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In vitro mass production of entomopathogenic nematodes on solid media: A review

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Abstract
Entomopathogenic nematodes, obligate insect pathogens, are widely used as biocontrol agents against economically important insect pests in different farming systems because they provide environmentally safe and sustainable crop protection. Hence the most important thing for the successful and reasonable usage of EPNs in crop protection is their production on large scale at competitive cost within a short time. In parallel to this, EPNs can be easily cultured either *in-vivo* or *in vitro* in the laboratory. The *in vivo* production is quite simple process as it involves the culturing on live insect host on the White Trap method, which involves the natural migration of IJs away from the infected host cadaver into the surrounding w+** ater layer. But the commercial production is quite impracticable due to high production costs, lacks of economies of scale and low nematode yields per gram of insect biomass. In place of that, the *in vitro* solid technology gives higher nematode yields per gram of solid media, which is based on introducing nematodes to a pure culture of the symbiotic bacteria into a nutritive, non-living medium that contains the sterile ingredients.

Keywords: Entomopathogenic nematodes, crop protection, *in vitro*, solid medium, production

Introduction
The nematodes are belonging to the genus *Heterorhabditis* and *Steinernema* has huge potential for management of insect pests of agriculture. They are using to manage many soil dwelling insect pests [28]. These EPNs are symbiotically associated with bacteria of the genera *Photorhabdus* and *Xenorhabdus* that belongs to Enterobacteriaceae. It has obtaining popularity due to ease of application using conventional liquid application equipment's Grewal (2002) [14]. EPNs have broad host range and exempted from the registration requirements in several countries [26]. The most important attribute is that they can be applied in conjugation with fertilizers, soil amendments and insecticides [46]. Tank mixed EPNs and pesticide combinations offers the cost-effective alternative in integrated pest management, as EPNs are safe and environmentally friendly. EPNs are required in bulk to apply in field level and multiplied in laboratory or by commercial companies' *in vivo* or *in vitro* solid and liquid media.

The most common insect host used for *in vivo* production is the last instar of the greater wax moth *Galleria melonella* (L.) (Lepidoptera: Pyralidae). It occurs naturally in beehives and nurtured using artificial diets. More than 40 countries are working to develop EPNs as biocontrol, which has now commercially sold in US, Japan, Europe and China for the control of insect pests in high value horticulture, agriculture and home kitchen gardens [48]. EPNs has been made since the first discovery of infection of Japanese beetle (*Popilla japonica* Newman) by *Steinernema glaseri* Steiner (1929) [52]. In India, first entomopathogenic nematode (DD-136), a commercial product of *S. carpocapsae* [59]. EPNs have a global distribution in the broadest geographic sense and exhibit differences in suitability of host range, virulence and environmental tolerance [15]. EPNs have been isolated from soil samples using insect baiting technique [4]. Indigenous EPNs may be more suitable for inundative release against local insect pests because of adaptation to local climate and other population regulators.

In field research, improved insect-nematode matches may become established. Deplorably EPNs have yet to be discovered which are effective against several of the most important soil insects, including wireworms, grape-vine Phylloxera, fire ants or corn root worms. The EPNs are constraint to specific conditions like other biocontrol agents as they are effective within a narrower temperature ranges than chemicals and more impacted by sub-optimal soil type, depth and irrigation frequency [10]. Hence there is now a tremendous need for the discovery of new nematode strains adapted to the local environmental conditions and pests especially in

India, in association with growth and reproduction of nematodes that are affected by ecological parameters.

Based on the attractive traits described above, the identified indigenous species are to be cultured *in vivo* or *in vitro* for large-scale commercial production as well as for laboratory experimentation and field-testing [49]. The technology of *in vitro* mass production of EPNs has been given by Glaser (1940) [13]. They have commercially been produced by more than 10 countries in Asia, Europe and North America. Thirteen different species have reached a commercial development such as *H. bacteriophora* Poinar (1976) [39]. *H. indica*. Poinar (1992) [42], *H. marelatus* Liu and Berry (1996) [31], *H. megidis* Poinar 1987 [40], *H. zealandica* Poinar (1990) [41], *S. carpocapsae*, *S. glaseri*, *S. kushidai* Mamiya (1988) [32], *S kraussei* Steiner (1923) [51], *S. longicaudatum* Shen and Wang (1992) [50], *S. riobrave* and *S. scapterisci* Nguyen and Smart (1990) [36].

For the biocontrol agent to be successful it should be amenable for mass production on a large scale, the ready availability of the organism in required quantity and at competitive cost makes them acceptable among entrepreneurs and farmers.

Entomopathogenic nematodes salutary effects

Biological control of pests by using EPNs is getting popularity in the recent past, as it has long-term benefits without harmful effects on non-targets, high virulence and high reproduction rate [11]. Though these nematodes are highly pathogenic, but are facultative parasitic due to their compatibility to complete their life cycle on the symbiotic bacteria. This high degree of safety means that unlike chemicals, nematode application does not need masks or other safety equipment. Most of the biocontrol agents take more days or even weeks to kill their host but EPNs in association with symbiotic bacteria kill insects within 24 to 48 hours. Nematodes are compatible with many insecticides and can be easily applied through standard agrochemical equipment's including pressurized mists, aerial sprayers, electrostatic fan etc [9]. Application of EPNs through irrigation system has improved grower acceptance and have the potential to recycle in the environment. EPNs are lethal to broad range of insect pests in the laboratory where host contact is assured, environmental conditions are optimum and no ecological and behavioral barriers to infection exist. EPNs have no competition from other biocontrol agents for control of plant boring and soil inhabiting insects.

Entomopathogenic nematodes life cycle

EPNs relies on the symbiotic partnership between fatal insect, bacterium and the host seeking nematode. The third stage juveniles called as infective juvenile (IJ) that are non-feeding, developmentally arrested stage and have attributes of both insect parasitoids and predators. They have a good tendency to locate host and nematodes penetrate into the insect body cavity usually via natural body openings (mouth, anus and spiracles) or areas of thin cuticle. *Steinernema* penetrate into the insect larvae primarily through body openings and *Heterorhabditis* also penetrate through the intersegmental membranes of the cuticle Burnell and Stock (2000) [8]. Inside the body cavity, symbiotic bacteria (*Xenorhabdus* for Steinernematids and *Photorhabdus* for Heterorhabditis) that are motile, gram negative, facultatively anaerobic rods in the family Enterobacteriaceae are released from the nematode gut, which multiply rapidly on haemolymph and cause rapid insect mortality due to septicemia [41]. The nematodes feed on the host tissues that are made edible by bacteria and complete 2-3 generations. The IJs of Steinernematids become males or females whereas Heterorhabditids develop into self-fertilizing hermaphrodites. They take a week or a couple of weeks to complete life cycle and thousands of IJs emerge from the cadaver in search of new hosts.

Mass multiplication of EPNs on solid mediums

The *in vitro* solid culture has several advantages over liquid culture like the effects of phase variation in yields is less than in liquid culture [18] and less monetary investments. It requires high cost of labour and highly vulnerable to contaminants, inability to timely monitor the process is its limitation's. Hence, Bedding (1990) [6] recommended the use of this technology where the labour cost is low and expertise is easily available like in developed countries. *In vivo* culture of entomopathogenic nematodes is satisfactory for maintaining strains or species and small-scale experiments, but for the field experiments, *in vitro* culture is required in bulk.

Glaser (1932) [12] was the first to culture *Neoaplectana* now *S. glaseri in vitro* using veal infusion agar, dextrose and baker's yeast. This method produced a moderate number of nematodes and did not yield sufficient quantity for field release. Later a variety of materials consisting of potato mash, ground veal pump, peptone-glucose agar and pork kidney, homogenized animal tissue, dog food, homogenate of chicken offal, modified dog biscuits medium, egg yolk, soy flour and cholesterol medium, etc have been employed for this type of studies. McCoy and Glaser (1936) [34] devised an improved technique where fermented potato mash medium was used to culture *S. glaseri*. This method produced about 4 million nematodes per 200 mm Petri plate. A further improvement has described by Mc Coy and Girth McCoy (1938) [33]. They used ground extracted veal pulp in liquid culture. This produced robust and larger nematodes and the yield was 9000-12000 nematodes per cm^2 of culture area.

In the mass production of the nematodes, one or more associated species of organisms were usually present. However, Glaser (1940) [13]; Stoll (1959) [54] EPNs have cultured on an axenic or bacteria-free, liquid medium, showing that the presence of associated bacteria was not essential for the development of the nematodes. Stoll (1953) [53] maintained an axenic culture for 7 years, during which 180 to 195 generations were cultured *in vitro*. Discovery of the symbiotic association between bacteria and nematodes there was considerable improvement in the *in vitro* culture techniques. The associated bacteria serve as food source for the nematodes but apparently do not supply all the nutrients that are needed.

The first medium used for EPNs production contained dog food as a base multiplication of *S. carpocapsae*, 3-7 X 106 IJs per Petri dish in about 20 days after inoculation [22]. Hansen *et al.* (1970) [19] cultured *S. carpocapsae* in a medium containing heroin supplemented with α-globulins at 30 °C. Hara *et al.* 1981 multiplied *S. carpocapsae* on a dog food agar medium (100 g powdered dog food + 500 ml of 1% agar). The initial inoculum of 100-200 IJs yielded 2 million IJs in about 20-30 days after inoculation. The knowledge that the bacterium *Xenorhabdus nematophila* [43]. Thomas and Poinar (1979) [55] had a primary and secondary form Akhurst RJ. (1980) [2] was very important in improving the *in vitro* production techniques of the nematode. A major breakthrough in the mass production of the nematode was accomplished by

Bedding (1981) [7]. Who devised a simple medium for culturing *Steinernema* spp. and *Heterorhabditis* spp. monoxenically and economically in large quantities. He has provided a large surface area by using the sterilized polyether-polyurethane sponge thinly coated with a homogenate of animal fats and kept in conical flasks (70 g/flask). Maximum yields were obtained after 2-3 weeks for *Steinernema* and 4-5 weeks for *Heterorhabditis* yielding half a million IJs per gram of medium.

The porous foam afforded an outstanding area to volume for growth by providing adequate gas exchange. Bedding (1984) [5] improved the mass production technique by coating the sponge with the homogenate of chicken offal and by using autoclavable plastic bags as containers. Wout's (1981) [60] reared *H. heliothidis* monoxenically on an artificial medium consisting of commercially available nutrient broth, yeast extract and vegetable oil coated on the polyether-polyurethane sponge along with a bacterial symbiont and obtained yield of about 10 million IJs in one month after inoculation.

They have adapted the medium to the availability of ingredients. The soy flour which serves as binding agent was replaced with ground millet, wheat bran, and fine corn meal. Agar has not found to be suitable binding agent during the sterilization in autoclave as it gets liquefied and drips out of the sponge. Abe 1987 [1] standardized a medium containing wheat bran, rice shell and salad oil for culturing *S. feltiae* which yielded 107 IJs per kg of the medium after one month of inoculation. Ritter (1988) [45] observed that minimum 0.0025% cholesterol in the lipid defined artificial media is necessary to support the development of *N. carpocapsae* to the dauer stage. Xu *et al.* (1989) [61] reported that media composed of 71% chicken offal, 8% sponge, 10% lard and 5% water yielded 10-26 X 106 IJ of *S. glaseri* in 70 g media with a greater virulence and retaining more symbiotic bacteria. A comparison of the growth and propagation of *S. carpocapsae*, *S. feltiae* and *S. glaseri* on the dog food and nutrient agar medium revealed that dog food agar is better than nutrient agar since EPNs need other nutrients too for their growth and multiplication. *S. glaseri* and *S. feltiae* develop better than *S. carpocapsae* [27].

The nematode growth can be increased by increasing the size of inoculum and decreasing the culture time [17, 18]. Ogura and Haraguchi (1993) [37] successfully cultured *S. kushidai* on the artificial medium containing dog food –peptone, agar and pig intestine agar medium. The initial inoculum of 1000 IJs yielded 3, 44,300 & 2, 44,000 IJs in both media, respectively. Yang *et al.* (1997) [64] proved this as they successfully cultivated *S. carpocapsae* on three media of plant, animal and both plant-animal protein origin by following the method developed by Bedding (1975) [4]. The highest nematode yield, 5.8 X 105 IJs/g medium was from animal protein medium followed by medium with both plant and animal proteins, while plant protein gave the lowest yield, 3.6 X 105 IJs/g medium. Yadav *et al*. (2015) [62] observed that the animal protein-based media was found to give higher multiplication of IJs of *S. carpocapsae* than plant protein-based media when studies were carried out on different artificial media *i.e.*, Nutrient broth, Wheat flour, Maize flour, Lipid and Modified Wout's media.

Wang and Bedding (1998) [58] examined the final population development of *Heterorhabditis bacteriophora* and *S. carpocapsae in vitro* in solid media a range of initial inoculation levels. The highest yield of *H. bacteriophora* was observed with initial inoculum of 10^6 IJs/flask which was 10-fold of the optimum inoculum for *S. carpocapsae*. Also, at the highest initial inoculum of 10^7 nematodes per flask, population of *H. bacteriophora* doubled but *S. carpocapsae* halved.

EPNs have reproduced more rapidly on LH than IH medium but population was higher on IH than LH after 20 days of incubation at 25 ºC [27]. They reproduced more rapidly on the media containing more liver, but the final populations were higher on the media containing more intestine. The growth and reproduction of *S. glaseri* and its associated bacteria were affected by temperature. *S. glaseri* could not survive above 35 ºC on dog food peptone medium and *Galleria mellonella* larvae but at 25 ºC the progeny production was higher and maximum bacterial growth was observed at 30º to 33 ºC [63]. Kranti and Kumar (2018) [29] conducted studies on two local isolates of entomopathogenic nematodes (EPNs) from Haryana namely *Steinernema abbasi* and *Heterorhabdus indica* were mass multiplied on eight culture media compromising of Wout's medium, Modified Wout's medium I, Modified Wout's medium II, Modified Wout's medium III, Egg yolk medium I, Egg yolk medium II, Modified egg yolk medium and Modified dog biscuit medium and harvested at two different time intervals *i.e.* at 30 days and 60 days interval. Along with this, studies were also conducted to know the effect of temperature *i.e*. at 15 ºC, 20 ºC and 30 ºC on the infective juvenile (IJ) yields. Of the eight media tested, *S. abbasi* and *H. indica* both multiplied maximum on modified dog biscuit medium. Wout's medium was second best in IJs yield.

Vyas *et al.* (2001) [57] used 21 animal and plant protein media for mass production of the native *Steinernema* spp. and recorded the maximum production in hen-egg yolk medium which was economically better than universally used dog food biscuit agar. Hussaini *et al*. (2000) [25] mass multiplied the *Steinernema* sp. (SSL2) PDBC EN.13.21 in four combinations of dog food biscuit media and Wout‟s medium. The maximum yield (30.58 X105 IJs) was obtained in Wout‟s medium followed by the dog biscuit + peptone + beef extract (24.5 X 105 IJs), dog biscuit + peptone (12.20 X 105 IJs) and dog biscuit + bacterial culture (10.14 X 105 IJs), also the cost of production for 10 lakh IJs was highest in dog biscuit +bacterial culture. Hussaini *et al*. (2002) [24] evaluated different plant and animal protein media *in vitro* for the production of *S. carpocapsae*, *S. bicornutum*, and *H. indica*.

Narkhedkar *et al*. (2005) [35] recommended the composition of new medium as: Bacto nutrient broth-16 ml, pork fat 50 g, agar agar 12 g and distilled water 1000 ml for *in vitro* mass multiplication of *H. indica*. Prabhu *et al*. (2006) [44] made attempts to develop low cost innovative mass production technology for *S. glaseri* in animal /plant origin media. The animal origin media gave more yield and among plant origin media, bengal gram and maize oil yielded more nematodes whereas bengal gram flour and cotton oil had negative effect on the nematode multiplication. Oil and protein sources too affect the yield in EPNs propagation [23]. Further Umamaheswari *et al*. 2008 mass produced native strains of *H. indica* and *S. siamkayai in vitro* using ten various media containing various plant proteins and oils.

Pervez and Ali (2010) [38] *in vitro* mass multiplied entomopathogenic nematodes (*S. masoodi*, *S. seemae* and *S. mushtaqi*) in six different artificial media. Maximum yield was obtained on egg yolk medium followed by Wout's medium in all the three species tested. Anjum and Prabhuraj (2007) [3] mass produced *H. indica* on different artificial

media such as Wout's medium, modified egg yolk medium, modified wheat flour medium and modified dog biscuit medium with different inoculation levels *viz*. 1000, 2000 and 3000 IJs per flask. Salma and Shahina (2012) [47] mass produced eight strains of EPNs *viz*. *S. pakistanense*, *S. asiaticum*, *S. abbasi*, *S. siamkayai*, *S. carpocapsae*, *S. feltiae*, *H. indica* and *H. bacteriophora in vitro* on soy flour, wheat flour, lipid medium and corn flour and obtained highest yield in soy flour medium and minimum in corn flour medium. Leite *et al.* (2017) [30] assessed the effect of nematode inoculum age on the production of *Steinernema feltiae* in liquid, solid and biphasic processes.

References

1. Abe Y. Culture of *Steinernema feltiae* (DD-136) on bran media. Japanese Journal of Nematology. 1987; 17:31-4.
2. Akhurst RJ. Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. Microbiology. 1980; 121(2):303-309.
3. Anjum S, Prabhuraj A. Mass production of *Heterorhabditis indicus* in different artificial media. Karnataka Journal of Agricultural Sciences. 2010; 20(4):859- 860.
4. Bedding RA, Akhurst RJ. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica. 1975; 21(1):109-110.
5. Bedding RA. Large scale production, storage and transport of the insect-parasitic nematodes *Neoaplectana* spp. and *Heterorhabditis* spp. Annals of applied biology. 1984; 104(1):117-120.
6. Bedding RA. Logistics and strategies for introducing entomopathogenic nematode technology into developing countries. Entomopathogenic nematodes in biological control. 1990; 22:233-246.
7. Bedding RA. Low cost *in vitro* mass production of *Neoaplectana* and *Heterorhabditis* species (Nematoda) for field control of insect pests. Nematologica. 1981; 27(1):109-114.
8. Burnell A, Stock SP. *Heterorhabditis*, *Steinernem*a and their bacterial symbionts-lethal pathogens of insects. Nematology. 2000; 2(1):31-42.
9. Ehlers RU, Peters A. Entomopathogenic nematodes in biological control: feasibility, perspectives and possible risks. , in Biological Control- Benefits and Risks (eds H. M. T. Hokkanen and J. M. Lynch) Cambridge University Press, Cambridge. 1995, 119-136.
10. Georgis R, Gaugler R. Predictability in biological control using entomopathogenic nematodes. Journal of economic entomology. 1991; 84(3):713-720.
11. Georgis R, Kaya HK, Gaugler R. Effect of steinernematid and heterorhahditid nematodes (Rhahditida: Steinernematidae and Heterorhahditidae) on nontarget arthropods. Environmental Entomology. 1991; 20(3):815-822.
12. Glaser RW. A pathogenic nematode of the Japanese beetle. Journal of Parasitology, 1932; 18:119.
13. Glaser RW. Continued culture of a nematode parasite in a Japanese beetle. Journal of Experimental Zoology. 1940; 84:1-12.
14. Grewal PS. Formulation and Application Technology. Entomopathogenic nematology, 2002, 265.
15. Griffin CT, Downes MJ. Low temperature activity in *Heterorhabditis* sp. Nematoda: *Heterorhabditis* spp. (Nematoda: Heterorhabditidae). Nematologica. 1991; 37:83-91.
16. Han R, Cao L, Liu X. Effects of inoculum size, temperature and time on *in vitro* production of *Steinernema carpocapsae* Agriotos. Nematologica. 1993; 39:366-375.
17. Han R, Cao L, Liu X. Relationship between media composition, inoculumn size, temperature and culture time in the yields of *Steinernema* and *Heterorhabditis* nematodes. Fundamental and Applied Nematology. 1992; 15:223-229.
18. Han RC, Ehlers RU. Effect of *Photorhabdus luminescens* phase variants on the *in vivo* and i*n vitro* development reproduction of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. FEMS Microbiology/Ecology. 2001; 35:239-247.
19. Hansen EL, Yarwood EA, Buecher EJ. Growth of nematodes in defined medium containing hemin and supplemented with commercially available proteins. Nematologica. 1970; 16(3):403-409.
20. Hara AH, Lindegren, Kaya HK. Monoxenic mass production of the entomopathogenic nematode, *Neoaplectana carpocapsae* Weiser on dog food-agar medium. U.S. Department of Agriculture. 1981, 16-18.
21. Hashimoto H. The effect of transferring methods on successive axenic culture of *Steinernema kushidai* (Nematoda: Steinernematidae). Japanese Journal of Nematology. 1997; 26:23- 28.
22. House HL, Welch HE, Cleugh TR. Food medium prepared of dog biscuit for the mass production of the nematode DD-136 (Nematoda: Steinernematidae). Nature. 1965; 206:847.
23. Hussaini SS, Nagesh M, Rajeshwari R, Hussaini MD. Effect of protein and lipid sources in the Wout's medium on the yield and pathogenicity of *Steinernema carpocapsae* and *Steinernema tami*. Indian Journal of Plant protection. 2007; 35:93-96.
24. Hussaini SS, Singh SP, Parthasarathy R, Shakeela V. *In vitro* production of entomopathogenic nematodes in different artificial media. Indian Journal of Nematology. 2002; 32:44- 46.
25. Hussaini SS, Singh SP, Parthasarathy R, Shakeela V. Infectivity of native populations of *Steinernema* spp. and *Heterorhabditis indica* in sand and sandy loam soils columns against *Agrotis ipsilon* (Hufnagel). Annals of Plant protection Sciences. 2000; 8:200-205.
26. Kaya HK, Gaugler R. Entomopathogenic nematodes. Annual Review of Entomology. 1993; 38:181-206.
27. Kondo E, Ishibashi N. Dependency of three Steinernematid nematodes on their symbiotic bacteria for growth and propagation. Japanese Journal of Nematology. 1991; 21:11- 17.
28. Koppenhöfer AM. Nematodes. In Field manual of techniques in invertebrate pathology. 2007, 249-264. Springer, Dordrecht.
29. Kranti KVVS, Narendra KG. Mass Multiplication of Entomopathogenic Nematodes on *in vitro* Solid Media. International Journal of Current Microbiology and Applied Science. 2018; **7**(07):3282-3292.
30. Leite L, Shapiro-Ilan D, Hazir S, Jackson M. Effect of inoculum age and physical parameters on in vitro culture of the entomopathogenic nematode *Steinernema feltiae*. Journal of Helminthology. 2017; 91(6):686-695.

31. Liu J, Berry RE. *Heterorhabditis marelatus* n. sp. (Rhabditida: Heterorhabditidae) from Oregon. Journal of Invertebrate Pathology. 1996; 67:48-54.
32. Mamiya Y. *Steinernema kushidai* n. sp. (Nematoda: Steinernematidae) associated with *scarabaeid* beetle larvae from Shizuoca, Japan. Applied Entomology and Zoology. 1988; 23:313- 320.
33. McCoy EE, Girth HB. The culture of *Neoaplectana glaseri* on veal pulp. New Jersey Department of Agriculture, Circular. 1938, 285.
34. McCoy EE, Glaser RW. Nematode culture for Japanese beetle control. New Jersey Department of Agriculture, Circular. 1936, 265.
35. Narkhedkar NG, Panchbhai NV, Mukewar PM, Singh P. Development of new substrate for mass multiplication of *Heterorhabditis indica* in the control of cotton bollworm *Helicoverpa armigera*. International Journal of Nematology. 2005; 15:191-193.
36. Nguyen KB, Smart GC. *Steinernema scapterisci* n. sp. (Steinernematidae: Nematoda). Journal of Nematology. 1990; 22:187-199.
37. Ogura N, Haraguchi N. Artificial media for xenic culture of *Steinernema kushidai* (Nematoda: Steinernematidae). Nematologica. 1993; 4:613- 616.
38. Pervez R, Ali SS. *In vitro* mass production of entomopathogenic nematodes in different artificial media. Current Nematology. 2010; 21:9-12.
39. Poinar GO. Jr. Description and biology of a new insect parasitic rhabditoid, *Heterorhabditis bacteriophora* n. gen., n. sp. (Rhabditida; Heterorhabditidae n. fam.). Nematologica. 1976; 21:463- 470.
40. Poinar GO. Jr. Jackson T. Klein M. *Heterorhabditis megidis* sp. n. (Heterorhabditidae: Rhabditida) parasitic in the Japanese beetle, *Popillia japonica* (Scarabaeidae: Coleoptera), in Ohio. Proceedings of the Helminthological Society of Washington. 1987; 54:53-59.
41. Poinar GO. Jr. Taxonomy and biology of Steinernematidae and Heterorhabditidae. Entomopathogenic nematodes in biological control (eds R. Gaugler and H. K. Kaya), CRC Press, Boca Raton, Florida, U.S.A. 1990, 23-61.
42. Poinar GO, Karunakar G, David H. *Heterorhabditis indicus* (Rhabditida: Nematoda) from India: separation of *Heterorhabditis* spp. by infective juveniles. Fundamentals of Applied Nematology. 1992; 15:467-472.
43. Poinar GO, Thomas GM. A new bacterium, *Achromobacter nematophilus* sp. nov. (Achromobacteriaceae: Eubacteriales), associated with the nematode. International Bulletien of Bacteriological Nomenclature and Taxonomy, 1965; 15:249-252.
44. Prabhu S, Rajendran G, Subramanian S. *In vitro* mass production technology for the entomopathogenic nematode, *Steinernema glaseri.* Indian Journal of Nematology. 2006; 36:142- 144.
45. Ritter KS. *Steinernema feltiae* (Neoaplectana: Carpocapsae): effect of sterols and hypolipidemic agents on development. Experimental Prasitology. 1988; 67:257- 267.
46. Rovesti L, Deseö KV. Compatibility of chemical pesticides with entomopathogenic nematodes. *Steinernema carpocapsae* Weiser and *S. feltiae* Filipjev (Nematoda: Steinernematidae). Nematology. 1990; 36:237-245.
47. Salma J, Shahina F. Mass production of eight Pakistani strains of EPNs (Steinernematidae & Heterorhabditidae). Pakistan Journal of Nematology. 2012; 30:1- 20.
48. Shapiro-IIan DI, Gough DH, Piggott SJ, Fife JP. Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. Biological Control. 2006; 38:124-133.
49. Shapiro-Ilan DI, Gouge DH, Koppenhofer AM. Factors Affecting Commercial Success: Case Studies in Cotton, Turf and Citrus. (ed. R. Gaugler), CABI Publication, CAB International, Wallingford, Oxon, UK. 2002, 335.
50. Shen CP, Wang GH. Description and studies of an entomopathogenic nematode: *Steinernema longicaudatum* sp. nov. In: Proceedings of the first national academy symposium of young and middle-aged science and technology workers on plant protection, Beijing, China. Chinese Science and Technology Press. 1992, 230-223.
51. Steiner G. *Neoplectana kraussei* n. sp. Steiner in der Blattwespe *Lyda* sp. parasitierende Nematodenform Zentralblatt fur. Bakteriologie Zweite Abteilung. 1923; 59:14-18.
52. Steiner G. *Neoplectana glaseri* n. g. n. sp. (Oxyuridea), a new endemic parasite of the Japanese bettle (*Popilla japonica* Newman). Journal of Washington Academic Science. 1929; 19:436-440.
53. Stoll NR. Infectivity of Japanese beetle grubs retained by *Neoaplectana glaseri* after seven years axenic culture. Journal of Parasitology. 1953; 39:33.
54. Stoll NR. Conditions favoring the axenic culture of *Neoaplectana glaseri*, a nematode parasite of certain insect grubs. Annals of the New York Academy of Sciences. 1959; 77(2):126-136.
55. Thomas GM, Poinar GO. *Xenorhabdus* gen. nov., a Genus of Entomopathogenic, Nematophilic Bacteria of the Family *Enterobacteriaceae.* International Journal of Systematic and Evolutionary Microbiology. 1979; 29(4):352-360.
56. Umamaheshwari R, Sivakumar M, Subramanyan S. *In vitro* production of native isolates of *Heterorhabditis indica* and *Steinernema siamkayai.* Indian Journal of Nematology. 2008; 38:134-137.
57. Vyas RV, Yadav P, Gheelani YH, Chaudhary RK, Patel NB, Patel DJ. *In vitro* mass production of native *Steinernema* sp. Annals of Plant Protection Sciences. 2001; 9:77- 80.
58. Wang JX, Bedding RA. Population dynamics of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae in vitro* monoxenic solid culture. Fundamental and Applied Nematology. 1998; 21:165-171.
59. Weiser J. *Neoplectana carpocapsae* n. sp. (Anguillulata: Steinernematidae) Novycizopasnik housenik Oválese jableeneho, *Carpocapsa pomonella* L. *Vest.* Ceskoslovenske Zoologicke Spolecnosti. 1955; 19:44-52.
60. Wouts WM. Mass production of entomogenous nematode, *Heterorhabditis heliothidis* (Nematoda: Heterorhabditidae) on artificial media. Journal of Nematology. 1981; 13:467-469.
61. Xu JL Yang P, Lu XL. Effect on the productivity and the virulence of the insecticidal nematode, *Steinernema glaseri* in different culture media. Acta Entomologica Sinica. 1989; 32:317-321.

62. Yadav S, Sharma HK, Siddiqui AU, Sharma SK. *In vitro* mass production of *Steinernema carpocapsae* on different artificial media. Indian Journal of Nematology. 2015; 45(1):123-124.
63. Yamanaka S, Tanabe H, Takeuchi K. Influence of temperature on growth and propagation of *Steinernema glaseri* (Nematoda: Steinernematidae). Japanese Journal of Nematology. 2000; 30:47-50.
64. Yang H, Jian H, Zhang S, Zhang G. Quality of the entomopathogenic nematodes, *Steinernema carpocapsae* produced in different media. Biological control. 1997; 10:193-198