GMR
The Original

INFLUENCE OF SOIL MOISTURE ON VIRULENCE OF HETERORHABDITIS INDICA AGAINST BRAHMINA CORIACEA (HOPE)

Akshita[1], Harish kumar J[2], Damodhara GN[3], RS Chandel[4], Ayush Sharma[5], Suman Sanjta[6], Aditi Sharma[7], K.S. Verma[8], Gokul Kumar*[9]

[1,2,3,4,5,6,7,8,9] Department of Entomology, College of Agriculture CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176062, India.
*Corresponding Author: Gokul kumar, Lakhnaragokul@gmail.com

ABSTRACT
Soil moisture has significant effects on the infectivity and persistence of entomopathogenic nematodes (EPN). The research work describe the impact of soil moisture on the virulence of Heterorhabditis indica Poinar against I-III instar grubs of B. coriacea. In the present study, H. indica was tested at three different soil moisture levels of 55, 60 and 65 per cent against B. coriacea. There was gradual decrease in LC_{50} values of H. indica with increase in soil moisture percentage in all instars of B. coriacea indicating positive impact of soil moisture on its virulence. The LC_{50} values against first instar grubs of B. coriacea were computed to be 303.55, 254.42 and 231.09 IJs/ml at 55, 60 and 65 per cent soil moisture, respectively. In second instar, the LC_{50} values of H. indica at different soil moisture levels were calculated to be 329.12, 307.41 and 268.98 IJs/ml, respectively. The LC_{90} values were computed to be 2332.77, 2159.39 and 1723.52 IJs/ml. Against third instar, the LC_{50} values were found to be 394.08, 361.10 and 312.91 IJs/ml, and LC_{90} values were computed to be 3136.46, 2619.32 and 2234.69 IJs/ml. It was observed that LC_{50} values of H. indica decreased with increase in soil moisture and the results of the study iterated the role of soil moisture in dispersal of entomopathogenic nematodes and host infection.

KEYWORDS: Soil moisture, Heterorhabditis indica, Brahmina coriacea

INTRODUCTION

One of the largest family of Coleoptera, the Scarabaeidae, comprises more than 30,000 species worldwide (Fincher 1981). It is a vast distinct group of highly specialised beetles, and its lamellate antennae make them easy to recognize (Gardner 1935). Scarab beetle larvae, sometimes known as "white grubs," are most frequently seen in grasslands where they feed on the roots of various plants (Mehta et al. 2010). They consume both cultivated and wild vegetation since they are polyphagous. They remain undetected and abruptly expand their number in areas with ample food and minimal soil disturbance. According to Misra and Chandla (1989), white grubs among India's "five national pests" and have been reported in every state as they inflict harm to a wide range of cultivated crops. Important white grub species in Himachal Pradesh include Brahmina coriacea (Hope), Holotrichia longipennis Blanchard, Anomala dimidiata Hope, Anomala lineatopennis Blanchard, Melolontha indica Blanchard, and Lepidiota stigma (Fabricius), which cause damage to various crops ranging from 10 to 90 percent (Chandel et al. 2015). The scarabaeids are polyphagous pest that severely damages a variety of fruit and forest trees, as well as their nurseries, vegetables, grasslands, and field crops (Chandel and Kashyap 1997). The feeding behaviour of third instar grubs is the main factor contributing to the economic relevance of chafers (Chandel et al 2015). In endemic areas of Uttrakhand, Himachal Pradesh, and Jammu & Kashmir white grubs are reported to cause 40–90% production (Misra 2000). Many entomopathogenic nematodes naturally infect white grubs, killing their host and affecting 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 (Hominick 2002). Almost 200 species of insects from various orders have been shown to be infected by the entomopathogenic nematodes (Sharmila et al. 2018). Entomopathogenic nematodes have a potential in inundative and inoculative releases and have insignificant effects on non- target organisms (Bathon 1996). They have been most efficacious for insects in soil or cryptic habitats where there is protection from rapid dessication and UV radiation (Nagesh et al. 2015). The parasitic nematodes have been exempted from registration requirements greatly facilitating their development and distribution for insect control. Like other biopesticides, they can legally be used on all crops without restriction (Vashisht 2014). Entomopathogenic nematodes have many advantages over other control programmes. They can be applied with conventional spraying equipment (Ehlers and Peters 1995), and can tolerate

short period exposure to many chemicals (insecticides, fungicides, herbicides and fertilizers) in integrated pest management systems (Koppenhofer and Grewal 2008). Like microbial agents nematodes can easily be mass produced in liquid culture and stored maintaining their control potential for considerable time (Ehlers 1996). The pathogenicity or virulence of a microbial insecticide is a function of many interacting factors and is relative to a specific set of conditions which exist between microbial agent and a particular host (Dhoj et al. 2008). Motility and persistence of entomopathogenic nematodes is influenced by numerous interacting abiotic factors such as temperature, soil moisture, soil texture, relative humidity and UV radiations (Smits 1996). The most important factor is soil moisture, because nematodes need a water film for effective propulsion (Grant and Villani 2003). Therefore, the present study has been planned to further elucidate the effect of soil moisture on efficacy of entomopathogenic nematodes so as to utilize them more effectively in integrated management of white grubs

MATERIALS AND METHODS

Collection of beetles and grubs from field and maintenance in laboratory

Adults of B. coriacea were collected from field at night, and the beetles were maintained in glass jars having soil for egg laying. The eggs were separated with the help of moist Camel's hair brush and were placed in the Petri plates containing moist soil. In each Petri plates about 25 eggs were kept, and these Petri plates were maintained at room temperature. The first instar grubs were maintained on 4-5 days old maize seedlings, whereas second and third instar grubs were fed on small potato tubers in paper cups individually.

Maintenance of Heterorhabditis indica culture in laboratory

Heterorhabditis indica Poinar species of entomopathogenic nematode were procured from FARMER, Ghaziabad and Khandelwal Bio Fertilizers Pvt. Limited, Karnataka. In laboratory, the culture of H. indica was maintained on Galleria melonella larvae. White trap (White 1927) was used in harvesting of infective juveniles from host by placing moist filter paper on a concave side up of the watch glass surrounded with water in a large Petri plate. The harvesting was done daily up to 4-5 days or till the production continued. After harvesting, the nematodes were washed several times with 0.1 per cent formalin solution at 5-10^0 C before their use in various experiments. Dilution method was used for counting of infective juveniles.

Effect of soil moisture on efficacy of H. indica

Procedure for soil moisture determination

Soil was collected from the potato field in District Shimla from where adults of B.coriacea were collected. The aluminium container was weighed with its lid, and the weight of the empty aluminium container with lid was labelled as W_1. The soil sample was placed in the container and weighed again and labelled as W_2. Then, the sample was placed in an oven that was maintained at temperature of 105±5 °C. After 24 hours, the soil sample was removed from the oven and placed in the dessicator to cool the sample. After cooling the sample, the container was weighed again it was labelled as W_3. Gravimetric moisture content was determined by using the formula described by Hillel (1980).

$$\text{Water content (\%)} = \frac{W_2\text{-}W_3}{W_3\text{-}W_1} \text{ x } 100$$

Where,

W_1 = Weight of container + lid

W_2= Weight of container + lid+ moist soil

W_3= Weight of container +lid+ oven dry soil

Efficacy of H. indica was tested against first, second and third instars of white grubs in plastic pots filled with 350g of soil maintained at field capacity. Different graded concentrations ranging from 100-1600 IJs/ml were prepared. For each treatment 100, 200, 400, 800 and 1600 infective juveniles of nematodes were put into the soil directly in the cups containing grubs. The experiment was carried out three different soil moisture levels i.e. 55, 60 and 65 per cent, 10, 20 and 30 ml water was added daily in respective pots. The experiment was replicated 3 times. The data were recorded on the mortality of grubs after 24 hours up to a period of 6 days. The data on mortality were analyzed by using probit analysis.

To ascertain that the desired soil moisture contents were attained and sustained throughout the assay period, the amount of water necessary to attain 55, 60 and 65% gravimetric moisture contents in 350 g oven-dry soil samples was estimated using gravimetric procedure of Hillel (1980). This amount was added into the pots prior to introduction of grubs and nematode inoculum. Daily weighing of pots and replacement of water due to evaporation/soil surface exchange in order to maintain the initial/predetermined soil weight in the pots was then carried out. Daily irrigation of 10, 20 and 30 ml was enough to replace the evaporative loss occurring at the 55, 60 and 65% moisture contents, respectively, based on daily weight losses observed in the calibration trial. This daily irrigation procedure ensured that

the pot soil moisture contents were sustained at ±2% of the target moisture contents for six days as verified by gravimetric sampling.

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

Relative toxicity was calculated by dividing highest LC_{50} value of H. indica to a population with lowest LC_{50} value of the H. indica among the population.

The first instar grubs were fed on 4-5 days old maize seedlings, while second and third instar grubs were maintained on potato tubers. To record observations, the soil was tipped out of cup on a paper, and the grubs were observed carefully for mortality. A grub was considered to be dead, if it is 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 (Abbott 1925).

RESULTS AND DISCUSSIONS

The effect of soil moisture on virulence of H. indica against grubs of B. coriacea was determined at 55, 60 and 65 per cent soil moisture levels. There was gradual decrease in LC_{50} values of H. indica with increase in soil moisture percentage in all instars of B. coriacea indicating positive impact of soil moisture on its virulence. The LC_{50} values against first instar grubs of B. coriacea were computed to be 303.55 IJs/ml with 95 per cent fiducial limits ranging from 1503.51 and 2431.17 IJs/ml at 55 per cent soil moisture level. The value of regression line was calculated to be 1.59 with χ^2 value of 2.41 indicating homogeneity of data. The LC_{50} and LC_{90} values were calculated to be 254.42 IJs/ml (95% fiducial limits: 192.37 and 316.48 IJs/ml) and 1262.71 IJs/ml (95% fiducial limits: 1246.25 and 1954.74 IJs/ml) in soil having moisture percentage of 60 per cent. The probit kill (Y) was linearly related with log concentration (x) Y=1.09+1.62x**.** The data were homogenous as calculated value of χ^2= 0.95 was less than the tabulated value. The LC_{50} value was worked out to be 231.09 IJs/ml with 95 per cent fiducial limits of 179.23 and 282.94 IJs/ml at 65 per cent moisture. The LC_{90} value was computed to be 1262.71 IJs/m with 95 per cent fiducial limits varying between 1025.36 and 1500.06 IJs/ml. The regression line calculated with a slope of 1.51 and the value of χ^2 was determined to be 0.54, indicating homogeneity of data. In second instar, the LC_{50} values of H. indica at different soil moisture levels were calculated to be 329.12 IJs/ml, 307.41 IJs/ml and 268.98 IJs/ml, respectively. The LC_{90} values were computed to be 2332.77IJs/ml, 2159.39 IJs/ml and 1723.52 IJs/ml. The slope of regression line was calculated to be 1.51, 1.52 and 1.60 with χ^2 value of 4.36, 3.10 and 2.51 indicating homogeneity of data at different soil moisture levels. Against third instar, the LC_{50} values were found to be 394.08, 361.10 and 312.91 IJs/ml, and LC_{90} values were computed to be 3136.46, 2619.32 and 2234.69 IJs/ml. It was observed that LC_{50} values of H. indica decreased with increase in soil moisture.

Bioassays are indispensable to determine the pathogenicity of EPNs to screen virulent isolates or species as potential bio control agents. All instars of B. coriacea were found susceptible to infection by H. indica. 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. Nagesh et al. (2015) also reported that entomopathogenic nematodes kill host quickly within 24-48 hours. The white grubs infected with H. indica became flaccid, and their colour changed as brownish-red to brick red. The infected grubs showed faint luminescence in the dark. Similar, colour change in white grubs following infection by entomopathogenic nematodes have been reported by various workers (Ansari et al. 2003, Karimi and Kharazi-Pakdel, 2007). The internal tissues were disintegrated to a mass of gummy consistency and infective juveniles were clearly visible inside body under microscope**.** After about six day, the infective juveniles of H. indica came out of cadavers in large numbers (Gaugler 1988).

Table 1 Mortality response of all instars of B. coriacea to H. indica at different soil moisture levels

Moisture levels	LC_{50} values	Fiducial limits	LC_{90} values	Fiducial limits	Regression equation	χ^2
First instar						
≈55%	303.55 IJs/ml	228.87 and 378.23 IJs/ml	1967.34 IJs/ml	1503.51 and 2431.17 IJs/ml	1.05+1.59x	2.41
≈60%	254.42 IJs/ml	192.37 and 316.48 IJs/ml	1600.50 IJs/ml	1246.25 and 1954.74 IJs/ml	1.09+1.62x	0.95
≈65%	231.09 IJs/ml	179.23 and 282.94 IJs/ml	1262.71 IJs/ml	1025.36 and 1500.06 IJs/ml	0.84+1.76x	0.54
Second instar						
≈55%	329.12 IJs/ml	244.69 and 413.56 IJs/ml	2332.77 IJs/ml	1727.13 and 2938.41 IJs/ml	1.19+1.51x	4.36

≈60%	307.41 IJs/ml	228.55 and 386.28 IJs/ml	2159.39 IJs/ml	1609.31 and 2709.48 IJs/ml	1.21+1.52x	3.10
≈65%	268.98 IJs/ml	203.56 and 334.39 IJs/ml	1723.52IJs/ml	1336.90 and 2110.14 IJs/ml	1.10+1.60x	2.51
Third instar						
≈55%	394.08 IJs/ml	285.24 and 502.91 IJs/ml	3136.46IJs/ml	2174.51 and 4098.40 IJs/ml	1.31+1.42x	2.30
≈60%	361.10 IJs/ml	267.48 and 454.71 IJs/ml	2619.32 IJs/ml	1909.59 and 3329.06 IJs/ml	1.18+1.49x	2.00
≈65%	312.91 IJs/ml	232.66 and 393.17 IJs/ml	2234.69 IJs/ml	1664.09 and 2805.29 IJs/ml	1.23+1.51x	2.88

The LC_{50} value of H. indica in the first instar of B. coriacea as compared to second instar was 1.08 to 1.20 times lesser at soil moisture regime of 55-65 per cent. There was 1.16 to 1.19 times decrease in LC_{50} value of H. indica in second instar of B. coriacea as compared to third instar by changing soil moisture from 55 to 65 per cent. In third instar, increment of LC_{50} value of H. indica is 1.29-1.41 times as compared to first instar at different moisture regimes. Comparison of LC_{50} value of H. indica at different soil moisture levels in first instar of B. coriacea revealed 1.19 and 1.10 times decrease in LC_{50} value when the percentage of moisture was increased from 55 to 60 per cent and 60 to 65 per cent, respectively. Comparison of LC_{50} value obtained at 55 per cent moisture level with 65 per cent moisture level displayed 1.31 times decrease in LC_{50} value of H. indica in first instar. Similarly, in case of second instar, decrease in LC_{50} value was 1.07 times when virulence at 55 per cent soil moisture is compared with 60 per cent. Comparison of 60 per cent soil moisture with 65 per cent soil moisture showed decrease by 1.14 times, and for 55 per cent soil moisture with 65 per cent soil moisture, the decrease was calculated to be 1.22 times in second instar of B. coriacea. In third instar grubs, the decrease in LC_{50} value was 1.09 times by increasing soil moisture from 55-60 per cent, and 1.15 times by comparing 60 per cent soil moisture with 65 per cent soil moisture. It has been observed that LC_{50} values of H. indica decreased with increase in soil moisture content. Comparison of LC_{50} values obtained for I-III instar grubs of B. coriacea when H. indica was applied at different moisture levels.

Entomopathogenic nematodes need high relative humidity to survive and require free water for movement. They may become dormant at very low soil moisture (Grant and Villani 2003). Therefore, moisture conditions have been recognized as one of the most important factors in the soil environment affecting survival and virulence of nematode (Klein and Georgis 1992). The aim of our experiment was to investigate the impact of soil moisture on virulence of H. indica against I-III instar grubs of the B. coriacea. Comparison of LC_{50} values of H. indica in soil having different moisture level (55-65%) revealed differential susceptibility of I-III instars of B. coriacea. There was gradual decrease in LC_{50} value with increase in soil moisture percentage in all instars of B. coriacea indicating positive impact of soil moisture on the virulence of H. indica.

The response of second and third instar grubs of B. coriacea at different tested moisture levels was more or less similar to first instar. The virulence of H. indica to second instar grubs was recorded to be minimum when infective juveniles were applied at soil moisture ≈ 55 per cent. In second instar grubs of B. coriacea, H. indica showed 1.08 times higher virulence at soil moisture ≈ 60 per cent, and 1.22 times higher virulence at soil moisture ≈ 65 per cent by comparing their LC_{50} values with that obtained at soil moisture ≈ 55 per cent. On the basis of 95 per cent fiducial limits of LC_{50} values, there exist non-significant differences in efficacy of H. indica at different tested moisture levels (55-65 %) against second instar grubs of B. coriacea.

The virulence of H. indica to third instar grubs was recorded to be minimum when infective juveniles were applied at 55 per cent soil moisture. On the basis of LC_{50} values, the virulence of H. indica against I-III instar grubs, was 1.09 times more when infective juveniles were applied at soil moisture ≈60 per cent by comparing LC_{50} values as obtained at 55 per cent soil moisture. Comparison of LC_{50} values as calculated at 55 per cent soil moisture with 65 per cent soil moisture revealed 1.25 times higher efficacy of H. indica in third instar grubs. However, 95 per cent fiducial limits of LC_{50} values indicated non-significant differences in virulence at different moisture levels in the present study.

CONCLUSION

Entomopathogenic nematodes need high soil moisture for their survival, hence affecting their virulence. There is gradual increase in virulence with increase in soil moisture content within the tested limits of 55-65 per cent. Typically, environmental conditions were static in laboratory, but soil moisture levels vary naturally in the field especially under rainfed conditions. Further information needs to be gathered on the effects of soil moisture on nematodes. There is need to investigate if entomopathogenic nematodes in low moisture soil do not infect insects because they have entered a quiescent stage, are immobile, or for other reasons.

REFERENCES

1. Abbott WS (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.
2. Ansari MA, Tirry L and Moens M (2003). Entomopathogenic nematodes and their symbiotic bacteria for the biological control of Hoplia philanthus (Coleoptera: Scarabaeidae). Biological Control 28: 111-117.
3. Bathon H (1996). Impact of entomopathogenic nematodes on non-target hosts. Biocontrol Science and Technology 6: 421-434.
4. Chandel RS and Kashyap NP (1997). About white grubs and their management. Farmer and Parliament 37(10): 29-30.
5. Chandel RS, Pathania M, Verma KS, Bhattacharyya B, Vashisth S and Kumar V (2015). The ecology and control of potato white grubs of India. Potato Research 58: 147-164.
6. Dhoj GCY, Keller S, Nagel P and Kafle L (2008). Virulence of Metarhizium anisopliae and Beauveria bassiana against common white grubs in Nepal. Formosan Entomologist 28: 11-20.
7. Ehlers RU (1996). Current and future use of nematodes in biocontrol: Practice and commercial aspects in regard to regulatory policies. Biocontrol Science and Technology 6: 303-316.
8. Ehlers RU and Peters A (1995). Entomopathogenic nematodes in biological control: Feasibility, perspectives and possible risks. In: Biological Control - Benefits and Risks (Hokkanen HMT and Lynch JM, eds.). Cambridge University Press, Cambridge, 119-136.
9. Fincher GT (1981). The potential value of dung beetles in pasture ecosystem. Journal of the Georgia Entomological Society 16: 316-333.
10. Gardner JCM (1935). Immature stages of Indian Coleoptera (196). Indian Forest Records 1: 1-33.
11. Gaugler R (1988). Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. Agriculture, Ecosystems and Environment 24: 351-360.
12. Grant JA and Villani MG (2003). Soil moisture effects on entomopathogenic nematodes. Environmental Entomology 32: 80-87.
13. Hillel D (1980). Applications of Soil Physics. Academic Press, New York.
14. Hominick WM (2002). Biogeography. In: Entomopathogenic Nematology (Gaugler R, ed.). CAB International, Wallingford, UK, 115-143.
15. Karimi J and Kharazi-Pakdel A (2007). Incidence of natural infection of white grub Polyphylla olivieri (Coleoptera: Scarabaeidae) with entomopathogenic nematodes in Iran. IOBC/WPRS Bulletin 30: 39-42.
16. Klein MG and Georgis R (1992). Persistence of control of Japanese beetle (Coleoptera: Scarabaeidae) larvae with steinernematid and heterorhabditid nematodes. Journal of Economic Entomology 85: 727-730.
17. Koppenhöfer AM and Grewal PS (2008). Compatibility and interaction with agrochemicals and other biochemical agents. In: Nematodes as Biocontrol Agents (Grewal PS, Ehlers RU and Shapiro-Ilan DI, eds.). CAB International, Wallingford, Oxfordshire, UK, 363-382.
18. Mehta PK, Chandel RS and Mathur YS (2010). Status of white grubs in North-Western Himalaya. Journal of Insect Science 23: 1-14.
19. Misra SS (2000). White grubs. In: Diseases and Pests of Potato: A Manual (Khurana SMP, ed.). Central Potato Research Institute, Shimla, 57-60.
20. Misra SS and Chandla VK (1989). White grubs infesting potatoes and their management. Journal of the Indian Potato Association 16: 29-33.
21. Nagesh M, Verghese A and Bhatnagar A (2015). Importance of entomopathogenic nematodes in the management of soil arthropod pests. Proceedings of the XVIII Group Meeting of the All India Network Project on Soil Arthropod Pests, 46-49.
22. Sharmila S, Subramanian S and Poornima K (2018). Host range of entomopathogenic nematodes. Journal of Entomology and Zoology Studies 6: 1310-1312.
23. Smits PH (1996). Post-application persistence of entomopathogenic nematodes. Biocontrol Science and Technology 6: 379-387.
24. Vashisth S (2014). Distribution and biocontrol potential of entomopathogenic nematodes against some lepidopteran pests. PhD Thesis. Department of Entomology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India.
25. White GF (1927). A method for obtaining infective nematode larvae from cultures. Science 66: 302-303.