentine eta al, 1990 and Kaya *et al*, 1986) where adverse ecological conditions are much less important (Gaugler, 1998 and Gaugler and Kaya, 1990).

Attempts to optimize field performance of entomopathogenic nematodes, focused mainly on inundative release rates (Georgis and Gaugler, 1991). Unfortunately, this strategy was associated with inconsistent performance. Most failures were attributed to unsuitable nematode species or unfavorable ecological conditions (Georgis and Hague, 1991). Although inundative rates and avoidance of adverse conditions (i.e. by modified dates of application, effective delivery methods and irrigation) may partially enhance effectiveness, good performance may be achieved only if such tactics are combined with genetically improved nematode species. Steinernematids and Heterorhabditids are biological control agents and can be used only as **populations.** Each population consists of **diverse** and **dynamic** group of interbreeding individuals. These concepts are very important in pest management, and failure to understand their significance, may have a devastating effect on nematodes release strategies. Performance may be improved by not only the use of inundative rates (**quantitative**), but also by genetically enhancing desirable **quantitative** traits such as virulence and tolerance to adverse ecological conditions (Kaya, 1985). Magnitudes of the quantitative characteristics of nematodes may be negligible in their natural habitats where low nematode and insect populations occur. But with inundative commercial rates and greater host accessibility, host-pathogen interactions, become more operative and therefore, the significance of their qualitative characteristics becomes worthy of consideration in relation to their performance.

Steinernematids and Heterorhabditids have been recovered from various geographical regions (Akhurst and Brooks, 1984, Akhurst and Bedding 1986). They possess a wide range of genetic variabilities (Gaugler *et al*, 1989), including cold-tolerance (Finney-Crawley, 1985), drought tolerance (Glazer and Novan, 1990), adaptation to warm weather (Molyneux 1985 and Elawad *et al*, 1996) and virulence (Bedding *et al*, 1983). Genetic improvement may be achieved by rigorous screening and selection from naturally occurring local nematode populations and exotic world collections. Sampling for useful traits would be most effective in extreme habitats where natural selection pressures have enhanced desirable traits (Gaugler, 1989). The collection of nematodes from the geographical origins of migratory insects or insects that have recently invaded new regions, such as locust or the red palm weevil may lead to the recovery of more adaptive and effective nematode strains against such insects.

One of the best examples of genetic improvement through screening of naturally occurring nematode populations, was reported by Shapiro *et al*. (1985) who was able to enhance the virulence of *S. carpocapsae* by 2.5 folds after only two passages into gypsy moth larvae. Selection for host-finding efficiency resulted in a 72-fold increase against scarabaeid larvae (Gaugler *et al*, 1989 and 1991). Selection for virulence in *S. carpocapsae* resulted in the identification of certain strains which increased mortality of grape berry moth larvae from 68% and that of red palm weevils from 60% to 100%, 72 hrs after inoculation (Fig. 2) in the laboratory (Hanounik, 1994 a and b).

Once strains with desired traits have been selected, they can either be directly used or their traits incorporated into other well adapted nematode species, or else by genetic engineering if desired traits could be cloned into sexually incompatible species. Regardless of the method of genetic improvement, screening and selection should always be conducted by bringing target insect species and genetically improved nematodes, together under artificially simulated ecological pressure including various levels of moisture, heat or other factors in small micro plots in the field. Micro plots should be uniformly infested with target insects and then treated with the enhanced nematode to be tested. The level of ecological pressures, under which selection is to be conducted, must be **standardized**. Ecological pressures must be just enough to hamper the performance of local nematode species which serve as a control. Normally, standard nematode control species are chosen from locally used nematodes, which are known for their satisfactory performance under local conditions. Any other genetically enhanced species that outperforms such standard controls, under the given experimental pressures, are considered more efficient. Because useful nematode traits, such as the ability to seek and infect the host, complete the life cycle rapidly, lay large number of eggs, and survive longer are qualitative in nature, they must be quantified, and rating scales established, before efficient and inefficient species can be made. Such quantitative information may not only help identify efficient species, but also explain reasons underlying failures of inefficient nematode species or release strategies in the field.

Although the use of inundative rates (Georgis and Hague, 1991) of genetically improved nematode species, may enhance nematode performance against soil inhabiting insects, these strategies alone may not be sufficient to reverse the debilitating effects of rapid desiccation (Kamionek *et al*, 1974), solar radiation (Gaugler and Boush, 1978 and 1979) or temperature

extremes (Molyneux, 1986), when insects of aerial plant parts are targeted on exposed surfaces. Attempts to simulated humid conditions on aerial plant parts, where rapid desiccation hampers nematode performance, have focused on the use of anti-desiccants to slow rates of evaporation, extend water persistence and, as a result enhance nematodes survival and performance after application. Glazer *et al.* (1990 and 1992) achieved 85% mortality in *Heliothis armigera*, a cotton fruit borer, by the application of a drought-resistance *S. carpocapsae* 'Mexican' strains at the rate of 250 infective juveniles / ml, with the antidesiccant Folicot (Paraffin wax 40%), in a microplot test in the field. Similarly the use of the antidesiccant 'WPO' with a genetically enhanced Steinernematid strain 'S-GBM' extended its survival within wine grapes dense clusters, up to 96 hrs, and resulted in 85% mortality in grape berry moth larvae, compared to only 65% in chemical treated plots in the field (Hanounik, 1995). The world's first massive scale use of nematodes as biological control agent took place in China against the apple fruit borer *Carposina nipponensis* (Lepidoptera) and the shade tree stem borer moth *Holicocercus insularis*, with outstanding results (Bedding 1990). Soil application of *S. carpocapsae*, around the base of apple stems, gave excellent results against the apple fruit borer *C. nipponesis* compared to other methods of application or chemical insecticides. The life cycle of the shade tree stem borer moth is similar to that of the red palm weevil. Larvae, which hatch from eggs laid on the stem, penetrate into the wood, boring extensive internal tunnels and galleries. Injection of *S. carpocapsae* into infected stems, resulted in nearly 100% kill of larvae of *H. insularis* and tree recovery. The ability of these nematodes to seek target insects by travelling throughout the galleries inside the trunk gave excellent control (Bedding, 1990).

In laboratory test, conducted in United Arab Emirates in 1993 and Kingdom of Saudi Arabia in 1994, the application of certain genetically enhanced strains of *Steinernema* and *Heterorhabditis* species to control *R. ferrugineus*, resulted in 95 - 100% moralities in various larval stages of the insect, 72 hrs after inoculation (Fig. 3). Application of the enhanced strain 'S-RPW' of *Steinernema sp.* with the antidesiccant 'WPO', to larvae of *R. ferrugineus*, inside their galleries, in naturally infested date palm trunks, in the field, resulted in 50% mortality compared to only 20% with the un-enhanced population and no mortalities with rotenone, five days after application (Fig. 4). Temperature inside the galleries ranged between 26 - 28°C and relative humidity between 90 - 98% at time of application in the month of August 1993 in Abu-Dhabi, United Arab Emirates. These results showed that only selection for virulence in *S. carpocapsae* was sufficient to decrease the 50% efficacy gap between laboratory and field tests by 20%. Performance can be enhanced further by increasing the rate and improving the method of application to place nematodes in direct contact with or in the vicinity of their target host inside the galleries. Similar laboratory findings were reported with a local *Heterorhabditis* species in Egypt (Shamseldean and Abd-Elgawad, 1994).

In general, entomopathogenic nematodes perform better when:

a. Steinernematids are used compared to Heterorhabditids.
b. Genetically enhanced virulent strains are used compared to nematodes before improvement (Deseo and Miller, 1985).
c. Nematodes are injected into insect galleries in fig trees (Lindegren and Barnet, 1982), alter trees (Kaya and Brown, 1986) shade trees (Bedding, 1990) and Peach trees (Cossentine *et al*, 1990) compared to surface trunk application.
d. Nematodes are applied to soil around the base of trees to control apple fruit borer (Bedding, 1990).
e. Nematodes are applied into combination with anti-desiccants (Glazer *et al*, 1990 and 1992 and Hanounik, 1995).
f. Nematodes are appropriately delivered, in such a manner as to establish a direct contact or to be placed in the near vicinity of target insects in the soil or inside their galleries in tree trunks.

An integrated system approach, exploiting the quantitative and qualitative characteristics of entomopathogenic nematodes, time and methods of their application, and use of antidesiccants, when insects attacking aerial plant parts are targeted, would be most efficient in the improvement of their performance as biological control agents in the field.

Nematode Production

Nematode production may be carried out either in natural hosts (in-vivo) or in artificial cultures (in-vitro).

IN-VIVO PRODUCTION: In-vivo production of Steinernematids and Heterorhabditids uses *G. Mellonella* or other suitable caterpillars as a natural host for the production of infective juveniles. This method yields enough nematodes to cover the needs of laboratory and small-scale experimental field plots. Yields as high as 150,000 *S. feltiae* (Dukty *et al*, 1964) and 350,000 *H bacteriophora* infective juveniles (Milstead and Poinar, 1978) have been reported per larvae of *G. Mellonella*. Although nematodes produced

by the in-vitro system are generally of a better quality compared to those produced on artificial cultures, this method has failed to meet the economy requirements of commercial mass production.

In general, the efficiency of the in-vivo production system depends on various factors, including host resistance and its larval size, nematode inoculum density and virulence which may impact the rate of reproduction and eventually the yield and quality of infective juveniles.

IN-VITRO PRODUCTION: Successful commercialization of nematodes as bio-control agent requires a well-established mass-production technology. Georgis and Hague (1991) reported that 7.5 x 10^9 juveniles of *S. carpocapsae* per hectare would be required for the control of Japanese beetles in ornamentals. Based on our preliminary results, an estimated 2 x 10^8 infective juveniles per hectare would be required to control red palm weevils in date palms. The cost of in-vivo production on *Galleria* larvae, was estimated at US$1.0 per million (Poinar, 1972). These high costs made it economically impossible to use nematodes in large-scale commercial applications.

Attempts to scale up nematode production have resulted in the development of efficient in-vitro, liquid and solid culture production technologies. At present, Steinernematids may be economically produced, in a liquid culture, in 15,000 to 80,000 litres bio-reactors, with yields as high as 100,000 infective juveniles per ml every 16 days (Georgis and Hague, 1991). This liquid culture consists of various vitamins, minerals, fats and proteins (Friedman, 1989). Heterorhabditids can not be produced in liquid cultures due to their sensitivity to such systems. Their production is possible on solid cultures but at a cost of 1 - 3 times higher than that of Steinernematids in liquid culture (Georgis and Hague, 1991).

The solid-culture mass production technique utilizes foam cubes impregnated with homogenates of ox kidney (Bedding, 1981) or chicken offal (Bedding, 1984 and 1988b) with an average yield of 300 thousand infective juveniles per gram of medium every 2 - 3 weeks with Steinernematids and 3 - 4 weeks with Heterorhabditids species. The cost of production, per million, in the solid culture technique is estimated at about US$0.02 to 0.05. The most important characteristics of Bedding's solid culture technique is its capacity to provide a large surface to volume ratio which favors nematode development and reproduction. However, nematode production by Bedding's solid culture technique was recently improved further by an average of 20 - 30% by modifying the design of the production apparatus (Hanounik, 1996a).

In mass production, both qualitative and quantitative aspects of nematodes must be addressed. Gaugler and Georgis (1991) reported that *S. carpocapsae* produced by the in-vivo or the in-vitro techniques were equally effective against larvae of Japanese beetles. On the other hand, *H. bacteriophora* produced by the in-vitro liquid culture technique was less effective compared to that produced by the in-vivo or in-vitro solid culture techniques.

Formulation

Steinernematids and Heterorhabditids are perishable biological control agents and therefore, their successful use depends on their tolerance to storage before application. After production and prior to formulation, nematodes may be stocked for 2 - 3 months, in an aqueous aerated suspension, in large tanks, at 5 - 8°C for Steinernematids and 10 - 12°C for Heterorhabditids, without affecting their qualitative characteristics.

Generally, warm temperatures (20 - 30°C) enhances nematodes activity and metabolism, which in turn, reduces their energy reserves, survivability and field efficiency. A successful formulation must arrest nematode's metabolism while preserving their viability and virulence (Georgis, 1990). This can be achieved either by **refrigeration, immobilization on a solid carrier or dehydration.** Nematodes formulated on moist foam, peat or vermiculite need continuous refrigeration to arrest their activity. This type of formulation may serve the small scale home-garden market but not large scale commercialization. To improve their shelf life without refrigeration, nematodes have recently been formulated with solid carriers such as clay (Bedding 1984, 1988a), activated charcoal (Biotechnology, Australia 1985), polyachrylamide gels (Pruitt, 1988), alginate (Kaya and Nelsen ,1985 and Poinar *et al*, 1985), or dehydrated (Friedman, 1989), with a shelf life of 3 - 5 months at room temperature and 6 - 10 months with refrigeration.

Conclusion

Steinernematids and Heterorhabditids are important biological control agents. Advances made in nematode production and restrictions enforced on the use of chemical pesticides have contributed to the commercialization of those nematodes as biological control agents. However, additional progress is needed to improve mass-production and formulation technologies, virulence, tolerance to adverse ecological conditions, and release strategies before these nematodes can be commercialized as biological control agents for red palm weevils and other cryptic insects occupying aerial plant parts in the Arabian Gulf Region.

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