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Dadhe CG
Section of Agricultural Entomology, College of Agriculture, Nagpur, Dr. PDKV, Akola, Maharashtra, India

Lavhe NV
1Assistant Professor, Section of Agricultural Entomology, College of Agriculture, Nagpur, Dr. PDKV, Akola, Maharashtra, India

Borkar SL
Assistant Professor, Section of Agricultural Entomology, College of Agriculture, Nagpur, Dr. PDKV, Akola, Maharashtra, India

Bhoyar ES
Section of Agricultural Entomology, College of Agriculture, Nagpur, Dr. PDKV, Akola, Maharashtra, India

Corresponding Author:
Dadhe CG
Section of Agricultural Entomology, College of Agriculture, Nagpur, Dr. PDKV, Akola, Maharashtra, India

Bio-efficacy of different EPN isolates against fall armyworm, *Spodoptera frugiperda* S.L. Smith

Dadhe CG, Lavhe NV, Borkar SL and Bhoyar ES

Abstract
The overall results on bio-efficacy of different isolates EPN tested, *Heterorhabditis spp*. (CICR-Brown) isolate showed high infectivity and mortality of different instars of *S. frugiperda*. 2nd instar larvae showed highest mortality 91.42% when it was treated with the concentration 50IJs/20µl. However, in case of 3rd instar larvae of *S. frugiperda* the highest larval mortality observed was 97.14% from treatment concentration and 50IJs/20µl. Whereas 4th instar larvae showed highest mortality 92.42% when it was treated with the concentration of 50IJs/20µl in case of 5th instar larvae, the highest mortality observed was 82.85% when it was treated with treatment concentration 50IJs/20µl. However, the 6th instar larvae showed highest mortality 85.71% at treatment concentration 50IJs/20µl. *Heterorhabditis spp*. (PKV-1) and Steinernema spp. (CICR-White) showed highest mortality 85.71% at treatment concentration 50IJs/20µl in case of 2nd and 3rd instar larvae and highest mortality of 74.28% was observed in 50IJs/20µl concentration of *Heterorhabditis spp*. (PKV-Guava).

Keywords: Bio-efficacy, isolates, EPN, mortality, *Heterorhabditis*, fall armyworm, instars

Introduction
The fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) [14] (Lepidoptera: Noctuidae) is native to the tropical region of the western hemisphere from the United States to Argentina considered as a most important pest of corn in Brazil, after making its way to Africa in 2016, it now appears to have found its home in India. First infestation of this pest in India is found on maize crop in Karnataka July 2018. The voracious pest, known to devastate a one-acre field in a week, could endanger the agricultural output of India. The insect "has not only invaded the maize crop in Maharashtra, the area adjoining the borders of Odisha and Chhattisgarh, West Bengal and Gujarat but also sorghum and other millet crops in Telangana and the northern part of Karnataka (Chormule *et al*., 2019) [7].

Nematodes that parasitize insects, known as entomopathogenic nematodes (EPNs), have been described from 23 nematode families (Koppenhofer, 2007) [12]. 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) [10]. Both entomopathogenic nematode and their associate bacterial symbionts are non-pathogenic to warm-blooded vertebrates, animals and human (Boemare, *et al.*, 1996) [5]. 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) [11]. 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 Method
Collection and rearing of test insect
Larvae of *Spodoptera frugiperda* were collected from field and reared in the laboratory of Insectary premises of Entomology Section. The *S. frugiperda* were reared in the laboratory conditions on castor leaves.

Initial culture as egg mass/larvae were collected from the infested crop and reared on healthy castor leaves. The leaves were kept in the plastic container containing moist filter paper to keep it fresh. These were served as immediate source of food for the first instar larvae. Leaves along with the egg mass were transferred to pre sterilized transparent plastic containers and covered with muslin cloth. The leaves were changed when the larvae enter into the third instar. There after fresh castor leaves were given every day till the larvae enter into the last instar larval stage. These late larval instars were collected from containers and will be released in to another plastic container having saw dust for pupation. Pupae thus obtained collected and kept in small plastic jars covered with muslin cloth.

Bioefficacy of different EPN isolates against *Spodoptera frugiperda*

In order to study the infectivity and bioefficacy of isolated samples against fall armyworm, all the instars of the larva of *Spodoptera frugiperda* (excluding 1st instar) were exposed to 0 (Untreated check), 5, 10, 15, 20, 25, 30 and 50 IJs /larvae concentration of each EPN isolates *Heterorhabditis spp*. (PKV-1), *Heterorhabditis spp.* (CICR-Brown), *Steinernema spp*. (CICR- White), and *Heterorhabditis spp.* (PKV-Guava) (Yadav and Lalramliana, 2012) [16]. Mortality was observed regularly up to 20 days from the day of inoculation of isolates. The EPN isolates were inoculated against all instars of *Spodoptera frugiperda* (excluding 1st instar) under similar set of conditions.

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 isolate *Heterorhabditids spp.* (PKV-1) against *S. frugiperda*

The results depicted in table 1 revealed that the EPN isolate *Heterorhabditis spp*. (PKV-1) showed remarkable pathogenecity against all instars larvae of *S. frugiperda* in laboratory condition. All the treatment concentrations prepared showed significantly high mortality than control against all instar larvae. 2nd instar larvae showed highest mortality 85.71% when it was treated with the concentration 50IJs/20µl after which 80%, 80%, 80%, 68.57%, 65.71% and 37.14% mortality observed from 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. However, in case of 3rd instar *S. frugiperda* the highest larval mortality observed was 85.71% at two treatment concentration 30IJs/20 µl and 50IJs/20µl and 74.7%, 68.57%, 64.17%, 60%, 43.23% mortality was observed at treatment concentration 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. Whereas 4th instar larvae showed highest mortality 82.85% when it was treated with the concentration of 50IJs/20µl and 77.14%, 73.37%, 68.57%, 55.09%, 57.14% and 50.14% mortality at treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. In case of 5th instar larvae, the highest mortality observed was 77.14% when it was treated with treatment concentration 50IJs/20µl and 73.37%, 68.67%, 62.85%, 52.23%, 54.28%, 45.27% mortality was observed at treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. However, the 6th instar larvae showed highest mortality 67.14% at treatment concentration 50IJs/20µl and 60%, 56.92%, 57.14%, 50.23%, 51,14%, 37.14% mortality was observed from treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. 2nd and 3rd instar larvae showed highest susceptibility against the EPN isolate *Heterorhabditis spp*. (PKV-1) than 4th, 5th and 6th instar larvae.

Table 1: Pathogenecity of EPN isolate *Heterorhabditids spp* (PKV1) against *S. frugiperda*

Treatment concentration	Larval mortality of *S. frugiperda (%)*				
	2nd instar	**3rd instar**	**4th instar**	**5th instar**	**6th instar**
5IJs/20µl	37.14 (37.52)	43.23 (41.09)	50.14 (45.05)	45.27 (42.26)	37.14 (37.52)
10IJs/20µl	65.71 (54.14)	60 (50.76)	57.14 (49.08)	54.28 (47.23)	51.14 (45.63)
15IJs/20µl	68.57 (55.88)	64.17 (53.21)	55.09 (47.90)	52.23 (46.26)	50.23 (45.11)
20IJs/20µl	80 (63.42)	68.57 (55.88)	68.57 (55.88)	62.85 (52.42)	57.14 (49.08)
25IJs/20µl	80 (63.42)	74.7 (59.57)	73.37 (58.91)	68.67 (55.88)	56.92 (48.96)
30IJs/20µl	80 (63.42)	85.71 (67.77)	77.14 (61.42)	73.37 (58.91)	60 (50.75)
50IJs/20µl	85.71 (67.83)	85.71 (67.77)	82.85 (65.53)	77.14 (61.42)	77.14 (61.42)
Control (distilled sterile water)	5.71 (13.81)	5.71 (13.81)	5.7 (13.8)	2.85 (9.71)	2.85 (9.715)
SE(m)	0.949	0.812	0.729	0.651	0.671
C.D.	2.908	2.486	2.234	1.994	1.889
C.V.	3.682	3.508	2.542	3.41	2.501

(Figures in the parenthesis are Arc sin transformed values)

Pathogenecity of EPN isolate *Heterorhabditids spp* (CICR-Brown) against *S. frugiperda*

The results depicted in table 2 revealed that the EPN isolate *Heterorhabditis spp.* (CICR-Brown) showed remarkable pathogenecity against all instars larvae of *S. frugiperda* in laboratory condition. All the treatment concentrations prepared showed significantly high mortality than control against all instar larvae. 2nd instar larvae showed highest mortality 91.42% when it was treated with the concentration 50IJs/20µl after which 77.14%, 74.37%, 71.42%, 69.18%, 68.57% and 44.32% mortality observed from treatment concentration 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. However, in case of 3rd instar *S. frugiperda* the highest larval mortality observed was 97.14% from treatment concentration and 50IJs/20µl and 91.42%, 89.72%, 85.71%, 83.17%, 77.14% and 62.54%

mortality were observed at treatment concentration 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively. Whereas 4th instar larvae showed highest mortality 92.42% when it was treated with the concentration of 50IJs/20µl and 78.14%, 75.37%, 70.42%, 68.18% and 71.57%, mortality at treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. In case of 5th instar larvae, the highest mortality observed was 82.85% when it was treated with treatment concentration 50IJs/20µl and 77.14%, 73.27%, 74.28%, 70.14%, 68.57%, 56.15%, mortality was observed at treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. However, the 6th instar larvae showed highest mortality 85.71% at treatment concentration 50IJs/20µl and 82.85%, 78.28%,71.42%, 70.15%, 66.57%, 62.65% mortality was observed from treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. 2nd, 3rd and 4th, instar larvae showed highest susceptibility against the EPN isolate *Heterorhabditis spp.* (PKV-1) than 5th and 6th instar larvae.

Table 2: Pathogenecity of EPN isolate *Heterorhabditis spp.* (CICR-Brown) against *S. frugiperda*

Treatment concentration	Larval mortality of *S. frugiperda*				
	2nd instar	3rd instar	4th instar	5th instar	6th instar
5IJs/20µl	44.32 (41.72)	62.54 (52.24)	62.65 (52.31)	56.15 (48.51)	47.23 (43.39)
10IJs/20µl	68.57 (55.88)	77.14 (61.42)	71.57 (57.76)	68.57 (55.88)	66.57 (54.66)
15IJs/20µl	69.18 (56.26)	83.17 (65.77)	70.15 (56.868)	70.14 (56.86)	68.18 (55.64)
20IJs/20µl	71.42 (57.66)	85.71 (67.77)	74.28 (59.513)	70.42 (57.03)	71.42 (57.66)
25IJs/20µl	74.37 (59.58)	89.72 (71.32)	78.28 (62.21)	75.37 (58.85)	73.27 (58.85)
30IJs/20µl	77.14 (61.42)	91.42 (73.1)	82.85 (65.53)	77.14 (61.42)	78.14 (65.53)
50IJs/20µl	91.42 (73.1)	97.14 (80.7)	92.42 (74.07)	82.85 (65.53)	85.71 (67.79)
Control (DW)	8.57 (17.01)	5.7 (13.8)	8.57 (17.01)	2.85 (9.71)	5.71 (13.81)
SE(m)	0.732	1.239	0.811	0.747	0.283
C.D.	2.242	3.793	2.483	2.289	0.865
C.V.	2.678	2.576	2.576	2.502	0.943

(Figures in the parenthesis are Arc sin transformed values)

Pathogenecity of EPN isolate *Steinernema spp.* (CICR-White) against *S. frugiperda*

The results depicted in table 3 revealed that the EPN isolate *Steinernema spp.* (CICR-White) showed remarkable pathogenecity against all instars larvae of *S. frugiperda* in laboratory condition. All the treatment concentrations prepared showed significantly high mortality than control against all instar larvae.

2nd instar larvae showed highest mortality 91.42% when it was treated with the concentration 50IJs/20µl after which 77.14%, 50.09%, 51.42%, 42.37%, 37.14% and 33.14% mortality observed from treatment concentration 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. However, in case of 3rd instar *S. frugiperda* the highest larval mortality observed was 68.57% from treatment concentration and 50IJs/20µl and 68.57%, 64.32%, 57.14%, 56.82%, 57.14%, and 42.73% mortality was observed at treatment concentration.

30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively. Whereas 4th instar larvae showed highest mortality 67.57% when it was treated with the concentration of 50IJs/20µl and 66.57%, 62.32%, 70.42%, 56.14%, 53.82%, 55.14%, 40.73 mortality at treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. In case of 5th instar larvae, the highest mortality observed was 57.14% when it was treated with treatment concentration 50IJs/20µl and 54.28%, 51.23%, 48.57%, 38.27%, 31.42%, 30.87%, mortality was observed at treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20 µl, 5IJs/20 µl respectively. However, the 6th instar larvae showed highest mortality 48.57% at treatment concentration 50IJs/20µl and 42.85%, 42.82%, 37.14%, 37.04%, 31.42%, 31.32% mortality was observed from treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl,15IJs/20µl, 10IJs/20 µl, 5IJs/20 µl respectively. 2nd, 3rd and 4th, instar larvae showed highest susceptibility against the EPN isolate *Heterorhabditis spp.* (PKV-1) than 5th and 6th instar larvae.

Table 3: Pathogenecity of EPN isolate *Steinernema spp.* (CICR-White) against *S. frugiperda*

Treatment concentration	Larval mortality of *S. frugiperda (%)*				
	2nd instar	3rd instar	4th instar	5th instar	6th instar
5IJs/20µl	37.14 (37.52)	43.23 (41.09)	50.14 (45.05)	45.27 (42.26)	37.14 (37.52)
10IJs/20µl	65.71 (54.14)	60 (50.76)	57.14 (49.08)	54.28 (47.23)	51.14 (45.63)
15IJs/20µl	68.57 (55.88)	64.17 (53.21)	55.09 (47.90)	52.23 (46.26)	50.23 (45.11)
20IJs/20µl	80 (63.42)	68.57 (55.88)	68.57 (55.88)	62.85 (52.42)	57.14 (49.08)
25IJs/20µl	80 (63.42)	74.7 (59.57)	73.37 (58.91)	68.67 (55.88)	56.92 (48.96)
30IJs/20µl	80 (63.42)	85.71 (67.77)	77.14 (61.42)	73.37 (58.91)	60 (50.75)
50IJs/20µl	85.71 (67.83)	85.71 (67.77)	82.85 (65.53)	77.14 (61.42)	77.14 (61.42)
Control (DW)	5.71 (13.81)	5.71 (13.81)	5.7 (13.8)	2.85 (9.71)	2.85 (9.715)
SE(m)	0.949	0.812	0.729	0.651	0.671
C.D.	2.908	2.486	2.234	1.994	1.889
C.V.	3.682	3.508	2.542	3.41	2.501

(Figures in the parenthesis are Arc sin transformed values)

Pathogenecity of EPN isolate *Heterorhabditids spp.* (PKV-Guava) against *S. frugiperda*

The results depicted in table 4 revealed that the EPN isolate *Heterorhabditis spp.* (PKV-Guava) showed remarkable pathogenecity against all instars larvae of *S. frugiperda* in laboratory condition. All the treatment concentrations prepared showed significantly high mortality than control against all instar larvae. 2nd instar larvae showed highest mortality 74.28% when it was treated with the concentration 50IJs/20µl after which 60%, 58.92%, 57.14%, 55.62%, 54.28% and 45.72% mortality observed from treatment concentration 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. However, in case of 3rd instar *S. frugiperda* the highest larval mortality observed was 94.28% from treatment concentration and 50IJs/20µl and 88.57%, 74.37%, 68.57%, 52.23%, 57.14%, and 51.48% mortality was observed at treatment concentration 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl and 5IJs/20µl respectively. Whereas 4th instar larvae showed highest mortality 80% when it was treated with the concentration of 50IJs/20µl and 77.14%, 67.37%, 62.85%, 52.23%,56.18%, 51.48 mortality at treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. In case of 5th instar larvae, the highest mortality observed was 77.14% when it was treated with treatment concentration 50IJs/20µl and 60%, 57.92%, 57.14%, 53.09%, 54.28%, 51.78%, mortality was observed at treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20 µl, 5IJs/20 µl respectively. However, the 6th instar larvae showed highest mortality 77.14% at treatment concentration 50IJs/20µl and 68.57%, 62.85%, 54.28%, 53.57%, 51.14%, 50.78% mortality was observed from treatment concentration of 30IJs/20µl, 25IJs/20µl, 20IJs/20µl, 15IJs/20µl, 10IJs/20µl, 5IJs/20µl respectively. 2nd, 3rd and 4th, instar larvae showed highest susceptibility against the EPN isolate *Heterorhabditis spp.* (PKV-1) than 4th and 6th instar larvae.

Table 4: Pathogenecity of EPN isolate *Heterorhabditids spp.* (PKV-Guava) against *S. frugiperda*

Treatment concentration	Larval mortality of *S. frugiperda*				
	2nd instar	3rd instar	4th instar	5th instar	6th instar
5IJs/20µl	45.72 (42.52)	51.48 (45.83)	51.48 (45.83)	51.78 (46.0	50.78 (45.42)
10IJs/20µl	54.28 (47.44)	57.14 (49.08)	56.18 (48.53)	54.28 (47.43)	51.14 (45.63)
15IJs/20µl	55.62 (48.21)	52.23 (46.26)	52.23 (46.26)	53.09 (46.75)	53.57 (47.02)
20IJs/20µl	57.14 (49.08)	68.57 (55.88)	62.85 (52.42)	57.14 (49.08)	54.28 (47.43)
25IJs/20µl	58.92 (50.12)	74.37 (59.58)	67.37 (55.14)	57.92 (49.54)	62.85 (52.42)
30IJs/20µl	60 (50.75)	88.57 (70.25)	77.14 (61.42)	60 (50.75)	68.57 (55.88)
50IJs/20µl	74.28 (59.52)	94.28 (76.27)	80 (63.45)	77.14 (61.42)	77.14 (61.42)
Control (distilled sterile	8.57	8.57	5.71	2.85	5.71
water)	(17.01)	(17.01)	(13.81)	(9.71)	(13.81)
SE(m)	0.84	0.673	0.82	0.677	0.63
C.D.	2.571	2.062	2.512	2.074	1.931
C.V.	4.72	2.582	3.964	3.828	3.456

(Figures in the parenthesis are Arc sin transformed values)

Discussion

Present findings are in corroboration with Andalo *et al*., (2010) [3] who confirms evaluated *Heterorhabditis spp.* and *Steinernema spp.* against *Spodoptera frugiperda* and found virulent. Salvadori *et al.,* (2012) [13] from Brazil and Caccia *et al.,* (2014) [6] confirms the potentially effectiveness of EPN against *Spodoptera frugiperda* in their studies, which is in line with the findings of the present studies. Various authors *viz*. Divya and Sankar (2009) [8], D. Andiroubane *et al.,* (2010) [2], Kary *et al.,* (2012) [9], Vasisth *et al.,* (2013) [15], (Yadav and Lalramliana, 2012), Atwa *et al.,* (2013) [4] 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

In present investigations, among various entomopathogenic isolates, *viz*., *Heterorhabditis spp*. (PKV1), *Heterorhabditis spp.* (CICR-Brown), *Steinernema spp*. (CICR- White), and *Heterorhabditis spp* (PKV-Guava), *Heterorhabditis spp.* (CICR-Brown) was found to be most virulent isolate in checking the population of fall armyworm under laboratory conditions. *Heterorhabditis spp.* (CICR-Brown) also show highest average percent mortality in all the instar (excluding 1st instar) of *S. frugiperda.*

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