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Pathogenicity of indigenous EPN isolates of entomopathogenic nematode against rice meal moth, *Corcyra cephalonica* and greater wax moth *Galleria mellonella* under laboratory conditions

Dadhe CG, Lavhe NV and Wargane VS

Abstract
The results on pathogenicity of indigenous EPN isolates *viz*., *Heterorhabditis spp*.(PKV-1), *Heterorhabditis spp*.(CICR Brown), *Steinernema spp*. (CICR-White) and *Heterorhabditis spp*. (PKV-Guava) from Nagpur against the 5th instar larvae of *Corcyra cephalonica* and *Galleria mellonella* revealed that highest mortalities observed in EPN sample CICR-Brown in the treatment concentration of 50IJs/20µl which were 94.28%, in 30IJs/20µl were 82.85% and 74.28%, 74.28%, 68.57%, 60% and 57.14% in the treatment concentration 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. However, in case of CICR-white the highest mortality was observed in 50IJs/20µl that was 68.57% and 65.71%, 62.85%, 62.85%, 60%, 57.14%, 54.28% in the treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. In case of greater wax moth, the highest mortalities observed in EPN sample CICR-Brown in the treatment concentration of 50 IJs/20 µl which were 97.14%, and 91.42%, 82.67%, 68.57%, 68.57%, 67.56%, 60.36% mortality were observed in the concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl,15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively.

Keywords: *Heterorhabditis*, *Steinernema*, EPN, Pathogenicity, Nematode, Isolates

Introduction
Nematodes that parasitize insects, known as entomopathogenic nematodes (EPNs), have been described from 23 nematode families (Koppenhofer, 2007) [8]. The most commonly found entomopathogenic nematode species belong to the families Allantonematidae, Mermithidae, Steinernematidae, and Heterorhabditidae. The entomopathogenic nematode in the families of Steinernematidae and Heterohabditidae are potential virulent agents because of their symbiotic association with bacteria *Xenorhabdus spp*. and *Photorhabdus spp*. respectively (Kaya *et al*., 2006) [6]. These bacteria are gram negative, anaerobes, non-spore former, do not have environmentally resistant stages, generally non-pathogenic when ingested by a host and are also incapable of penetrating a host. They provide nutrients to the entomopathogenic nematodes, produce antibiotics that inhibit competing microbes, kill the host through septicemia and cause bioconversion of the host into a nutrient soup that is ideal for nematode development. Both entomopathogenic nematode and their associate bacterial symbionts are non-pathogenic to warm- blooded vertebrates, animals and human (Boemare *et al*., 1996) [3]. Biological control of pests using entomopathogenic nematodes is an ideal alternative, is economical, and has long term control, without risk to non-target organisms. The EPNs are potential agents as they serve as vectors of bacteria, achieve a quick kill of target insect pests, have a broad host range, highly virulent, possess chemoreceptor, can be cultured easily *in vitro*, have a numerical but no functional response, are safe to vertebrates, plants and non-targets, have been exempted from registration in USA, are easily applied using standard application equipment, are compatible with many chemical pesticides, and are amenable to genetic selection (Kaya and Gaugler, 1993) [7]. This intense interest is a function of the impressive attributes of these beneficial nematodes that include ease of mass production, ease of application, host specificity, high lethality and safety to non-target organisms. This research would help to determine in what situations entomopathogenic nematodes can be used and provide some insight into their effectiveness in various circumstances.

Materials and Methods

Collection and rearing of test insect

The laboratory hosts *C. cephalonica* and *Galleria mellonella* were reared in the Bio-control laboratory of the Entomology Section. The 5thinstar larvae of these two insects were used as host for EPN.

Rearing of *Corcyra cephalonica*

To obtain the larva of *Corcyra cephalonica* throughout the experimental period, rearing of rice moth was done in the laboratory. The culture was maintained on Sorghum based artificial diet with following ingredients for one tray (15x30 cm).

1. Crushed Sorghum grain (2.5 kg)
2. Groundnut kernel powder (100 g to enrich the diet with protein)
3. Yeast powder (5 g)
4. Wettable Sulphur 80% (5 g as a prophylactic measure against mites)
5. Streptomycin sulphate 0.05% (15 ml/tub)

Sorghum grain free from any infestation and infection were ground to make 3-4 pieces of each grain and heat sterilized in hot air oven at 100 ºC for 30 min. The various ingredients listed above were added and mixed well to obtain a homogenous mixture. The mixture was sprayed with 0.05% streptomycin sulphate. Wooden trays (15x30 cm) were used as rearing trays for these insects. Each tray was filled with 2.5 kg of diet material. These were inoculated with nucleus egg culture of *Corcyra cephalonica* (0.5 cc /tray) and secured with tight lids having fine mesh at the center. They were also covered with muslin cloth as precautionary measures for preventing the entry of any foreign insect in the culture.

The moths started emerging after 40 days of inoculation. They were collected daily by opening the trays with the help of specimen tubes and transferred in the mating chambers which were provided with 10% honey solutions as diet.

Rearing of *Galleria mellonella*

The greater wax moth, *Galleria mellonella* Larvae, was collected from BTC College of Agriculture and Research Station, Bilaspur, Chhattisgarh, India and these larvae were fed with diet by Singh 1994 [10].

Diet ingredients: Wheat flour: 350gm Maize flour: 200gm Milk powder: 130gm Yeast powder: 70gm Glycerin: 150ml Honey: 100ml

Diet prepared by well mixing of all these ingredients in a container. These diets were used to fed to neonates to 5th instars larvae. The adults were kept in Plastic jars size of 5cm x 30cm and cover with tissue paper for egg laying and fed with honey solution with the help of cotton swab. Female adults were laying eggs on the tissue paper, every 24 hours eggs were collected. These clusters of eggs were placed in small jars size of 5cmx 15cm and incubated at (28±20C, RH 70±5% for 24 hours) after adding 5 gm freshly prepared diet with different composition. Neonates were emerged after eggs hatched in 3-4 days, these neonates were developed in to 1stInstars, 2nd Instar, 3rd Instar, 4th Instar and finally 5th Instar larvae, in these larval stages also added 20 to 35 gm freshly prepared diet after consumed previous diet. Fully developed 5th Instars Larvae were collected and kept in to other plastic jars for adult formation. All 5th Instars Larvae were made cocoon developed in to adult stage. After adult formation one pair of male & female adults were separated and kept into other plastic jar to examine number of eggs laying by single female.

Pathogenecity of EPN Isolates

In order to know the infectivity and pathogenecity of all the EPN isolates were inoculated against 5th instar larvae of *C. cephalonica* and *G. mellonella* under similar set of laboratory conditions. Filter paper impregnation method was use by exposing host insects to nematode impregnated filter paper at the bottom of rearing tray. EPN juveniles at the rate of 0 (Untreated check), 5, 10, 15, 20, 25, 30 and 50 Infective Juveniles in 20µl for each dose were distributed over the filter paper and individual larvae of 5th instars of *C. cephalonica* and *G. mellonella* were put in nematode inoculated cell of insect rearing trays. Mortality was checked 24 and 48 hours after inoculation. The experiments were conducted in completely randomized design with 3 replications 8 treatments and the data so obtained was analyzed by standard statistical procedures. The percent mortality in each treatment was calculated and corrected by Abbott's formula (Abbott 1925) [1].

$$\text{Mortality (\%)} = \frac{t-c}{c} \times 100$$

Where,

t is the percent mortality in the treatments and c is the percent mortality in the control

Results and Discussion

Pathogenecity of EPN isolates against *Corcyra cephalonica* larvae

The results depicted in Table 1 revealed that the all four EPN isolates showed remarkable pathogenecity against 5th instar larvae of *C. cephalonica* in laboratory condition. All the treatment concentrations prepared showed significantly high mortality than control against 5th instar larvae. The highest mortalities observed in EPN sample CICR-Brown in the treatment concentration of 50IJs/20µl which were 94.28%, in 30IJs/20µl were 82.85% and 74.28%, 74.28%, 68.57%, 60% and 57.14% in the treatment concentration 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. However, in case of CICR-white sample the highest mortality was observed in 50IJs/20µl that was 68.57% and 65.71%, 62.85%, 62.85%, 60%, 57.14%, 54.28% in the treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. And in case of PKV-1 sample the highest mortality observed was 85.71% in the treatment concentration of 50IJs/20µl and 74.28%, 65.71%, 65.71%, 65.71%, 60%, 60% were observed in the concentrations of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. And EPN sample Sample-4 showed highest mortality at 50IJs/20 µl which was 80% and 71.42%, 68.57%, 65.71%, 51.42%, 51.42%, and 45.71% were observed in concentration treatments of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. Among all the samples CICR-Brown sample of EPN showed the highest mortality.

Table 1: Pathogenecity of EPN isolates against *Corcyra cephalonica* larvae

Concentration of EPN isolate used	Larval mortality of *C. cephalonica* (%)			
	***Heterorhabditis spp.* (PKV-1)**	***Hetrorhabditis spp.* (CICR Br)**	***Steinernema spp.* (CICR- White)**	***Heterorhabditis spp.* (PKV- Guava)**
5 IJs/20µl	60 (50.75)	57.14 (49.09)	54.28 (47.44)	45.71 (42.52)
10 IJs/20µl	60 (50.75)	60 (50.76)	57.14 (49.09)	51.42 (45.8)
15IJs/20µl	65.71 (54.14)	68.57 (55.88)	60 (50.77)	51.42 (45.8)
20IJs/20µl	65.71 (54.14)	74.28 (59.5)	62.85 (52.43)	65.71 (54.14)
25 IJs/20µl	65.71 (54.14)	74.28 (59.5)	62.85 (52.43)	68.57 (55.89)
30 IJs/20µl	74.28 (59.5)	82.85 (65.51)	65.71 (54.14)	71.42 (57.67)
50 IJs/20µl	85.71 (67.77)	94.28 (76.13)	68.57 (55.88)	80 (63.45)
Control (DW)	2.85 (9.715)	2.85 (9.715)	2.85 (9.715)	2.85 (9.715)
SE(m)	0.638	0.258	0.493	0.697
C.D.	1.953	0.789	1.51	2.135
C.V.	2.845	1.19	2.696	3.695

(Figures in the parenthesis are Arc sin transformed values)

Pathogenecity of EPN isolates against *Galleria mellonella*

The results depicted in Table 2 revealed that the all four EPN isolates Showed remarkable pathogenecity against 5th instar larvae of *Galleria mellonella* in laboratory condition. All the treatment concentrations prepared showed significantly high mortality than control against 5th instar larvae. The highest mortalities observed in EPN sample CICR-Brown in the treatment concentration of 50IJs/20µl which were 97.14%, and 91.42%, 82.67%, 68.57%, 68.57%, 67.56%, 60.36% mortality were observed in the concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20 µl, 5IJs/20 µl respectively. However, in case of CICR-white sample the highest mortality was observed in 50IJs/20µl that was 68.57% and 65.71%, 63.72%, 62.85%, 59.14% 57.14%, 54.72% in the treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. And in case of PKV-1 sample the highest mortality observed was 82.85% in the treatment concentration of 50 IJs/20µl and 82.18%, 74.95%, 74.28%, 65.71%, 63.41%, 57.47% were observed in the concentrations of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. And EPN sample Sample-4 showed highest mortality at 50IJs/20µl which was 85.71% and 82.85%, 73.78%, 68.57%, 53.72%, 45.71% and 43.62% were observed in concentration treatments of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. Among all the isolates CICR-Brown sample of EPN showed the highest mortality.

Table 2: Pathogenecity of EPN isolates against *Galleria mellonella*

Concentration of EPN isolate used	Larval mortality of *G. mellonella* (%)			
	***Heterorhabditis spp.* (PKV-1)**	***Heterorhabditis spp.* (CICR -Br)**	***Steinernema spp.* (CICR- White)**	***Heterorhabditis spp.* (PKV- Guava)**
5IJs/20µl	57.47 (49.28)	60.36 (50.96)	54.72 (47.69)	43.62 (41.31)
10IJs/20µl	63.41 (52.76)	68.57 (55.88)	57.14 (49.09)	45.71 (42.52)
15IJs/20µl	65.71 (54.14)	67.56 (55.6)	59.14 (50.24)	53.72 (47.11)
20IJs/20µl	74.28 (59.51)	68.57 (55.88)	62.85 (52.43)	68.57 (55.88)
25IJs/20µl	74.95 (59.94)	82.67 (65.39)	63.72 (52.95)	73.78 (59.18)
30IJs/20µl	82.18 (65.01)	91.42 (72.93)	65.71 (54.14)	82.85 (65.52)
50IJs/20µl	82.85 (65.53)	97.14 (80.7)	68.57 (55.88)	85.71 (67.78)
Control (distilled sterile water)	5.71 (13.82)	5.71 (13.82)	5.71 (13.82)	5.71 (13.82)
SE(m)	0.667	0.853	0.606	0.716
C.D.	2.044	2.614	1.854	2.192
C.V.	2.638	1.964	3.231	3.203

(Figures in the parenthesis are Arc sin transformed values)

Discussion

Present findings are in corroboration with Kalia *et al.,* (2014) [5] who also reportedthat LC_{50} against *Helicoverpa armigera* was 54.68 IJs/larva, *Spodoptera litura* was 85 IJs/larva and *Galleria mellonella* was 16.88 IJs/larva for *Steinernema thermophilum* along with ovicidal virulence up to 84 percent. Kalia *et al.,* (2014) [5] tested laboratory the biocontrol potential of *Steinernema thermophilum* against eggs and larval stages of *Helicoverpa armigera* and *Spodoptera litura*, as well as *Galleria mellonella* (used as a model host). In terms of host susceptibility of lepidopteran larvae to *S. thermophilum*, based on the LC_{50} 36 hour after treatment, *G. mellonella* (LC_{50} = 16.28 IJ/larva) was found to be more susceptible than *S. litura* (LC_{50} = 85 IJ/larva), whereas neither host was found to be significantly different from *H. armigera* (LC_{50} = 54.68 IJs/larva). In addition to virulence to the larval stages, ovicidal activity up to 84% was observed at 200 IJs/50 and 100 eggs of *H. armigera* and *S. litura*, respectively. Andalo *et al.,* (2010) [2] confirms the studies carried out during the present findings where he has evaluated *Heterorhabditis spp* and *Steinernema spp.* against *Spodoptera frugiperda* and found virulent. Salvadori *et al.,* (2012) [9] from Brazil and Caccia *et al.,* (2013) [4] confirms the potentially effectiveness of EPN against *Spodoptera frugiperda* in their studies, which is in line with the findings of the present studies. The variation in pathogenecity of EPN to different stages of insects have been reported by different workers as well. Remarkable differences in the pathogenecity of EPN isolates against *C. cephalonica* and *G. mellonella* larvae were also noticed in the present investigations.

Conclusion

Pathogenecity of Entomopathogenic nematode isolates against *Corcyra cephalonica* and larvae *G. mellonella*

revealed that among all the isolates *Heterorhabditis spp*. (CICR-Brown) sample of EPN showed the highest mortality.

References

1. Abbott WS. A method for computing the effectiveness of an insecticide. Journal of Economy Entomology. 1925;18:265-267.
2. Andalo V, Santos V, Furtado Moreira G, Costa Moreira C, Moino JA. Evaluation of entomopathogenic nematodes under laboratory and greenhouses conditions for the control of *Spodoptera frugiperda.* Ciencia Rural. 2010;40:1860-1866.
3. Boemare NC, Laumond, Mauleon H. The entomopathogenic Nematode-bacterium Complex: Biology life and vertebrate safety. Biocontrol Sci. Tech. 1996;6(3):333- 346.wqa1212.
4. Caccia MG, Valle ED, Dauset ME. Susceptibility of *S. frugiperda, H. gelotopoeon* to EPN *Steinernema diaprepesi* under laboratory conditions. Chilean J Agril. Research. 2014;74(1):123-126.
5. Kalia, VG, Sharma, Shapiro-IIan DJ, Ganguly S. Biocontrol potential of *Steinernema thermophilum* and its symbiont *Xenorhabdus indica* against lepidopteran pests; Virulence to egg and larval stages. J Nematology. 2014;46(1):18-26.
6. Kaya HK, Aguillera MM, Alfred A, Choo HY, Torre MD, Fodor A, *et al*. Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. Biological Control. 2006;38:134-155.
7. Kaya HK, Gaugler R. Entomopathogenic nematodes. Annual Review of Entomology. 1993;38:181-206.
8. Koppenhoper AM, Grewal PS, Fuzy EM. Differences in penetration routesand establishment rates of four entomopathogenic nematode species into four white grub species. Journal of Invertebrate Pathology. 2007;94:184-195.
9. Salvadori JDM, *et al.* Characterization of EPN and symbiotic bacteria active against *S. frugiperda* and contribution of bacterial urease to the insecticidal effect. Biological Control. 2012;63(2012):253-263.
10. Singh SP. Technology for production of natural enemies. Project Directorate of Biological Control, Bangalore, India. Tech. Bulletin, No, 1994, 221(4).