In vitro cost-effective culture technique for pure line production of facultative nematode parasites of insects and free-living nematodes

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Summary. Four nematode isolates, *Acrobeloides* spp. (isolates HNK19, HNK25), *Metarhabditis* sp. (HNK22) and *Distolabrellus* sp. (isolate HNK26), were recovered from the soils of different agriculture fields of Noida and Meerut regions of India. Pure line populations of these isolates were obtained through a new method, *i.e.*, agar trap technique, and then these populations were used to test the efficiency of this technique for mass culture of facultative nematode parasites of insects and free-living nematodes. This technique was also compared with the universal nematode mass culture method using the larvae of *Galleria mellonella*. Results of the present study revealed that all the isolates grew faster on agar trap rather than on *Galleria* larvae. At day 20 post-inoculation, the highest and lowest nematode yield in agar trap was recorded as 2.54×10^5 nematodes (trap)$^{-1}$ and 3.9×10^4 nematodes (trap)$^{-1}$ in HNK25 and HNK22, respectively, as compared to the *Galleria* method, the highest and lowest nematode yield was recorded as 2.19×10^4 nematodes (larva)$^{-1}$ and 1.25×10^3 nematodes (larva)$^{-1}$ in HNK25 and in HNK26, respectively.

Key words: *Acrobeloides*, *Distolabrellus*, *in vitro* mass culture, *Metarhabditis*, nematode production, nematode pure line population.

Nematodes are the most abundant, ubiquitous and diverse type of animals across the world. It is estimated that there are over a million nematodes species (Lambshead & Boucher, 2003), of which more than 28,000 species are described globally (Hodda, 2022). More than half of nematode species are free-living, reside in both aquatic and terrestrial habitats and feed on bacteria, fungi, algae, dead organisms, living tissue and other nematodes. Due to their abundance and omnipresence in ecosystems, nematodes also serve as elegant indicators of environmental disturbance (Bongers, 1990; Ferris *et al.*, 2001; Yeates, 2003; Höss *et al.*, 2004; Schratzberger *et al.*, 2006; Heininger *et al.*, 2007). They show various responses to stress factors where some species are sensitive to pollutants and others are tolerant (Korthals *et al.*, 1996; Ferris *et al.*, 2004; Tenuta & Ferris, 2004). Based on their feeding habits, they are grouped in four categories: *i*) bacterial feeder nematodes exclusively feeding on bacteria; *ii*) fungal feeder nematodes feeding on fungi; *iii*) predatory nematodes feeding on other nematodes; and *iv*) omnivore nematodes feeding on different type of food sources including bacteria, algae, fungi and nematodes. Other than free-living nematodes, there is a group of nematodes called parasitic nematodes that infect various organisms including insects, animals, humans and plants. In relation to insects, nematodes have established various types of associations. In facultative parasitism, nematodes opportunistically infect healthy insects as a facultative parasite, obtain nutrition from them and even can kill them, but they do not rely on any insect host to complete their life cycle. In the absence of an insect host, they feed on bacteria, fungi, algae or even higher plants and still retain their ability to reproduce and develop outside the host.

This study highlights the mass production of facultative nematode parasites of insects and free-living nematodes through agar trap technique. This is a very useful technique to produce large population of pure monogenic lines of some of the facultative nematode parasites of insects and free-living nematodes, which are frequently used for the experimental purposes. For mass culture, pure line populations (PLP) of four isolates were used to check the efficacy of the method. The agar trap technique was tested and compared with another method (*Galleria* baiting method) for mass

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production. The purpose of this study was: *i*) to establish an effective and useful low labour cost method for mass production of facultative nematode parasites of insects and free-living nematodes; and *ii*) to investigate the effectiveness of this technique in mass production of some species of facultative nematode parasites of insects and free-living nematodes.

MATERIAL AND METHODS

Rearing and maintenance of *Galleria mellonella*. Larvae of *Galleria mellonella* (Fabriciüs, 1798) were reared in the Nematology Laboratory, Department of Zoology, Ch. Charan Singh University, Meerut, India on artificial diet as suggested by David and Kurup (1988). Fifth and last instars of *G. mellonella* were used to perform the experiments.

Isolation of nematodes from soil samples. Four isolates were recovered from soils of different fields of Noida (28°32'37.65" N, 77°19'51.63" E) and Meerut (29°5'49.04" N, 77°55'13.78" E), Uttar Pradesh, India, using *Galleria* soil baiting technique (Bedding & Akhurst, 1975). The fifth instar of *G. mellonella* larvae were used to isolate nematodes from soil samples. For each soil sample, ten larvae of *G. mellonella* were placed at the bottom of a sterilised polystyrene plastic jar (250 ml) containing fine and moist soil. The prepared soil baits were placed in a BOD (Biological Oxygen Demand) incubator at 27 ± 1°C and checked daily up to 7 days for larval mortality. Cadavers from different soil baits were separately collected, washed three times with double distilled water (DDW), disinfected with 0.1% sodium hypochlorite and transferred onto White traps (White, 1927) for nematode emergence. The nematodes collected from White traps were stored in vented tissue culture flasks in a BOD incubator at 15°C for further use. Four isolates were each identified up to genus level based on morphological characters and designated as HNK19, HNK22, HNK25 and HNK26 (Table 1) and used in the experiments.

Preparation of agar trap. In the present study, agar traps were prepared by two methods.

Method 1. To prepare 12 agar traps, 1.5 g of regular grade agar powder (Sisco Research Laboratories Pvt. Ltd) was boiled with 100 ml of DDW using a laboratory hot plate at 60°C until the agar powder completely dissolved, and then the prepared solution was poured into 12 sterilised glass Petri dishes (Borosil, 3.8 cm diam.), and left for 4 to 5 h at room temperature to cool and solidify. After solidification, dry milk powder was poured on one side of each small Petri dish containing solidified agar medium. Then, the nematodes were placed onto the agar medium in the Petri dish. Finally, the prepared agar plate with nematodes was placed into a large, sterilised glass Petri dish (Borosil, 8.9 cm diam.), half-filled with double distilled water (DDW) and covered with a lid. The small Petri dish was carefully placed on one side of the large Petri dish so that the outer edge of the small Petri dish touched one side and formed a thin film of water between both Petri dishes. The prepared agar trap was incubated in the BOD at 27 ± 2°C. To harvest the nematode populations, the small Petri plate was removed carefully from the large Petri dish and the nematodes were harvested, washed twice with DDW and transferred into a vented tissue culture flask and kept in the BOD incubator at 15°C. After harvesting the nematodes, the small Petri plate was again placed into the large Petri dish with DDW to recover more nematodes.

Method 2. Agar plates were prepared as detailed in method 1 and each agar plate with nematodes was placed carefully over a sterile plastic tea strainer lined with double layer of tissue paper, which was already positioned on a sterilised plastic container (250-300 ml) filled with DDW. The plastic container was filled with DDW, so that the volume of water was only half that of the small Petri dish. The prepared agar trap was incubated in the BOD incubator at 27 ± 2°C for nematode emergence. To harvest the nematodes from the agar trap, the tea strainer together with the small Petri dish was removed from the top of the plastic container. The emerged population of nematodes was harvested from the plastic container, washed twice with DDW and transferred into a vented tissue culture flask and kept in the BOD incubator at 15°C. After harvesting, to obtain more nematode population, the tea strainer along with small Petri plate was again placed over the plastic container with DDW.

***In vitro* culture of pure line population through agar trap.** A pure line population of each isolate was obtained from a single female from the agar trap, which was then used to perform all the experiments. Four agar traps were prepared to obtain pure line populations of four isolates namely, HNK19, HNK22, HNK25 and HNK26. A single gravid female nematode from each isolate was handpicked using a picking needle and placed carefully onto the small Petri dish containing agar medium and 0.1 g of milk powder. Finally, the small Petri plate was placed carefully into large Petri dish, half filled with DDW. The lids of all the prepared agar traps were labelled for identification and then the traps were incubated in the BOD incubator at 27

± 2°C for emergence of nematodes. Within a week, pure line populations (PLP) were obtained from all agar traps. The population of nematodes starts moving from the small Petri dish towards the water in the large Petri dish through water film. Then, they were harvested and stored in the BOD incubator at 15°C for further experimentation.

***In vitro* mass culture of nematodes through agar trap.** To test the efficiency of the agar trap technique, three different concentrations of four isolates were used and different parameters were measured. For each isolate three different types of agar traps (trap 1, trap 2 and trap 3) were prepared. For trap 1, a single gravid female was selected from all isolates, while in trap 2 and trap 3, both adults and juveniles from each population were used to prepare agar traps. In trap 2, a total of 10 nematodes (n = 10) from each population were placed onto each agar trap, while in trap 3, a total of 50 nematodes (n = 50) were used in each.

Considering the milk powder as a parameter, two groups (group 1 and group 2) of each isolate were made in which 0.1 g of milk powder was used in group 1 and 0.5 g of milk powder was used in group 2. The number of nematodes (n = 10) was same in both groups.

All the prepared agar traps were incubated in the BOD incubator at 27 ± 2°C for the production and emergence of nematodes. Agar traps were checked on daily basis for the emergence of the nematodes. The emerged populations were harvested from all agar traps and stored in vented tissue culture flask in the incubator at 15°C for further use. For progeny count, emerging nematodes were collected up to 25 days from all the traps and then, the nematode density from all the agar traps was quantified separately by counting the number from each trap in a 25 µl volume with the help of counting dish under a stereomicroscope Nikon SMZ 645 (Tokyo, Japan).

Comparison between agar trap (*in vitro*) and *G. mellonella* (*in vivo*) techniques for mass culture of nematodes. Agar trap (*in vitro*) and *G. mellonella* (*in vivo*) technique were compared with each other to evaluate the most suitable and efficacious technique for mass production of facultative nematode parasites of insects and free-living nematodes. For this purpose, three pure line populations viz., HNK22 (*Metarhabditis* sp.), HNK25 (*Acrobeloides* sp.) and HNK26 (*Distolabrellus* sp.) obtained through agar trap technique were used. Two groups, group A and group B for each isolate were made where group A was for agar trap technique, in which 10 nematodes per trap with 0.5 g of milk powder were used and group B was for *Galleria* method where 10 nematodes per *Galleria* larvae were injected for progeny production. For mass production through agar trap technique (*in vitro*), ten nematodes from each isolate were placed on each agar trap, while in *G. mellonella* technique (*in vivo*), ten nematodes from each isolate were injected into fully grown single larva with the help of 1 ml insulin syringe (Dispo Van). Ten replicates of insect larvae were used for each isolate. Dead larvae were transferred to white trap for emergence of nematodes. The emerged nematodes from agar traps and white traps were collected separately up to 20 days, counted under stereomicroscope with the help of counting dish and stored in BOD at 15°C for further process.

Statistical analysis. All the experiments have been repeated five times and the nematode yield through *in vitro* (agar trap technique) and *in vivo* (*Galleria* technique) methods were analysed by analyses of variance (ANOVA) and a comparison of means was done using Duncan's Multiple Range Test (DMRT). All statistics and graphical representations were done using Microsoft Excel and GraphPad Prism 6.

Table 1. Locality and habitat of the nematodes recovered from Noida and Meerut, Uttar Pradesh, India.

No.	Genus	Isolate	Locality	Latitude, longitude and altitude	Habitat/Crop
1	*Acrobeloides*	HNK19	Raipur, sector-125, Noida	28°32'37.65" N, 77°19'51.63" E, 200.39 m a.s.l.	Soil of cauliflower field
2	*Metarhabditis*	HNK22	Kheemipura, Mawana, Meerut	29°5'49.04" N, 77°55'13.78" E, 231.94 m a.s.l.	Soil of forest area
3	*Acrobeloides*	HNK25	Kheemipura, Mawana, Meerut	29°5'49.04" N, 77°55'13.78" E, 231.94 m a.s.l.	Soil of forest area
4	*Distolabrellus*	HNK26	Jalalpur Jora, Mawana, Meerut	29°5'49.04" N, 77°55'13.78" E, 231.94 m. a.s.l.	Soil of sugarcane field

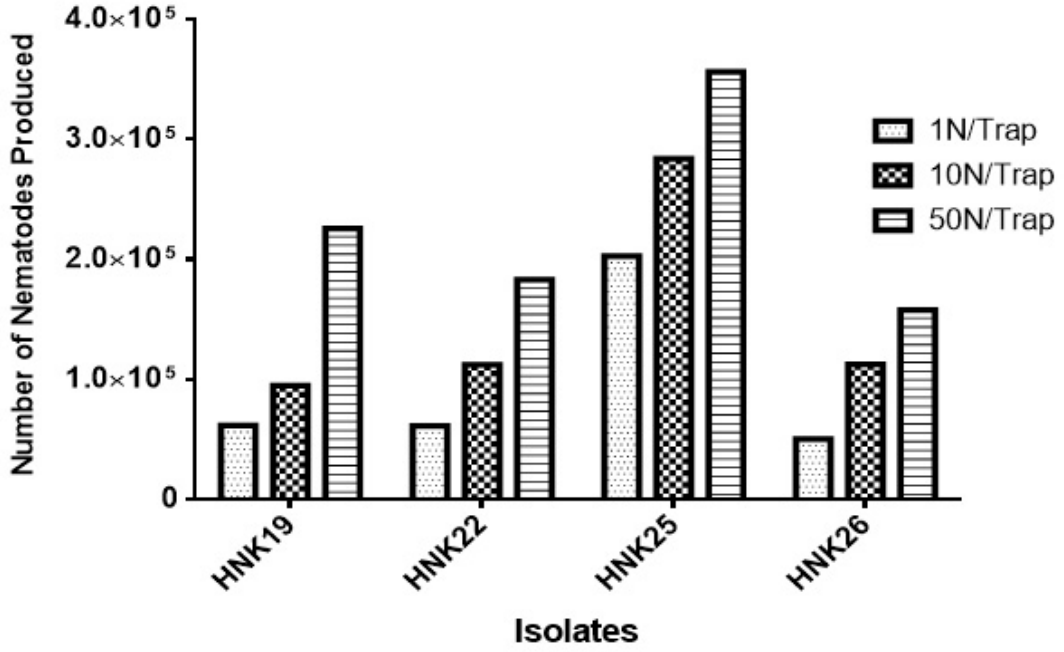

Fig. 1. Mean nematode production of *Acrobeloides* spp. (HNK19 and HNK25), *Metarhabditis* sp. (HNK22) and *Distolabrellus* sp. (HNK26) using the agar trap technique at day 25 post-inoculation with three different concentrations (1 nematode (trap)$^{-1}$, 10 nematodes (trap)$^{-1}$ and 50 nematodes (trap)$^{-1}$) of each isolate.

RESULTS

***In vitro* mass production of nematodes through agar trap.** The agar traps were prepared using two methods (method 1 and method 2). For both methods, there was no difference in preparation of agar plates, and there was no significant difference (F = 0.055, df = 1, *P* = 0.8293) in nematode yield among the method 1 and method 2. Although, three different concentrations (1 nematode (trap)$^{-1}$, 10 nematodes (trap)$^{-1}$ and 50 nematodes (trap)$^{-1}$) of four isolates (HNK19, HNK22, HNK25 and HNK26) were used to evaluate the efficiency of the agar trap technique in production of nematodes, and compared with each other. Statistically highly significant variation was found between the means of total nematode yield from trap 1, trap 2 and trap 3 (F = 46.39, df = 2, *P* = 0.0002) and between the total means of different isolates (F = 49.71, df = 3, *P* = 0.0001). The experimental data revealed that the production of nematodes using the agar trap technique was highest in HNK25 (*Acrobeloides* sp.) with 2.03 × 10^5 nematodes (trap)$^{-1}$, 2.84 × 10^5 nematodes (trap)$^{-1}$, 3.56 × 10^5 nematodes (trap)$^{-1}$, in trap 1 (1 nematode (trap)$^{-1}$), trap 2 (10 nematodes (trap)$^{-1}$) and trap 3 (50 nematodes (trap)$^{-1}$) respectively, while the lowest nematode count was recorded in HNK26 (*Distolabrellus* sp.) with 5.04 × 10^4 nematodes (trap)$^{-1}$ in trap 1 (1 nematode (trap)$^{-1}$) and 1.58 × 10^5 nematodes (trap)$^{-1}$ in trap 3 (50 nematodes (trap)$^{-1}$) and in HNK19 (*Acrobeloides* sp.) with 9.50 × 10^4 nematodes (trap)$^{-1}$ in trap 2 (10 nematodes (trap)$^{-1}$) (Fig. 1).

Two concentrations of milk powder were also applied and compared with the nematodes produced using the *G. mellonella* technique. Nematodes produced from group 1 (10 nematodes (trap)$^{-1}$ with 0.1 g milk powder) and group 2 (10 nematodes (trap)$^{-1}$ with 0.5 g milk powder) were compared with each other on 25th day post-inoculation. The difference between the mean nematode yield in group 1 and group 2 was not significant (F = 6.14, df = 1, *P* = 0.089). However, the nematode yield in group 2 was slightly higher (79.4%) than the other group. The highest nematode count was recorded in isolate HNK25 in both groups with 2.8 × 10^5 nematodes (trap)$^{-1}$ in group 1 and 6.9 × 10^5 nematodes (trap)$^{-1}$ in group 2, while the lowest nematode count was found in isolate HNK19 in both groups with 9.5 × 10^4 nematodes (trap)$^{-1}$ in group 1 and 1.3 × 10^5 nematodes (trap)$^{-1}$ in group 2 (Fig. 2).

Comparison between agar trap (*in vitro*) and *G. mellonella* (*in vivo*) techniques for mass culture of nematodes. Progeny produced from the agar trap (*in vitro*) and the *Galleria* technique (*in*

vivo) were collected and counted at different days post-inoculation, day 10, day 15 and day 20, and compared with each other (Fig. 3). There was a statistically significant difference between the total mean of nematode yield obtained from agar trap and *G. mellonella* techniques (F = 15.43, df = 1, *P* = 0.004). According to the results of the Duncan's multiple range test (with a confidence interval of 95%), the mean nematode yield in group A, was significantly (*P* = 0.023) different between the post-inoculation days (day 20 and day 10), (day 20 and day 15), (day 15 and day 10), while in group B, there was no significant (*P* = 0.060) difference in mean nematodes yields between the post-inoculation days (day 20 and day 15) and (day 15 and day 10). A significant difference was noted only between the yield at post-inoculation day 20 and day 10 in group B (Fig. 4).

The data revealed that the nematode yield at day 10 post-inoculation in group A was higher in isolate HNK22 with 1.07×10^5 nematodes $(trap)^{-1}$, followed by HNK26 (1.9×10^4 nematodes $(trap)^{-1}$), and HNK25 (8.52×10^3 nematodes $(trap)^{-1}$), whereas in group B, the highest nematode count was recorded in isolate HNK22 with 6.32×10^3 nematodes $(larva)^{-1}$, followed by HNK25 1.9×10^2 nematodes $(larva)^{-1}$), while in HNK26, no progeny was obtained. At day 15 post-inoculation, nematode yield in group A was higher in HNK25 with 1.73×10^5 nematodes $(trap)^{-1}$, followed by HNK26 1.52×10^5 nematodes $(trap)^{-1}$) and HNK22 (4.8×10^4 nematodes $(trap)^{-1}$) and in group B, higher nematode yield was recorded in HNK22 with 1.17×10^4 nematodes $(larva)^{-1}$, followed by HNK26 (7.37×10^3 nematodes $(larva)^{-1}$) and HNK25 (6.5×10^3 nematodes $(larva)^{-1}$). At day 20 post-inoculation, highest nematode yield in group A was recorded in HNK25 with 2.54×10^5 nematodes $(trap)^{-1}$, followed by HNK26 (with 8.78×10^4 nematodes $(trap)^{-1}$), and HNK22 (3.9×10^4 nematodes $(trap)^{-1}$), while in group B, the highest nematode count was recorded in HNK25 with 2.19×10^4 nematodes $(larva)^{-1}$, followed by HNK22 (1.46×10^4 nematodes $(larva)^{-1}$) and HNK26 (1.25×10^3 nematodes $(larva)^{-1}$).

In group A, the isolate HNK22 initially grew faster with 1.07×10^5 nematodes $(trap)^{-1}$ at day 10 post-inoculation and then, the population decreased at day 15 (4.8×10^4 nematodes $(trap)^{-1}$) and day 20 (3.9×10^4 nematodes $(trap)^{-1}$) post-inoculation. Isolate HNK25 had the lowest progeny count at the initial time period with 8.52×10^3 nematodes $(trap)^{-1}$ at day 10 post-inoculation and then the population increased by day 15 (1.73×10^5 nematodes $(trap)^{-1}$) and day 20 (2.54×10^5 nematodes $(trap)^{-1}$) post-inoculation. In isolate HNK26, the population was

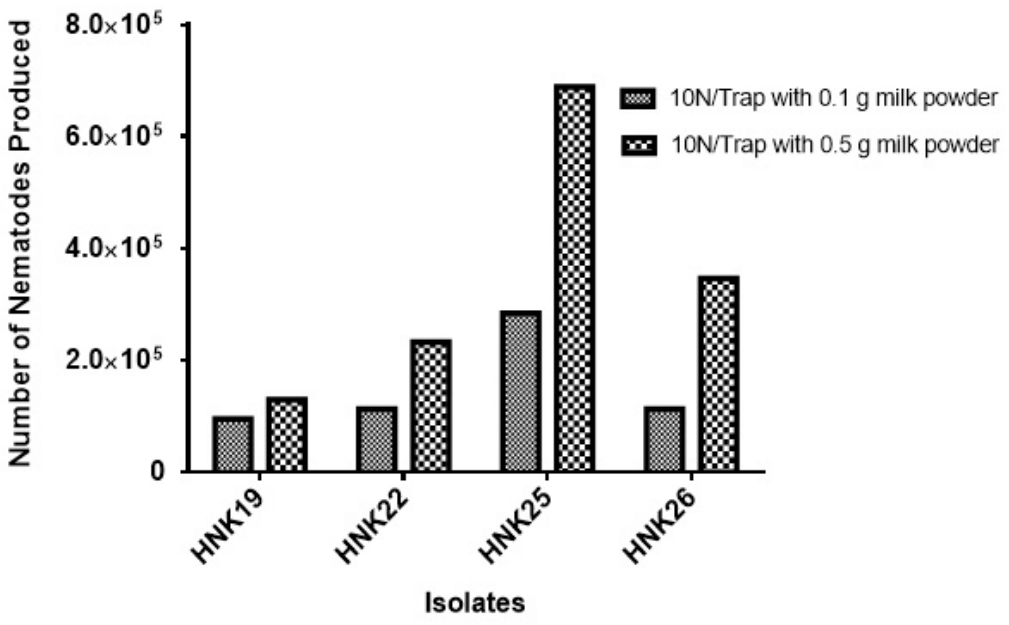

Fig. 2. Mean nematode production of *Acrobeloides* spp. (HNK19 and HNK25), *Metarhabditis* sp. (HNK22) and *Distolabrellus* sp. (HNK26) using the agar trap technique at day 25 post-inoculation with two different concentrations of milk powder (10 nematodes $(trap)^{-1}$ with 0.1 g milk powder and 10 nematodes $(trap)^{-1}$ with 0.5 g milk powder) for each isolate.

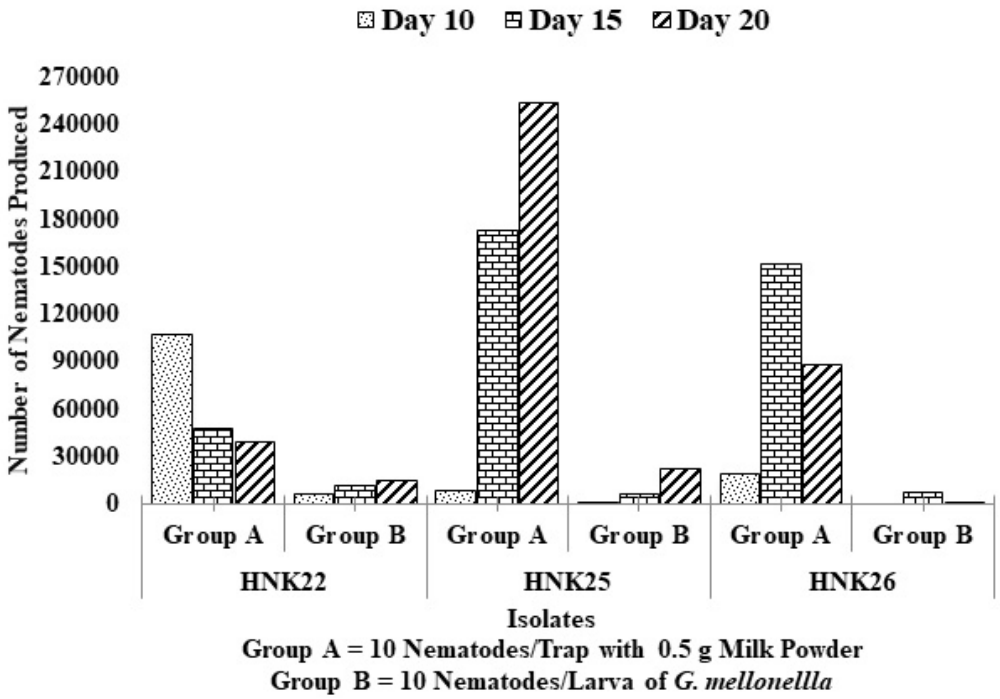

Fig. 3. Mean nematode yield of *Metarhabditis* sp. (HNK22), *Acrobeloides* sp. (HNK25) and *Distolabrellus* sp. (HNK26) through *in vitro* (agar trap – group A) and *in vivo* (*Galleria mellonella* – group B) methods at different post-inoculation days (day 10, day 15 and day 20).

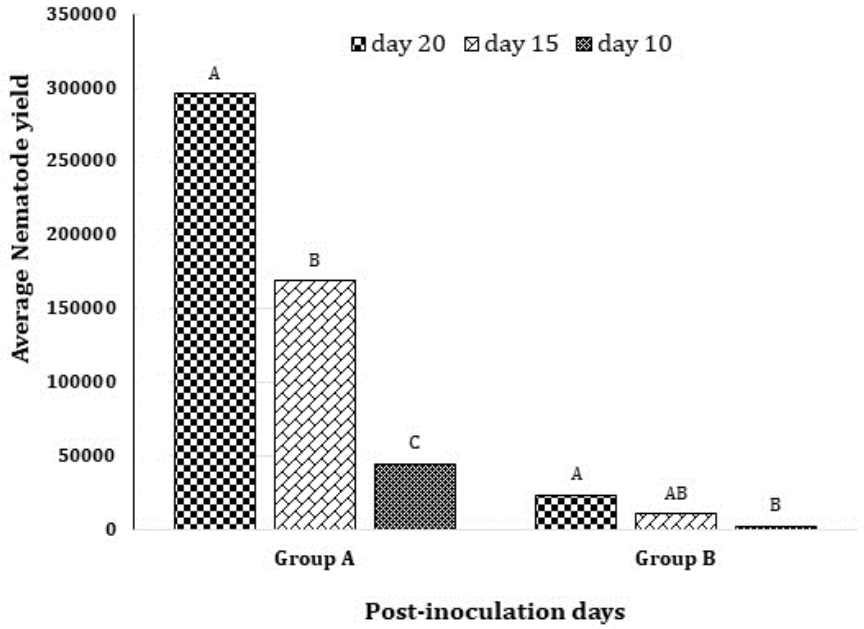

Fig. 4. Average nematode yield from group A (agar trap) and group B (*Galleria mellonella*) at different post-inoculation days (day 20, day 15 and day 10). Nematode yield among all post-inoculation days was significantly (P = 0.023) different in group A and non-significant (P = 0.060) in group B, according to Duncan's multiple range test.

lowest at day 10 (1.9 × 104 nematodes (trap)$^{-1}$) post-inoculation, increased at day 15 (1.52 × 10^5 nematodes (trap)$^{-1}$) and then decreased at day 20 (8.78 × 10^4 nematodes (trap)$^{-1}$) post-inoculation.

In group B, the isolate HNK22 had the lowest progeny count at day 10 (6.32 × 10^3 nematodes (larva)$^{-1}$), which then increased by day 15 (1.17 × 10^4 nematodes (larva)$^{-1}$) and day 20 (1.46 × 10^4 nematodes (larva)$^{-1}$) post-infection period. Growth rate of isolate HNK25 (*Acrobeloides* sp.) was lowest at day 10 (1.9 × 10^2 nematodes (larva)$^{-1}$), which increased at day 15 (6.5 × 10^3 nematodes (larva)$^{-1}$) and day 20 (2.19 × 10^4 nematodes (larva)$^{-1}$). In isolate HNK26 (*Distolabrellus* sp.), the population was not observed in the first 10 days, after which it was 7.38 × 10^3 nematodes (larva)$^{-1}$ on 15th day and decreased at day 20 to 1.25 × 10^3 nematodes (larva)$^{-1}$.

DISCUSSION

Nematodes associated with insects are considered as beneficial organisms in agriculture and gardening because some of the nematodes – facultative parasites – have the ability to control the insect pest populations by destroying their soil-dwelling larvae, and free-living nematode have the ability to improve the soil structure and growing process of plants by providing the nutrients to the soil. In aquaculture, several species of nematodes, such as *Panagrellus redivivus* (Linnaeus 1767), have received particular attention due to rapid growth rate and have been identified as suitable alternative to *Artemia* Leach, 1819 nauplii in recent years (Brüggemann, 2012). Biedenbach and his co-workers (1989) used the nematodes as live food to culture the Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) larvae and observed that the growth of the larvae fed on different densities of nematodes was faster or similar to *Artemia* diet. For *in vivo* production of nematodes, the primary expense includes the cost of insect hosts and labour (Shapiro-Ilan *et al.*, 2014), where the labour cost and availability of insects are the major problems on production of insect hosts (Ehlers & Shapiro-Ilan, 2005). However, for *in vitro* production of nematodes using the Agar Trap technique, there is no need to purchase or to rear insect hosts, thus saving costs. Also, it is a difficult task to inject the insect-parasitic and free-living nematodes in insect host larvae to produce these nematodes in large quantities. Therefore, an effective and productive technique (agar trap technique) was established for production of nematodes at low labour cost. The efficiency of the agar trap technique was tested on four isolates, HNK19 (*Acrobeloides* sp.), HNK22 (*Metarhabditis* sp.), HNK25 (*Acrobeloides* sp.) and HNK26 (*Distolabrellus* sp.). In addition, one isolate of *Panagrellus* species (HNK14), isolated from the common evening brown butterfly, *Melanitis leda* (Linnaeus, 1758), was also used to test the efficiency of the agar trap technique on mass production of *Panagrellus* and was successfully cultured using this technique. The data of nematode yield from the agar trap technique showed that this technique is highly effective in producing high number of nematodes in less time. The data also revealed that the milk powder affects the growth of nematodes; the trap with higher concentration of milk powder produced greater numbers of nematodes, showing a positive correlation between the concentration of milk powder and nematode yield. Nematodes grew faster on the agar trap in comparison to *Galleria* technique. However, the yield of nematode varies with nematode species and depends on nutrient status and other environmental factors, such as temperature, aeration, and moisture (Burman & Pye, 1980; Woodring & Kaya, 1988; Friedman, 1990; Grewal *et al.*, 1994; Shapiro-Ilan *et al.*, 2002; Dolinski *et al.*, 2007). *In vivo* production yield depends on nematode doses (Boff *et al.*, 2000), whereas in the agar trap (*in vitro*) production yield depends on nematode doses as well as concentration of milk powder. Nematode doses significantly affect the nematode yield where the milk powder provide the nutrient for nematodes and helps them to grow on agar medium for a long period. In the *G. mellonella* method, the growth of nematode population stops when the available food resources from the insect host are exhausted, but in the agar trap technique, a small amount of milk powder (0.1 g) can be added in the same trap to increase nematode production. The agar trap technique is the cost-effective and productive technique through which higher yield of some facultative nematode parasites of insects and free-living nematodes can be achieved in less time at low labour cost. Further experiments with other nematode species will provide more information.

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Heena and A.K. Chaubey. Экономически эффективный метод культивирования *in vitro* чистых линий нематод – факультативных паразитов насекомых и свободноживущих нематод.

Резюме. Четыре изолята нематод, а именно *Acrobeloides* spp. (изоляты HNK19 и HNK25), *Metarhabditis* sp. (HNK22) и *Distolabrellus* sp. (HNK26) были выделены из почв различных сельскохозяйственных полей регионов Нойда и Меерут. Чистые лини популяций этих изолятов были получены новым методом – методом агаровых ловушек, а затем на этих популяциях была проверена эффективность данного метода для массового культивирования нематод – факультативных паразитов насекомых и свободноживущих нематод. Проведено сравнительное изучение нового метода с универсальным методом массового культивирования нематод с использованием личинок *Galleria mellonella*. Результаты настоящего исследования показали, что все изоляты росли быстрее на агаровой ловушке, чем на личинках *Galleria*. На 20-й день после инокуляции самый высокий и самый низкий выходы нематод в агаровой ловушке были зарегистрированы как $2{,}54 \times 10^5$ нематоды на ловушку и $3{,}9 \times 10^4$ нематоды на ловушку у изолятов HNK25 и HNK22, соответственно. По сравнению с методом выращивания на особях рода *Galleria*, самый высокий и самый низкий выходы нематод были зарегистрированы как $2{,}19 \times 10^4$ нематоды на личинку и $1{,}25 \times 10^3$ нематоды на личинку у HNK25 и HNK26, соответственно.