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In vivo studies on biotic potential of EPN isolates in rice meal moth and greater wax moth

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Abstract
The highest emergence of infective juveniles observed from EPN sample CICR-Brown in the treatment concentration of 50IJs/20 µl which were 6381×10^2 from 30IJs/20µl were 5722×10^2 and 4943.5×10^2, 4729×10^2, 4416×10^2, 4066×10^2 and 4031×10^2 from 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively. However, from EPN sample PKV-1 the highest emergence of infective juveniles observed from the treatment concentration of 50 IJs/20µl were 4029×10^2, and 3947×10^2,3679×10^2,3566×10^2, 3382×10^2, 3136×10^2 and 2762×10^2 from 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively. The highest population of infective juveniles observed from EPN sample CICR-Brown from the treatment concentration of 50IJs/20 µl which were 6477.50×10^2 and from 30IJs/20µl were 6234.5×10^2, 6212.5×10^2, and 6125.5×10^2, 5694×10^2, 5566×10^2 and 3864×10^2 from 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively. However, from EPN sample PKV-1 the highest multiplication of infective juveniles observed at the treatment concentration of 50 IJs/20µl were 5064.5 and 4979×10^2,4943×10^2, 4420×10^2, 3964×10^2, 3873×10^2, 3521×10^2 from 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively.

Keywords: entomopathogenic nematodes, juveniles, CICR-brown, PKV-1, emergence

Introduction
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) [5]. 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. Nematodes that parasitize insects, known as entomopathogenic nematodes (EPNs), have been described from 23 nematode families (Koppenhofer, 2007) [6]. 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) [4]. 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 septicaemia 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) [2]. Biological control of pests using entomopathogenic nematodes is an ideal alternative, is economical, and has long term control, without risk to non-target organisms.

Materials and Methods
In order to get pure culture of the respective EPN isolates they were re-infected to the larvae of *C. cephalonica* and *Galleria mellonella* at ambient laboratory conditions. Initially measured amounts of suspension with standard count of IJs/20 µl from the respective EPN isolates were

taken and larvae of *C. cephalonica* and *Galleria mellonella* were inoculated by direct contact method. After the interval of 10-12 days the nematode suspension with infective juveniles from all four isolates were collected and re-infected to fresh *C. cephalonica* and *Galleria mellonella* larvae and this process of inoculation and re-infection of nematode suspension to larvae of *C. cephalonica* repeated until the pure culture of nematode populations with infective juveniles were obtained. *Galleria mellonella* larvae resulted better than *Corcyra cephalonica* for the multiplication. These pure cultures were used for preparation of different doses / concentration for treatments for further studies. The standard IJ counts were prepared by using double distilled water.

Reproduction of EPNs on *C. cephalonica*

4th instar larva of *C. cephalonica* were exposed to 5, 10, 15, 20, 25, 30 and 50 IJs /larvae concentration of each EPN isolates (Yadav and Lalramliana 2012) [9] in separate rearing tray and total number of IJs produced /larva upto a period of 20 days was counted. The nematode infected dead larvae were removed from tray, and transferred individually on to white trap for their emergence from the body (White, 1927) [8]. Larvae were collected daily for upto a period of 20 days till the emergence of IJs will stop from insect cadavers and total number of IJs /larva was determined.

Reproduction of EPNs on *Galleria mellonella*

5th instar larva of *Galleria mellonella* were exposed to 5, 10, 15, 20, 25, 30 and 50 IJs /larvae concentration of each EPN isolates (Yadav and Lalramliana 2012) [9] in separate rearing tray and total number of IJs produced /larva up to a period of 20 days were counted. The nematode infected dead larvae were removed from tray, and transferred individually on to white trap for their emergence from the body (White, 1927) [8]. Larvae were collected daily for upto a period of 20 days till the emergence of IJs will stop from insect cadavers and total number of IJs /larva was determined.

The experiments were conducted in completely randomized design with 3 replications 7 treatments and the data so obtained was analyzed by standard statistical procedures.

Results and Discussion

Reproduction of EPN Isolates

The reproduction / multiplicity of all four EPN isolatesPKV-1, CICR-Brown, CICR-White, Sample-4 EPN isolates on 5th instar larvae of *Corcyra cephalonica, Galleria mellonella* and *Spodoptera frugiperda* were studied in laboratory under ambient conditions of temperature and humidity and the results have been depicted in following tables under respective subheadings.

Reproduction on *Corcyra cephalonica* larvae

The result depicted in table 1 revealed the reproduction and recovery of infective juveniles obtained from mature instar larvae of *C. cephalonica*. The highest emergence of infective juveniles observed from EPN sample CICR-Brown in the treatment concentration of 50IJs/20 µl which were $6381\times10^2$from 30IJs/20µl were 5722×10^2,and 4943.5×10^2, 4729×10^2, 4416×10^2, 4066×10^2 and $4031\times10^2$from 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively. However, from EPN sample PKV-1 the highest emergence of infective juveniles observed from the treatment concentration of 50 IJs/20µl were 4029×10^2, and3947×10^2,3679×10^2,3566×10^2, 3382×10^2, 3136×10^2 and 2762×10^2 from 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively and in case of CICR-White highest multiplication of IJs observed at 50IJs/20µl were $3067\times10^2$and 3436.5×10^2, 3305×10^2, 3294×10^2, 2914×10^2, 2873×10^2 and $2762\times10^2$from treatment concentration 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively. And EPN isolate PKV-1 showed highest rate of emergence of EPN at treatment concentration 50IJs/20µl were 3829×10^2, and, 3781×10^2, 3621.5×10^2, 3543.5×10^2, 3521.5×10^2, 3166×10^2 and 2690×10^2 from treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively.

Table 1: Reproduction of EPNs on *Corcyra cephalonica* larvae

Concentration of EPN isolate	Emergence of Infective Juveniles emerged from single larvae×10^2			
	PKV-1	CICR-Brown	CICR-White	PKV-Guava
5IJs/20µl	2762(52.559)*	4031(63.495)	2762.5(52.563)	2690(51.869)
10Js/20µl	3136(56.004)	4066(63.77)	2873.5(53.609)	3166(56.271)
15IJs/20µl	3382(58.159)	4416(66.458)	2914(53.985)	3521.5(59.347)
20IJs/20µl	3566(59.72)	4729(68.772)	3294(57.398)	3543.5(59.532)
25IJs/20µl	3679(60.659)	4943.5(70.315)	3305(57.493)	3621.5(60.183)
30IJs/20µl	3947(62.83)	5722(75.649)	3436.5(58.626)	3781(61.494)
50IJs/20µl	4029(63.479)	6381(79.886)	3607(60.063)	3829(61.883)
SE(m)	1.635	1.389	1.565	1.65
C.D.	0.525	0.631	0.502	0.53

*Figures in the parenthesis are Square root transformed values

Reproduction on *Galleria mellonella* larvae

The result depicted in table 2 revealed the reproduction and recovery of infective juveniles obtained from mature instar larvae of *G. mellonella*. The highest population of infective juveniles observed from EPN sample CICR-Brown from the treatment concentration of 50IJs/20 µl which were $6477.50\times10^2$and from 30IJs/20µl were 6234.5×10^2, 6212.5×10^2, 6125.5×10^2, 5694×10^2, 5566×10^2, $3864\times10^2$from 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively. However, from EPN sample PKV-1 the highest multiplication of infective juveniles observed at the treatment concentration of 50 IJs/20µl were 5064.5, and4979×10^2,4943×10^2, 4420×10^2,3964×10^2, 3873×10^2, $3521\times10^2$from 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively. And in case of CICR-White highest emergence of infective juveniles was observed when the larvae treated at concentration 50IJs/20µl were 3964.5×10^2, 3483×10^2, 3333.5×10^2, 3010×10^2, 2916×10^2, 2873×10^2 and $2864\times10^2$from treatment concentration 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively. And EPN sample PKV-Guava showed highest rate of multiplication of EPN at treatment

concentration 50IJs/20µl were 3917.5×10^2, and 3873.5×10^2, 3673.5×10^2, 3581×10^2, 3549.5×10^2, 3357×10^2, and 2744×10^2 from treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively.

Among all the four EPNs isolates *Heterorhabditis spp* (CICR-Brown) having the highest rate of emergence of infective juveniles.

Table 2: Reproduction of EPNs on *Galleria mellonella* larvae

Concentration of EPN isolate	Emergence of Infective Juveniles emerged from single larvae×10^2			
	PKV-1	CICR-Brown	CICR-White	PKV-Guava
5IJs/20µl	3521.5(59.347)	3964.00(62.965)	2864.00(53.52)	2744(52.387)
10J_s/20µl	3873.5(62.242)	5566(74.61)	2873.5(53.609)	3357(57.944)
15IJ$_s$/20µl	3964(62.965)	5694(75.463)	2916(54.004)	3549.5(59.582)
20IJ$_s$/20µl	4420(66.488)	6125.5(78.272)	3010.5(54.872)	3581(59.846)
25IJ$_s$/20µl	4943.5(70.315)	6212.5(78.824)	3333.5(57.741)	3673.5(60.614)
30IJ$_s$/20µl	4979(70.567)	6234.5(78.963)	3483(59.021)	3873.5(62.242)
50IJs/20µl	5064.5(71.17)	6477.5(80.488)	3964.5(62.969)	3917.5(62.595)
S.E.(m)	0.471	0.393	0.547	0.475
C.D.	1.468	1.223	1.703	1.479

(Figures in the bracket are Square root transformation)

Kary *et al.*, (2012) [3], Vasisth *et al.*, (2013) [7], Atwa *et al.*, (2013) [1] studied various species of two EPN, (*Heterorhabditis spp* and *Steinernema spp.*) against different insects and their findings are in confirmation with the results of present studies carried out in Nagpur region of Maharashtra state.

Conclusion

Among all the four EPNs isolates *Heterorhabditis spp* (CICR-Brown) having the highest rate of emergence of infective juveniles. Rate of multiplication of various isolates of entomopathogenic nematode is more in greater wax moth as compared to rice meal moth.

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