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Effect of organic amendments on infectivity of entomopathogenic nematodes against *Brahminacoriacea* (hope)

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ABSTRACT

Background: *Motility and persistence of entomopathogenic nematodes areinfluenced by numerous interacting factors such as temperature, soil moisture, soil texture, relative humidity and UV radiations. The most important factor is soil texture, because soil particle size, composition and organic matter content in soil strongly influence the nematode motility, infectivity, development, reproduction and survival of entomopathogenic nematodes.*

Results: *The bioassay studies were done to study the effect of organic amendments on the infectivity of Heterorhabditis indica (Poinar) against I-III instar grubs of Brahminacoriacea (Hope) under laboratory conditions. The virulence of H. indica was tested in soil amended with different organic substrates,i.e. cocopeat, vermicompost, vermiculite and FYM, used in different combination ratios of 9:1, 8:2 and 7:3 of soil and substrates.In sand, the LC_{50} values of grubs (I-III instar) were computed to be 233.33-307.67 IJs/ml. H. indica displayed more or less the same virulence, irrespective of thesoil and sand media used for rearing of grubs. In soil:FYM ratios of 9:1, 8:2 and 7:3, the LC_{50} values of the respective instars ranged from 277.83-339.14, 243.54-290.26 and 216.08-250.36 IJs/ml, respectively. In soil:vermicompost ratio of 9:1, 8:2 and 7:3, the LC_{50} values of H. indica against I-III instar grubs of B. coriacea were calculated in the range of 256.37-345.98, 214.19-282.33 and 168.75-240.53 IJs/ml, respectively. It has been observed that the addition of vermicompost produced more or less similar effects as noticed with FYM. The LC_{50} values of H. indica against I-III instar grubs ranged from 337.54-545.77, 293.07-455.60 and 247.72-404.65 IJs/ml in cocopeat and soil ratios of 9:1, 8:2 and 7:3, respectively. The virulence of H. indica to I-III instar grubs decreased with the addition of cocopeat in soil. In different soil:vermiculite combinations, the LC_{50} values of H. indica against I-III instar grubs were calculated to be 530.83-905.79, 456.93-771.52 and 396.71-668.03 IJs/ml, respectively. Maximum virulence of H. indica was observed in vermicompost, followed by FYM and cocopeat.*

Conclusions: *The infectivity of entomopathogenic nematodes increases with an increasing amount of organic amendments*

Keywords: *Heterorhabdits indica, Brahminacoriacea, vermiculite, FYM, vermicompost, cocopeat.*

Introduction

Scarabaeidae is one of the largest families of Coleoptera, which contains more than 30,000 species throughout the world [7]. It constitutes a large, distinct group of highly specialised beetles, which could easily be identified by their lamellate antennae [9]. The larvae of scarab beetles are commonly known as "white grubs", which are chiefly found in grasslands feeding on roots of many plants [22]. Being polyphagous, they feed on a wide variety of cultivated as well as uncultivated plants and feed on almost all field crops grown during the rainy season, *viz.,* potato, vegetables, groundnut, sugarcane, maize, pearl millet, sorghum, cowpea, pigeon pea, cluster bean, soybean, rajmash, upland rice, and ginger are damaged [23]. White grubs cause damage ranging from 10 to 90 per cent in different crops [5].White grubs are naturally infected by various entomopathogenic nematodes, which kill their host and debilitate their future generations.

Entomopathogenic nematodes (EPNs) in the families Heterorhabditidae and Steinernematidae have received more attention, and they have very good potential in the management of insect pests, primarily of soil-dwelling insects [14].

The nematodes of genus *Heterorhabditis*actively seek out or hunt for their prey, sometimes several inches below the soil surface, and stay in one spot for an extended period of time [20][21]. The infective juveniles of *Heterorhabditis*gain entry to the host body by abrading the intersegmental membranes of the insect using a dorsal tooth[4].

This ability of *H. indica* is crucial for the success of entomopathogenic nematodes applications for insect control in soil. Particle size composition and organic matter content in soil strongly influence the availability of moisturein a given soil. Motility is influenced by numerous factors such as soil texture, soil particle, composition and organic matter. Soil texture is one of the important factors because soil strongly influences the nematode motility, activity and survival[19]. Nematode motility generally decreases as soil pores becomes smallertherefore, the present study aimed to investigate the effect of organic amendments on the efficacy of entomopathogenic nematodes so as to utilise them more effectively in integrated management of white grubs.

Methods

Culture of *B. coriacea*

Adults of *B. coriacea* were collected from different locations of the Rohru Shimla district with the help of a light trap. The collected beetles were transferred to glass jars (10.5x15.5 cm), which were half-filled with a mixture of moist sand, soil and FYM (1:1:1), and then the twigs of rose, peach and plum were fixed in

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the soil for the feeding and mating of beetles. After mating, female beetles lay eggs in soil and jars were daily examined for the presence of eggs. The eggs were separated with the help of a moist Camel's hair brush and were placed in the Petri plates containing moist soil.After hatching, the grubs were transferred to small paper cups filled with moist soil containing 4-5 days old maize seedlings, whereas second and third instar grubs were fed on small potato tubers in the paper cups individually.Field-collected grubs were acclimatised in plastic trays filled with the moist soil for 2-3 days before testing. The field collected grubs were fed on small potato tubers.

Culture of *H. indica*

H. indica species of entomopathogenic nematode was procured from FARMER, Ghaziabad and Khandelwal Bio Fertilisers Pvt. Limited, Karnataka. In the laboratory, the culture of *H. indica* was maintained on *Galleria mellonella* larvae. White trap[28] was used in harvesting of infective juveniles from the host by placing moist filter paper on a concave side up of the watch glass surrounded with water in a large Petri plate. Harvested infective juveniles of *H. indica* were used for treatments. Dilution method used for counting of infective juveniles under a stereo-zoom microscope.

Different organic amendments used in the present study

The virulence of *H. indica* was tested on first, second, and third instar grubs of *B. coriacea* in soil/sand amended with different organic substrates. In the present study, four different organic substrates,*i.e.* cocopeat, vermicompost, vermiculite and FYM, were used in different combinations. Different organic substrates,*i.e* cocopeat, vermicompost, vermiculite and FYM, were used in the soil:substrate ratio of 9:1, 8:2 and 7:3. After weighing a known quantity of soil and substrate, both were thoroughly mixed and filled in paper cups@100g per cup. In each cup, one grub was released, and a known quantity of infective juveniles ranging from 100-1600 IJs/ml was added to each cup with the help of a dropper. The first instar grubs were fed on 4-5 dayold maize seedlings, while second and third instar grubs were maintained on potato tubers. To record observations, the soil was tipped out of the cup onto paper, and the grubs were observed carefully for mortality. A grub was considered to be dead, if it failed to respond, when probed.

The mortality data were converted to per cent mortality, and per cent mortality was corrected by using Abbott's formula [2].The corrected per cent mortality was subjected to probit analysis [8] to calculate $LC_{50}$and LC_{90} values.

$$\text{Per cent corrected mortality} = \frac{\%\text{ mortality in treatment} - \%\text{ mortality in control}}{100 - \%\text{ mortality in control}} \times 100$$

Results

Against first instar grubs of *B. coriacea* in soil: FYM ratios of 9:1, 8:2 and 7:3, the LC_{50}andLC_{90} values were calculated to be 277.83, 243.54, 216.08 IJs/ml and 1314.13, 1176.63 and 976.92 IJs/ml, respectively. In different soil:vermicompost ratios of 9:1, 8:2 and 7:3, the $LC_{50}$of *H. indica*were computed to be 256.37, 214.19 and 168.75 IJs/ml and LC_{90}values were calculated to be 1421.03, 1170.56 and 915.18 IJs/ml, respectively. The slope of the regression line was 1.74, 1.76 and 1.78 with χ^2 values of 2.91, 2.86 and 2.79. In case of soil: cocopeat ratios of 9:1, 8:2 and 7:3, the LC_{50} values of *H. indica*were observed to be 337.54, 293.07 and 247.72 IJs/ml, and the LC_{90} values were computed to be 2390.05, 2136.87 and 1877.78 IJs/ml, respectively.When soil was amended with vermiculite, it indicated a negative impact on the infectivity of *H. indca.*

The LC_{50} values of *H. indica* against first instar grubs of *B. coriacea* in three different soils:vermiculite ratios of 9:1, 8:2 and 7:3 found to be 905.79, 771.52 and 668.03 IJs/ml with LC_{90} values of 8351.40, 8463.07 and 7973.08 IJs/ml (Table 1).

Against second instar, in soil:FYM combination ratios of 9:1, 8:2 and 7:3, the LC_{50} and LC_{90} values were calculated to be 297.04, 241.30 and 206.68 IJs/ml and 1607.19, 1256.81 and 1117.24 IJs/ml, respectively. When infective juveniles of *H. indica* were added in different soil:vermicompost combination ratios of 9:1, 8:2 and 7:3, the LC_{50}values were computed to be 297.04, 241.30 and 206.68 IJs/ml and with their respective LC_{90} values of 1641.02, 1556.68 and 1157.98 IJs/ml. In combination of soil:cocopeat ratios of 9:1, 8:2 and 7:3, the concentrations required to kill the 50 per cent of second instar grubs of *B. coriacea* were 432.15, 376.46 and 304.15 IJs/ml, respectively. The LC_{90} values were calculated to be 3642.91, 3318.55 and 2699.04 IJs/ml. Similarly, in soil vermiculite ratios of 9:1, 8:2 and 7:3, the LC_{50} and LC_{90} values were computed to be 706.12, 623.18, and 546.39 IJs/ml, and 6549.54, 6233.61 and 5820.02 IJs/ml, respectively (Table 2).

Against third instar, in soil:FYM ratios of 9:1, 8:2 and 7:3,*H. indica* produced 50 per cent kill at concentrations of 339.14, 290.26 and 250.36 IJs/ml, respectively. The LC_{90} values were calculated to be 1176.34, 1617.26 and 1242.83 IJs/ml.In different soil:vermicompost ratios of 9:1, 8:2 and 7:3, the LC_{50} values were estimated to be 345.98, 282.33, and 240.53 IJs/ml with the LC_{90} values of 2244.08, 1852.97 and 1597.39 IJs/ml, respectively. In case of soil:cocopeat ratios, the LC_{50} and LC_{90} values were calculated to be 545.77, 455.60, 404.65 IJs/ml and 4537.01, 3243.29, 3005.32 IJs/ml for 9:1, 8:2 and 7:3 combination, respectively.. When infective juveniles of *H. indica* were applied in soil:vermiculit ratios of 9:1, 8:2 and 7:3 (Table 3), the LC_{50} and LC_{90} values were found to be 905.79, 771.52, 668.03 and 8351.40, 8463.07, 7973.08 IJs/ml, respectively.

The relative toxicity was determined by dividing the LC_{50}value of *H. indica* as obtained in soil + vermiculite mixture (9:1), which was the least effective, by that of other tested treatment combinations. In the present study, *H. indica* exhibited the least virulence against first instar grubs when infective juveniles were applied in soil media consisting of soil + vermiculite in a 9:1 ratio. The order of virulence of *H. indica* on the basis of LC_{50} values as obtained in different soil media (Table 4) was soil + vermicompost-7:3 (3.15 times virulent) > soil + vermicompost-8:2 (2.48 times virulent) > soil + FYM-7:3 (2.46 times virulent) > soil + FYM-8:2 (2.18 times virulent) > soil + cocopeat-7:3 (2.14 times virulent) > soil (2.10 times virulent) > soil + vermicompost-9:1 (2.07 times virulent) > soil + FYM-9:1(1.91 times virulent) > soil + cocopeat-8:2 (1.81 times virulent) > soil + cocopeat-9:1 (1.27 times virulent) > soil + vermiculite-7:3 (1.34 times virulent) and soil + vermiculite-8:2 (1.16 times virulent).

The response of second and third instar grubs of *B. coriacea* in different tested combinations of soil and organic amendments was more or less similar to that of the first instar. The virulence of *H. indica* to second instar grubs was recorded to be minimum when infective juveniles were applied in soil + vermiculite having a ratio of 9:1 (Table 5). The order of virulence of *H. indica* against second instar grubs, on the basis of LC_{50} values as obtained in different soil media in relation to soil + vermiculite (9:1) was soil+vermicompost-7:3(3.42 times virulent) > soil + FYM-7:3(3.03 times virulent) > soil + vermicompost-8:2(2.93 times virulent) > soil + FYM-8:2 (2.70 times virulent) > soil(2.46 times virulent) > soil + vermicompost-9:1(2.38 times virulent) >

soil + cocopeat-7:3(2.32 times virulent) > soil + FYM-8:2(2.23 times virulent) > soil + cocopeat(1.88 times virulent) > soil + cocopeat(1.63 times virulent) > soil + vermiculite-7:3(1.29 times virulent) and soil + vermiculite-8:2(1.13 times virulent). On the basis of 95 per cent fiducial limits of LC_{50} values, the virulence of *H. indica* in soil treatment was statistically at par in all soil + organic amendment combinations at all tested doses except vermiculite. There was a significant decrease in virulence when infective juveniles of *H. indica* were applied in soil mixed with vermiculite in all tested soil+vermiculite combinations.

The virulence of *H. indica* to third instar grubs was recorded to be minimum when infective juveniles were applied in soil + vermiculite having a ratio of 9:1 (Table 6). The order of virulence of *H. indica* against third instar grubs on the basis of LC_{50} values as obtained in different soil media in relation to soil + vermiculite (9:1) was soil + vermicompost-7:3 (3.77 times virulent) > soil + FYM-7:3 (3.62 times virulent) > soil + vermicompost-8:2 (3.21 times virulent) > soil + FYM-8:2 (3.12 times virulent) > soil (2.83 times virulent) > soil + FYM-9:1 (2.67 times virulent) > soil + vermicompost-9:1 (2.62 times virulent) > soil + cocopeat-7:3 (2.24 times virulent) > soil + cocopeat-8:2 (1.99 times virulent) > soil + vermiculite-7:3 (1.36 times virulent) and soil + vermiculite-8:2 (1.17 times virulent).

Discussion

The effect of different organic amendments on infectivity of *H. indica* varied at different soil: substrate ratios and different instars of white grubs.The comparison of LC_{50} values obtained in soil versus soil + FYM revealed that, by the addition of FYM in soil, the susceptibility of I-III instars increased. By addition of FYM @ 100g in 900g soil, the susceptibility of I-III instar decreased by 1.05-1.10 times. There was an increase in susceptibility of I-III instar grubs to *H. indica* by 1.03-1.10 times when *H. indica* infective juveniles were applied in soil mixed with FYM in an 8:2 ratio. When the quantity of FYM was increased to 300g, a similar increase in virulence of *H. indica* (1.16-1.28 times) was recorded against I-III instar grubs of *B. coriacea*. The increase in susceptibility by the addition of FYM was more pronounced in the second instar as compared to the third instar (Fig 1).

The infective juveniles produced quick mortality, and grubs (I-III instar) began to die after 24 hours of treatment. Maximum mortality has been recorded within 48-72 hours of treatment. [24] He also reported that entomopathogenic nematodes kill the host quickly within 24-48 hours. The white grubs infected with *H. indica* became flaccid, and their colour changed from brownish-red to brick red. The infected grubs showed faint luminescence in the dark. Similarly, colour change in white grubs following infection by entomopathogenic nematodes has been reported by various workers [1][16]. The internal tissues were disintegrated to a mass of gummy consistency, and infective juveniles were clearly visible inside the body under the microscope (Fig 2)**.** After about six days, the infective juveniles of *H. indica* came out of cadavers in large numbers[10].

[3] studied the comparison of soil amendment with entomopathogenic nematodes. They reported that organic manure under field conditions resulted in increased densities of native populations of *S. feltiae.* With the addition of chemicals, they interfere with attempts to use nematodes and inoculative agents for long-term control, whereas organic manure used as fertilisers may encourage the establishment and recycling of entomopathogenic nematodes.

It is a well-established fact that FYM improves soil physical, chemical and biological properties [17]. Improvement in the soil structure due to FYM application also improves soil water holding capacity [6].

The fact that use of FYM improves water holding capacity, which in turn plays a species-specific role in survival, movement, infectivity and persistence of entomopathogenic nematodes [11][30]. Infectivity of many EPN species is highest at moderate soil moisture [26] with the thickness of the water film being approximately half the thickness of the nematodes' body. In soil, infective juveniles move through the water film that coats the interstitial space. [25] reported that nematode movement can also be restricted if the interstitial spaces are completely filled with water (in water-saturated soil) when the pore's diameter is much greater than that of the nematodes.

The LC_{50} values of *H. indica* in second instar of *B. coriacea* as compared to first instar were 1.12 -1.22 times higher in soil:vermicompost ratio of 9:1-7:3. There was 1.16-1.17 times increase in LC_{50} values of *H. indica* in third instar of *B. coriacea* as compared to second instar in soil: vermicompost ratio of 9:1-7:3. In third instar, increment in LC_{50} of *H. indica* was 1.31 – 1.42 times as compared to first instar in different vermicompost treatments.

Comparison of LC_{50} values of *H. indica* in different doses of vermicompost for first instar grubs of *B. coriacea* revealed a 1.19-1.51 times decrease in LC_{50} when the amount of vermicompost was increased from 100-200g and 200-300g, respectively. Comparison of 100g vermicompost with 300g vermicompost indicated a 1.51 times decrease in LC_{50} values of *H. indica* in first instar grubs. Similarly, in the case of the second instar, the decrease in LC_{50} was 1.23 times when 100g vermicompost was compared with 200g vermicompost. Comparison of 200g with 300g vermicompost recorded a decrease of 1.16 times, and for 100g vermicompost with 300g, the decrease in LC_{50} was found to be 1.43 times in second instar grubs of *B. coriacea*. For third instar grubs, the decrease in LC_{50} comes out to be 1.22 times by increasing vermicompost from 100g to 200g, and 1.17 times by equating 200g with 300g vermicompost. Comparison of LC_{50} values obtained for I-III instar grubs of *B. coriacea* when *H. indica* was applied in soil with those of soil+vermicompost (9:1,8:2, 7:3) is indicated in fig 3. It has been observed that the addition of vermicompost produced more or less similar effects as noticed with FYM. The virulence of *H. indica* remained more or less the same (showed a marginal increase of 1.01-1.07 times) at a dose of 100g vermicompost in 900g of soil as shown in fig.3.

By the addition of vermicompost at 100g in soil, the susceptibility of I-III instar decreased by 1.01-1.07 times. There was increase in susceptibility of I-III instar grubs to *H. indica* by 1.13-1.18 times when *H. indica* infective juveniles were applied in soil mixed with vermicompost in 8:2 ratio. When the quantity of vermicompost was increased to 300g, similar increase in infectivity of *H. indica* (1.33-1.49 times) was recorded against I-III instar grubs of *B. coriacea*. The increase in susceptibility by the addition of vermicompost was more pronounced in the second instar as compared to the third instar. The use of vermicompost as a carrier to deliver entomopathogenic nematode directly to the soil could therefore be useful for agricultural settings [13]. Application in the field by using vermicompost as a carrier might protect the nematodes against UV damage, buffer temperature extremes and promote contact between the nematodes and the pest insects [12].

The LC_{50} values of *H .indica* in second instar of *B.coriacea* as compared to first instar were 1.22-1.28 times higher in soil:cocopeat ratio of 9:1-7:3. There was 1.05-1.33 times increase in LC_{50} value of *H. indica* in third instar of *B.coriacea* as compared to second instar in soil to cocopeat ratio of 9:1-7:3. In third instar, increment in LC_{50} of *H. indica* was 1.55-1.63 times higher as compared to first instar in different cocopeat treatments. Comparison of LC_{50} values of *H. indica* in different doses of cocopeat for first instar grubs of *B. coriacea* revealed a 1.15 and 1.18 folddecrease in LC_{50} when the amount of cocopeat was increased from 100-200g and 200 to 300g, respectively. Comparison of 100g cocopeat with 300g cocopeat indicated 1.36 times decrease in LC_{50} value of *H. indica* in first instar grubs. Similarly, in case of the second instar, the decrease in LC_{50} was 1.14 times when 100g cocopeat was compared with 200g of cocopeat. Comparison of 200g with 300g cocopeat recorded decrease of 1.23 times, and for 100g cocopeat with 300g, the decrease in LC_{50} value was computed to be 1.42 times in second instar grubs of *B. coriacea.* For third instar grubs, the decrease in LC_{50} comes out to be 1.19 times by increasing cocopeat from 100g to 200 g, and 1.12 times by equating 200g with 300 g cocopeat. It has been observed that LC_{50} values of *H.indica* decreased with addition of cocopeat. Comparison of LC_{50} values obtained for I-III instar grubs of *B. coriacea* when *H. indica* was applied in soil with those of soil+ cocopeat (9:1,8:2, 7:3) is indicated in fig 4.

By addition of cocopeat @100-200g in soil, the virulence of *H. indica* to first instar grubs decreased by 1.02-1.33 times. In the second instar, the virulence of *H. indica* decreased by 1.06-1.50 times in soil mixed with cocopeat in 7:3, 8:2 and 9:1 ratios. The decrease in virulence of *H. indica* to third instar grubs by amending soil with cocopeat (100-300g) is by 1.26 -1.70 times. The decrease in virulence of *H. indica* by the addition of cocopeat was observed more in the second instar as compared to the first and third instar grubs. [18] have suggested that habitat quality (sand vs. peat) has important implications for the foraging behaviour of soil-transmitted parasites such as entomopathogenic nematodes in a closely related species. [15] reported lesser invasion of *Galleria mellonella* larvae by *S. carpocasae* in pure peat. When peat and soil were mixed to create different media, the moisture level may also change, and consequently, the observed responses of our experiments might be influenced by differences in humidity. [18] have reported that in peat, a greater percentage of *S. carpocapsae* tend to disperse from the point of application with significant taxis towards the host. In cocopeat, *H. megidis* showed no taxis towards hosts, which can be true for *H. indica* in the present study.

Comparison of LC_{50} values of *H. indica* in soil having different proportions of vermiculite indicated clear-cut differences in virulence against all instars when the amount of vermiculite was changed in the soil. There was a gradual decrease in LC_{50} with an increase in the amount of vermiculite in all the instars of *B. coriacea,* showing a positive impact of vermiculite on the virulence of *H. indica*. The LC_{50} value of *H. indica* in second instar of *B.coriacea* as compared to first instar were 1.33-1.37 times higher in soil:vermiculite ratio of 9:1-7:3. There was 1.22-1.28 times increase in LC_{50} value of *H. indica* in third instar of *B.coriacea* as compared to second instar in soil to vermiculite ratio of 9:1-7:3. In third instar, increment in LC_{50} of *H. indica* was 1.68-1.70 times higher as compared to first instar in different vermiculite treatments.

Comparison of LC_{50} values of *H. indica* in different doses of vermiculite for first instar grubs of *B. coriacea* revealed a1.16 and 1.15 times decrease in LC_{50} when the amount of vermiculite was increased from 100-200g and 200 to 300g, respectively. Comparison of 100g vermiculite with 300g vermiculite indicated a1.33 times decrease in LC_{50} values of *H. indica* in first instar grubs. Similarly, in the case of the second instar, the decrease in LC_{50} was 1.13 times when 100g vermiculite was compared with 200g vermiculite. Comparison of 200g with 300g vermiculite recorded a decrease of 1.14 times, and for 100g vermiculite with 300g, the decrease in LC_{50} value was computed to be 1.29 times in second instar grubs of *B. coriacea.* For third instar grubs, the decrease in LC_{50} comes out to be 1.17 times by increasing vermiculite from 100g to 200 g, and 1.15 times by equating 200g with 300g vermiculite. It has been observed that the LC_{50} values of *H. indica* decreased with the addition of vermiculite. Comparison of LC_{50} values obtained for I-III instar grubs of *B. coriacea* when *H. indica* was applied in soil with those of soil+vermiculite (9:1,8:2, 7:3) is indicatedin fig 5.

By addition of vermiculite @100-200g in soil, the virulence of *H. indica* to first instar grubs decreased by 1.56-2.10 times. In second instar, the virulence of *H. indica* decreased by 1.9-2.46 times in soil mixed with vermiculite in 9:1, 8:2 and 7:3 ratios. The decrease in virulence of *H. indica* to third instar grubs by amending soil with vermiculite (100-300g) is by 2.08-2.82 times. The decrease in virulence of *H. indica* by addition of vermiculite was observed more in second instar as compared to first and third instar grubs.

We found that vermiculite addition to soil reduces the virulence of *H. indica*, suggesting that the vermiculite formulation may have damaged the nematodes. Such formulations rely on partially dehydrating nematodes to make them inactive [29]. This is in contrast to findings of [27] who reported increase in mortality in termite, *Reticulitermes flavipes* (Kollar) exposed to *H. indica* in small containers with vermiculite and sand. Further investigations are required to determine how vermiculite reduces the virulence of *H. indica* against *B. coriacea* in the present study.

Comparison of LC_{50} values of *H. indica* in different doses of cocopeat for first instar grubs of *B. coriacea* revealed 1.15 and 1.18 fold decrease in LC_{50} when the amount of cocopeat was increased from 100-200g and 200 to 300g, respectively. Comparison of 100g cocopeat with 300g cocopeat indicated a 1.36 times decrease in the LC_{50} value of *H. indica* in first instar grubs. Similarly, in case of the second instar, the decrease in LC_{50} was 1.14 times when 100g of cocopeat was compared with 200g of cocopeat. Comparison of 200g with 300g cocopeat recorded a decrease of 1.23 times, and for 100g cocopeat with 300g, the decrease in LC_{50} value was computed to be 1.42 times in second instar grubs of *B. coriacea.* For third instar grubs, the decrease in LC_{50} comes out to be 1.19 times by increasing cocopeat from 100g to 200 g, and 1.12 times by equating 200g with 300 g cocopeat. It has been observed that the LC_{50} values of *H.indica* decreased with the addition of cocopeat. Comparison of LC_{50} values obtained for I-III instar grubs of *B. coriacea* when *H. indica* was applied in soil with those of soil+cocopeat (9:1,8:2, 7:3) is indicatedin fig 4.

By addition of cocopeat @100-200g in soil, the virulence of *H. indica* to first instar grubs decreased by 1.02-1.33 times. In second instar, the virulence of *H. indica* decreased by 1.06-1.50 times in soil mixed with cocopeat in 7:3, 8:2 and 9:1 ratios. The decrease in virulence of *H. indica* to third instar grubs by amending soil with cocopeat (100-300g) is by 1.26 -1.70 times.

The decrease in virulence of *H. indica* by the addition of cocopeat was observed more in the second instar as compared to the first and third instar grubs. [18] have suggested that habitat quality (sand vs. peat) has important implications on the foraging behaviour of soil-transmitted parasites such as entomopathogenic nematodes in a closely related species. [15] reported lesser invasion of *Galleria mellonella* larvae by *S. carpocasae* in pure peat. When peat and soil were mixed to create different media, the moisture level may also change, and consequently, the observed responses of our experiments might be influenced by differences in humidity. [18] have reported that in peat, a greater percentage of *S. carpocapsae* tend to disperse from the point of application with significant taxis towards the host. In cocopeat, *H. megidis* showed no taxis towards hosts, which can be true for *H. indica* in the present study.

Comparison of LC_{50} values of *H. indica* in soil having different proportions of vermiculite indicated clear-cut differences in virulence against all instars when the amount of vermiculite was changed in the soil. There was a gradual decrease in LC_{50} with an increase in the amount of vermiculite in all the instars of *B. coriacea,* showing a positive impact of vermiculite on virulence of *H. indica*. The LC_{50} value of *H. indica* in second instar of *B.coriacea* as compared to first instar were 1.33-1.37 times higher in soil:vermiculite ratio of 9:1-7:3. There was 1.22-1.28 times increase in LC_{50} value of *H. indica* in third instar of *B.coriacea* as compared to second instar in soil to vermiculite ratio of 9:1-7:3. In third instar, increment in LC_{50} of *H. indica* was 1.68-1.70 times higher as compared to first instar in different vermiculite treatments.

Comparison of LC_{50} values of *H. indica* in different doses of vermiculite for first instar grubs of *B. coriacea* revealed 1.16 and 1.15 times decrease in LC_{50} when the amount of vermiculite was increased from 100-200g and 200 to 300g, respectively. Comparison of 100g vermiculite with 300g vermiculite indicated 1.33 times decrease in LC_{50} values of *H. indica* in first instar grubs. Similarly, in case of second instar, the decrease in LC_{50} was 1.13 times when 100g vermiculite was compared with 200g vermiculite. Comparison of 200g with 300g vermiculite recorded a decrease of 1.14 times, and for 100g vermiculite with 300g, the decrease in LC_{50} value was computed to be 1.29 times in second instar grubs of *B. coriacea.* For third instar grubs, the decrease in LC_{50} comes out to be 1.17 times by increasing vermiculite from 100g to 200 g, and 1.15 times by equating 200g with 300g vermiculite. It has been observed that the LC_{50} values of *H. indica* decreased with the addition of vermiculite. Comparison of LC_{50} values obtained for I-III instar grubs of *B. coriacea* when *H. indica* was applied in soil with those of soil+ vermiculite (9:1,8:2, 7:3) is indicated in fig 5.

By addition of vermiculite @100-200g in soil, the virulence of *H. indica* to first instar grubs decreased by 1.56-2.10 times. In second instar, the virulence of *H. indica* decreased by 1.9-2.46 times in soil mixed with vermiculite in 9:1, 8:2 and 7:3 ratios. The decrease in virulence of *H. indica* to third instar grubs by amending soil with vermiculite (100-300g) is by 2.08-2.82 times. The decrease in virulence of *H. indica* by the addition of vermiculite was observed more in the second instar as compared to the first and third instar grubs.

We found that vermiculite addition to soil reduces the virulence of *H. indica*, suggesting that the vermiculite formulation may have damaged the nematodes. Such formulations rely on partially dehydrating nematodes to make them inactive [29]. This is in contrast to findings of [27] who reported an increase in mortality in the termite, *Reticulitermes flavipes* (Kollar) exposedto *H. indica* in small containers with vermiculite and sand. Further investigations are required to determine how vermiculite reduces the virulence of *H. indica* against *B. coriacea* in the present study.

Abbreviations

FYM	**Farm yard manure**
>	**Greater than**
IJs	**Infective juveniles**
/	**Per**
@	**At the rate**
LC	**Lethal concentration**
Ml	**Millilitre**
I-III	**First to third instar**
G	**Gram**

Table 1: Mortality response of first instar grubs of B. coriacea to H. indica in combination of different organic substrates

Soil: Substrates ratios		LC_{50} values	Fiducial limits	LC_{90} values	Fiducial limits	Regression equation	χ^2
FYM	9:1	277.83	220.40 and 335.26	1314.13	1081.80 and 1546.4	Y=0.32+1.90x	1.58
	8:2	243.54	191.51 and 295.56	1176.63	964.52 and 1388.75	Y=0.48+1.88x	2.02
	7:3	216.08	171.68 and 260.48	976.92	817.93 and 1135.91	Y=0.37+1.98x	3.13
Vermicompost	9:1	256.37	197.81 and 314.93	1421.03	1134.27and1707.79	Y=0.18+1.74x	2.91
	8:2	214.19	165.39 and 262.99	1170.56	951.05 and 1390.07	Y=0.88+1.76x	2.86
	7:3	168.75	129.41 and 208.08	915.88	753.00 and 1078.76	Y=1.0+1.78x	2.79
Cocopeat	9:1	337.54	250.91 and 424.17	2390.05	1762.75 and 3017.35	Y=1.17+1.51x	1.06
	8:2	293.07	216.99 and 369.15	2136.87	1587.10 and 2686.63	Y=1.30+1.49x	0.71
	7:3	247.72	181.27 and 313.89	1877.78	1402.26 and 2353.30	Y=1.46+1.47x	0.46
Vermiculite	9:1	530.83	375.53 and 686.14	4665.30	2966.57 and 6364.02	Y=1.35+1.34x	1.37
	8:2	456.93	316.63 and 597.22	4588.47	2852.19 and 6324.75	Y=1.62+1.27x	0.41
	7:3	396.71	275.28 and 518.14	3951.26	2521.65 and 5380.86	Y=1.67+1.28x	0.38

Table 2: Mortality response of second instar grubs of B. coriacea to H. indica in combination of different organic substrates

Soil: Substrates ratios		LC_{50} values	Fiducial limits	LC_{90} values	Fiducial limits	Regression equation	χ^2
FYM	9:1	317.02	248.89 and 385.14	1607.19	1292.08 and 1922.31	Y=0.43+1.82x	2.32
	8:2	261.08	206.65 and 315.50	1256.81	1037.65 and 1475.97	Y=0.42+1.89	2.01
	7:3	232.67	183.50 and 281.85	1117.24	925.21 and 1309.28	Y=0.49+1.90x	2.19
Vermicompost	9:1	297.04	230.80 and 363.28	1641.02	1308.59 and 1973.46	Y=0.70+1.74x	2.15
	8:2	241.30	182.06 and 300.54	1556.68	1213.25 and 1900.12	Y=1.17+1.60x	2.28
	7:3	206.68	158.68 and 254.68	1157.98	938.67 and 1377.30	Y=0.96+1.74x	1.13
Cocopeat	9:1	432.15	309.87and 554.44	3642.91	2448.12 and 4837.70	Y=1.37+1.38x	1.11
	8:2	376.46	269.08 and 483.84	3318.55	2257.22 and 4379.88	Y=1.50+1.36x	0.63
	7:3	304.15	216.34 and 391.96	2699.04	826.22 and 1872.82	Y=1.61+1.36x	0.53
Vermiculite	9:1	706.12	489.37 and 922.88	6549.54	3820.03 and 9279.06	Y=1.31+1.30x	0.68
	8:2	623.18	428.33 and 818.04	6233.61	3623.35 and 8843.87	Y=1.49+1.26x	0.21
	7:3	546.39	371.99 and 720.80	5820.02	3374.76 and 8265.28	Y=1.63+1.23x	0.05

Table 3: Mortality response of third instar grubs of B. coriacea to H. indica in combination of different organic substrates

Soil: Substrates ratios		LC_{50} alues	Fiducial limits	LC_{90} values	Fiducial limits	Regression equation	χ^2
FYM	9:1	339.14	265.66 and 412.61	1776.34	1414.76 and 2137.92	Y=0.48+1.79x	1.79
	8:2	290.26	224.13 and 356.39	1617.26	1279.33 and 1955.19	Y=0.74+1.73x	1.82
	7:3	250.36	196.48 and 304.24	1242.83	1017.15 and 1468.51	Y=0.53+1.86x	2.53
Vermicompost	9:1	345.98	261.10 and 430.87	2214.08	1667.21 and 2760.96	Y=0.95+1.59x	1.10
	8:2	282.33	212.49 and 352.17	1852.97	1417.13 and 2288.80	Y=1.12+1.58x	0.58
	7:3	240.53	180.40 and 300.67	1597.39	1235.08 and 1959.69	Y=1.23+1.58x	1.27
Cocopeat	9:1	545.77	391.02 and 700.53	4537.01	2942.33 and 6131.70	Y=1.23+1.38x	1.66
	8:2	455.60	338.23 and 572.98	3243.29	2304.50 and 4182.09	Y=1.02+1.50x	0.49
	7:3	404.65	298.20 and 511.10	3005.32	2133.23 and 3877.41	Y=1.17+1.47x	0.10
Vermiculite	9:1	905.79	623.72 and 1187.86	8351.40	4586.60 and 12116.21	Y=1.21+1.29x	0.77
	8:2	771.52	514.97 and 1028.07	8463.07	4402.61 and 12523.53	Y=1.55+1.20x	0.28
	7:3	668.03	437.23 and 898.83	7973.08	4070.68 and 11875.48	Y=1.72+1.17x	0.22

Table 4: Relative toxicity of H. indica against first instar grubs of B. coriacea in different combinations of organic amendments

Substrates	LC_{50} values (IJs/ml)	Fiducial limits (IJs/ml)	Relative toxicity
Soil +Vermicompost (7:3)	168.75	129.41 and 208.08	3.15
Soil +Vermicompost (8:2)	214.19	165.39 and 262.99	2.48
Soil +FYM (7:3)	216.08	171.68 and 260.48	2.46
Soil +FYM (8:2)	243.54	191.51 and 295.56	2.18
Soil +Cocopeat (7:3)	247.72	181.27 and 313.89	2.14
Soil	252.77	201.68 and 303.85	2.10
Soil +Vermicompost (9:1)	256.37	197.81 and 314.93	2.07
Soil +FYM (9:1)	277.83	220.40 and 335.26	1.91
Soil +Cocopeat (8:2)	293.07	216.99 and 369.15	1.81
Soil +Cocopeat (9:1)	337.54	250.91 and 424.17	1.57
Soil + Vermiculite (7:3)	396.71	275.28 and 518.14	1.34
Soil + Vermiculite (8:2)	456.93	316.63 and 597.22	1.16
Soil + Vermiculite (9:1)	530.83	375.53 and 686.14	1.00

Table 5: Relative toxicity of H. indica against second instar grubs of B. coriacea in different combinations of organic amendments

Substrates	LC_{50} values (IJs/ml)	Fiducial limits (IJs/ml)	Relative toxicity
Soil +Vermicompost (7:3)	206.68	158.68 and 254.68	3.42
Soil +FYM (7:3)	232.67	183.50 and 281.85	3.03
Soil +Vermicompost (8:2	241.3	182.06 and 300.54	2.93
Soil +FYM (8:2)	261.08	206.65 and 315.50	2.70
Soil	286.73	223.98 and 349.49	2.46
Soil +Vermicompost (9:1)	297.04	230.80 and 363.28	2.38
Soil +Cocopeat (7:3)	304.15	216.34 and 391.96	2.32
Soil +FYM (9:1)	317.02	248.89 and 385.14	2.23
Soil +Cocopeat (8:2)	376.46	269.08 and 483.84	1.88
Soil + Cocopeat (9:1)	432.15	309.87 and 554.44	1.63
Soil +Vermiculite (7:3)	546.39	371.99 and 720.80	1.29
Soil + Vermiculite (8:2)	623.18	428.33 and 818.04	1.13
Soil + Vermiculite (9:1)	706.12	489.37 and 922.88	1.00

Table 6: Relative toxicity of H. indica against third instar grubs of B. coriacea in different combinations of organic amendments

Substrates	LC_{50} values (IJs/ml)	Fiducial limits (IJs/ml)	Relative toxicity
Soil +Vermicompost (7:3)	240.53	180.40 and 300.67	3.77
Soil +FYM (7:3)	250.36	196.48 and 304.24	3.62
Soil +Vermicompost (8:2	282.33	212.49 and 352.17	3.21
Soil +FYM (8:2)	290.26	180.40 and 300.67	3.12
Soil	320.56	224.13 and 356.39	2.83
Soil +FYM (9:1)	339.14	252.28 and 388.84	2.67
Soil +Vermicompost (9:1)	345.98	265.66 and 412.61	2.62
Soil +Cocopeat (7:3)	404.65	261.10 and 430.87	2.24
Soil +Cocopeat (8:2)	455.6	298.20 and 511.10	1.99
Soil +Cocopeat (9:1)	545.77	338.23 and 572.98	1.66
Soil + Vermiculite (7:3)	668.03	391.02 and 700.53	1.36
Soil + Vermiculite (8:2)	771.52	437.23 and 898.83	1.17
Soil + Vermiculite (9:1)	905.79	514.97 and 1028.07	1

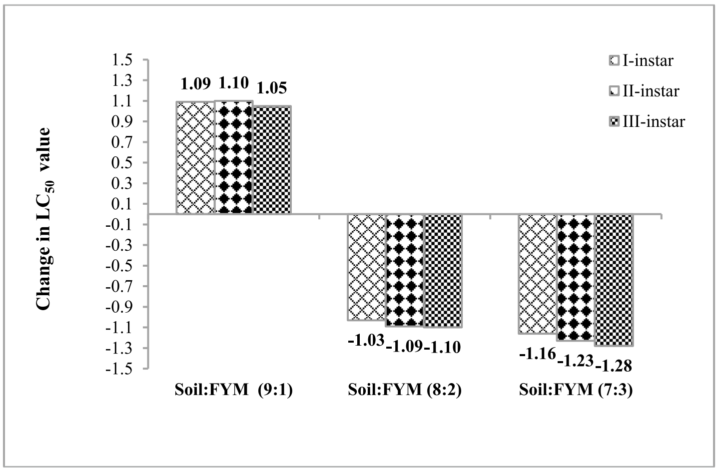

Fig 1: Comparison of Lc_{50} value of H. indica applied in soil versus soil+ FYM for I-III instars

Fig 2 a) White grubs infected with H. indica b) Infective juveniles coming out from the body of a white grubsc) Infective juveniles seen inside the body of grubs under microscope

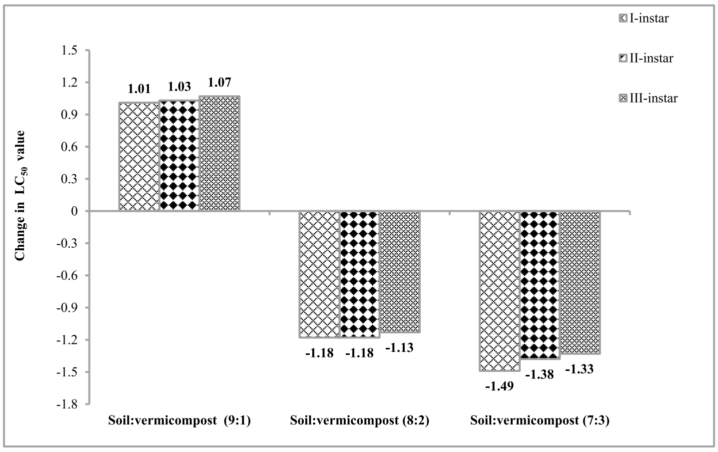

Fig 3: Comparison of LC_{50} value of H. indica applied in soil versus soil+ vermicompost for I-III instars

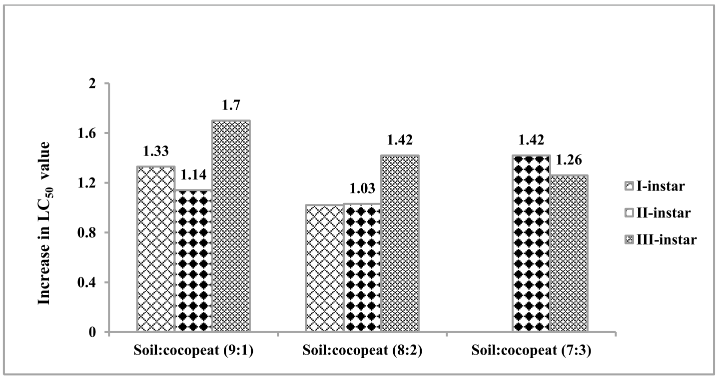

Fig 4: Comparison of LC_{50} value of H. indica applied in soil versus soil+ vermiculite for I-III instars

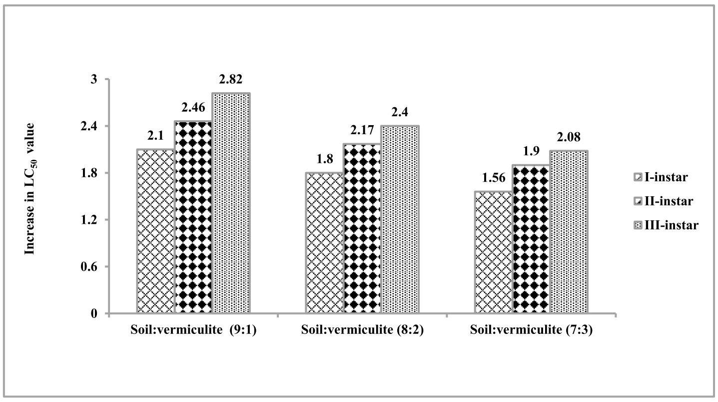

Fig 5 Comparison of LC_{50} value of H. indica applied in soil versus soil+ vermiculite for I-III instars

Conclusion: In conclusion our data and other studies show that different organic amendments have an effect on the virulence of entomopathogenic nematodes. It seems that nematodes tend to desiccate by the addition of vermiculite, whereas by the addition of cocopeat, the infective juveniles of *H. indica* didn't show taxis towards the hosts, thereby affecting the host searching ability of infective juveniles. Contrary to vermiculite and cocopeat, the virulence of *H. indica* increased with the addition of FYM and vermicompost. Among all tested soil amendments, vermicompost showed the maximum increment in virulence. There is improvement in soil structure due to the addition of FYM/ vermicompost, which improves soil water holding capacity, which played a significant role in their survival, movement and infectivity. These findings hold great promise for the use of EPN-inoculated vermicompost/FYM for integrated pest management of white grubs. Further investigations under field conditions would be the next step to ensure the results we have found hold true under greater biotic and abiotic stresses.

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