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Influence of dry heat, hot water and UV radiation on host infectivity of entomopathogenic nematode, *Heterorhabitis indica* (Poinar)

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ABSTRACT: Entomopathogenic nematodes are considered as potential biocontrol agents of major insect pests of cultivated crops. The lepidopteron pests such as *Helicoverpa armigera* and *Spodoptera litura* as well as few coleopterans like *Myllocerus* spp. and *Holotricha* sp. are effectively managed by entomopathogenic nematodes. An *in vitro* experiment was conducted to enhance the efficacy of *Heterorhabditis indica* using physical stressors. Physical stressors *viz.,* dry heat, hot water and UV radiation exposure were used in this experiment.These stressors were imposed on final instar larvae of rice moth, *Corcyra cephalonica*. The results showed that exposure of *C. cephalonica* larvae to 35°C dry heat for 10 minutes increased the infection rate to an extend of 10 per cent more compared to control. The infection rate of *H. indica* was also influenced by exposure of insect larvae to hot water. Out of four temperatures tested, immersion of insect larvae in hot water at 40°C for 10 minutes showed an increase in infection rate (51% increase over control). There was no significant influence on infectivity rate of *H.indica* due to UV radiation exposure.

Keywords: *Corcyra cephalonica*, dry heat, *Heterorhabditis indica*, hot water, UV radiation

INTRODUCTION

Insect pests, pathogens and nematodes are known to cause more economic damage in cultivated crops (Thomas*,* 1999). Some insecticides/pesticides are used to control many insect pests, pathogens and nematodes. Insecticides cause more residual toxicity in environment. Many insecticides were banned because of their residual effect. Some bioagents were used to control many pests and pathogens. Entomopathogenic nematodes act as a good biocontrol agents to manage lepidopteron and coleopteran pests (Gaugler and Han, 2002). Effectiveness of entomopathogenic nematodes, *Steinernema feltiae* was tested against Colorado Potato Beetle, *Leptinotarsa decemlineatea* (Say*)* and the results showed that agar gel formulation (1%) showed highest reduction of *L. decemlineatea* (Hany *et al.,*2012). *Heterorhabditis* spp., *Steinernema* spp. and *Neosteinernema* spp. are potential and widely studied entomopathogenic nematodes. Third stage juvenile (J3) is the infective stage of entomopathogenic nematodes. *Xenorhabdus* and *Photorhabdus* are the bacteria associated with the intestine of *Heterorhabditis* and *Steinernema.* Infective juveniles (IJs) yield vary between each genus of pests. Normally Infective Juveniles (IJs) yield was more from wax moth, *Galleria mellonella.* Infectivity of entomopathogenic nematodes was enhanced by physical and chemical stressors in *Tenebrio molitar* (Brown *et al*., 2006). Influence of different Physical stressors on infectivity of *Heterorhabditis indica* was studied and results are discussed in this paper.

MATERIALS AND METHODS

Culturing of rice moth, *Corcyra cephalonica*

Rice moth, *Corcyra cephalonica* was cultured using cumbu grains and groundnut medium. About 2kg of cumbu grains and 200g of broken groundnut were taken in a plastic tray. Eggs of *C. cephalonica* were obtained from the Department of Entomology, TNAU, Coimbatore. One cc of *C. cephalonica* eggs were inoculated in 2.25 kg medium in a plastic tray. This plastic tray was covered with a cotton cloth. The final instar larvae of *C. cephalonica* were collected 25 days after egg hatching and used for further experiments.

Preparation of White's trap

White's trap (White, 1927) was used to harvest infective juveniles of *H. indica*. White's trap was prepared using glass Petri plates with 5 cm and 9 cm diameter (Borosil make). A small (5cm) Petri plate was inversely placed in a 9 cm Petri plate. A Whatmann No.1 filter paper was taken and cut in to two halves. One half was again cut in to small stripes (2cm) and placed over the small Petri plate. The filter paper was made wet by placing small drops of sterile distilled water on the filter paper. Then, 20 ml of sterile water was poured in the large Petri plate. After this process, *H. indica* infected cadavers were placed on filter paper. This setup was maintained for 6-8 days at room temperature (27 ±2°C).

Mass culturing of entomopathogenic nematodes

Culture of entomopathogenic nematode, *H. indica* infective juveniles (IJs) was obtained from the Department of Nematology, TNAU, Coimbatore. A Whatmann No.1 filter paper was placed in the bottom of 9cm Petri plate. About one ml (200 IJs) of *H. indica* IJs suspension was added on the filter paper. After addition of nematodes, the rice moth, *C. cephalonica* larvae were placed on the filter paper. These plates were sealed with Klinflim and incubated for 5 days. *H. indica* infected larvae were transferred to White's trap after 5 days of inoculation. Infective juveniles (IJs) of *H. indica* emerged out of the infected insect larvae on the 8th day of inoculation. IJs were washed with the sterile distilled water 3-4 times, decanted and nematode suspensions was stored in a BOD incubator (Genuine model) at 20°C. A drop of Triton X was added to *H. indica* suspension to avoid the sticking of nematodes together.

Inducing Physical Stressors to Rice moth, *C. cephalonica*

Dry heat

The larvae (5 no) of rice moth, *C. cephalonica* were incubated at different temperatures *viz*., 30°C, 35°C, 40°C and 45°C in a BOD incubator (Remi Instruments) for 15 minutes. The dry heat stressed *C. cephalonica* larvae were inoculated with *H.indica* (100 IJs) and incubated for 5 days. A control was maintained by incubating *C. cephalonica* under room temperature (27± 2°C). The dead larvae were transferred to White's trap. The experiment was conducted in a Completely Randomized Design with six replications.

Hot Water

The larvae (5 no) of rice moth, *C. cephalonica* were immersed in hot water at different temperatures *viz.,* 35°C, 40°C and 45°C for 10 minutes. A control was maintained by immersing larvae in tap water (29 °C) for 10 minutes. The hot water stressed larvae were exposed to IJs (100 IJs) of *H. indica.* The dead larvae were transferred to White's trap. The experiment was conducted in a Completely Randomized Design with six replications.

UV radiation

Final instar larvae of *C. cephalonica* (5 no) were exposed to UV radiation in a Laminar Air Flow chamber (Clean air Instruments) at different time intervals *viz.,*10, 20 and 30 minutes. The larvae were maintained at normal light (room temperature) served as control. UV stressed larvae were exposed to IJs (100IJs) of *H. indica*. The dead larvae were transferred to White's trap. The experiment was conducted in a Completely Randomized Design with six replications.

Statistical analysis

The data obtained from above mentioned experiments were subjected to statistical analysis following the method formulated by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

Exposure of dry heat on *C. cephalonica*

Among four different dry heat treatments, exposure of *C.cephalonica* larvae at 35°C for 15minutes increased the infectivity of *H.indica.* The infection rate (4.40 insects) in 35 ° C compared to control. About 4.40larvae was infected when exposed to dry heat @ 35°C which was 10% increase over control under room temperature (Table 1). Other temperatures *viz.,* 30°C, 40° C and 45°C did not show any positive influence on *H. indica* infectivity as yield of infective juveniles.

The results of present study indicate that the efficacy of *H.indica* can slightly be enhanced by physical stressors. Only mild heat was applied to the final instar larvae of *C. cephalonica*. Results of present study are agreement with the findings of Hansen *et al.* (1990). They have reported that the larvae of Oriental fruit fly *Dacus dorsalis* (Hendel), couldn't survive, when centre temperature of papaya fruit was above 45.6° C. Studies of Armer *et al*. (2004) indicated that the haemolymph of Colorado potato beetle, *L. decemlineatea* was heated up to 60°C for 10 minutes inactivate the bacterium, *Photorhabdus luminescens* of *H. marelatus* and in turn indicates reduction the infection. At 50°C, the larvae of *C. cephalonica* were not alive. Up to 40 - 45°C only the larvae of *C. cephalonica* were alive under dry heat. Mild heat probably loosens the cuticle of *C. cephalonica* final instar larvae which might have helped the IJ of *H. indica* to enter insect body. Similar results were recorded in the infection of *T. molitor* by *H. bacteriophora* where the dry heat @ 40° C for 30 minutes enhanced infectivity (Brown *et al*., 2006).

Exposure of hot water on *C. cephalonica:*

Immersing *C. cephalonica* larvae in hot water increased the infection rate of *H. indica.* The highest infection was observed at 40°C. About 5 larvae were infected when exposed to hot water @ 35°C which was 51% increase over control under room temperature (Table 2). Number of *H. indica* infected *C. cephalonica* larvae (5 insects)

Table 1. Infection of *H. indica* on dry heat induced *C. cephalonica*

Treatment	Means of six replications	
	Number of *H. indica* infected larvae	Number of infective juveniles in 20 ml of *H. indica* suspension
30°C	2.17 [e] (1.41)	3353.4 [c] (3.41)
35°C	4.40 [a] (2.05)	4176.6[b] (3.59)
40°C	2.50 [d] (1.52)	1826.6 [e] (3.15)
45°C	2.84 [c] (1.64)	3518.4 [d] (3.44)
Control (27+2°C)	4.00 [b] (1.97)	7685 [a] (3.90)
SEd	0.228	0.171
CV (%)	22.36	8.48
CD (p=0.01)	0.621	0.478

Figures in parentheses are square root and log transformed value respectively.

In a column, means followed by common letter are significantly different from each other at 1% level by DMRT.

Table 2. Infection of *H. indica* on hot water induced *C. cephalonica*

Treatment	Means of six replications	
	Number of *H. indica* infected larvae	Number of infective juveniles in 20 ml of *H. indica* suspension
35°C	3.70 [c] (2.04)	4743.4 [a] (3.64)
40°C	5.00 [a] (2.34)	4654.4 [b] (3.62)
45°C	4.00 [b] (2.12)	3505 [d] (3.45)
30 °C	3.30 [d] (1.94)	4378 [c] (3.62)
SEd	0.1022	0.210
CV (%)	8.91	25.60
CD (p=0.01)	0.29	0.597

Figures in parentheses are square root and log transformed value respectively.

In a column, means followed by common letter are significantly different from each other at 1% level by DMRT.

Table 3. Infection of *H. indica* on UV exposed *C. cephalonica*

Treatment	Means of six replications	
	No. of *H. indica* infected larvae	**No. of infective juveniles in 20 ml of *H. indica* suspension**
UV exposure for 10 minutes	4.8 (2.3)	1325.5[d] (3.11)
UV exposure for 20 minutes	4.5 (2.23)	2232.5[c] (3.31)
UV exposure for 30 minutes	4.8 (2.30)	2155[b] (3.34)
No UV exposure	4.6 (2.25)	3370.5[a] (3.44)
SEd	0.0830	0.1138
CV (%)	6.73	0.3238
CD (p=0.01)	0.236 (NS)	5.96

Figures in parentheses are square root and log transformed value.
In a column, means followed by common letter are significantly different from each other at 1% level by DMRT.
NS - Non significant

were more in 40° C but the infective juveniles yield was more in 35°C of hot water immersion.The infective juveniles of *H. indica* were more (4743 per 20 ml in 35°C) followed by 40°C of hot water immersion (4654 IJs in 20 ml).

Inducing stress on *C. cephalonica* by immersing in hot water slightly improved the infection rate. The final instar larvae of *C. cephalonica* were inactive immediately after immersing in hot water. Immersion of larvae in hot water with temperatures above 45° C resulted in mortality. Hence, the present study was restricted up to 45° C but the results obtained by (Brown *et al.,* 2006) is contradictory that heat treatment in hot water at 65°&70° C increased *Tenebrio molitor* infection by *H. bacteriophora.* The variation in cuticle property of *C. cephalonica* and *T. molitor* might be the reason for temperature tolerance.

Exposure of UV on *C. cephalonica*

Exposure of *C. cephalonica* larvae to UV radiation slightly increased the infection rate of *H. indica* but it is statistically non-significant. The UV exposure for 10 minutes as well as 30 minutes showed higher infection rate (4.8 insect) compare to control. Infective juveniles were more in control. 3370 IJ yielded in UV unstressed *C. cephalonica* larvae (Table.3). Exposure of *C. cephalonica* larvae to UV radiation reduced body width of third instar larvae (Herlin *et al.,* 2015). The above finding supports the results of current study where in UV radiation exposure of *C. cephalonica* had positively influenced the infectivity of *H. indica* to some extent.

To conclude, the findings of present investigation revealed that the physical stressors *viz.,* dry heat, hot water immersion and UV radiation exposure have positive influence on the infectivity of *H. indica*. Even though the physical stress increased the infectivity, number of infective juveniles were either less or equal to control. The effect of physical stressors on the second generation infective juveniles and effect on mass multiplication are also to be studied to identify the best physical stressor to enhance entomopathogenic nematode's biocontrol potential.

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