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In Vitro Efficacy of Entomopathogenic Nematodes (EPNS) against Economically Important Insect-Pests of Cauliflower

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Abstract— *Cabbage butterfly, Pieris brassicae (Linnaeus), Tobacco caterpillar, Spodoptera litura (Fabricius) and Plusia orichalcea (Fabricius) causes considerable yield loss in economically important crops such as cabbage, cauliflower, cotton, tobacco, castor, and pulses etc. The nation has been using more pesticides to combat these insects, which has increased environmental pollution, pesticide resistance, pest resurgence, and residue in food, soil, and water. The present study was assessed to susceptibility of P. brassicae, S. litura and P. orichalcea to entomopathogenic nematodes (EPNs), Metarhabditis amsactae and mass multiplication of infective juveniles (IJs) in all three insects.* Two strains, *HAR-St-II and HAR-Ht-III of* M. amsactae *were tested against all three insects, at four inoculum levels i.e. 5, 10, 20 and 40 IJs /insect larva, under laboratory conditions at Department of Nematology, CCS Haryana Agricultural University, Hisar during 2021-2022. Results revealed that in both the strains of* M. amsactae, *as the observation time and level of IJs increased, there was a significant increase in per cent mortality of all three insects. Observation on recovery of M. amsactae was less from cadaver of P. orichalcea than P. brassicae and S. litura.*

Keywords— ***Metarhabditis amsactae, Spodoptera litura, Pieris brassicae, Plusia orichalcea, inoculum level, strain, mortality***

I. INTRODUCTION

Entomopathogenic nematodes (EPNs) are those nematodes that kill insects within 24 to 72 hours by inducing septicemia. So, these are important component of biological control of several insect- pests in agricultural crops. They are members of genus *Steinernema*, *Heterorhabditis* and *Rhabditis* (*Oscheius*) comes under the families Steinernematidae, Heterorhabditidae and Rhabditidae. These nematodes have a symbiotic association with insect pathogenic bacteria belonging to the genera *Photorhabdus, Xenorhabdus* and *Serratia* respectively. The third stage juveniles called as infective juveniles (IJs) are stunted, non-feeding, and possess characteristics of both predators and insect parasitoids. They have strong tendency to find their host (insect) and enter its body cavity mostly through places with weak cuticles or natural body apertures. Temperature affects the life cycle and development of EPNs, which varies between species and strains and lasts from the time IJs enter the host until new IJs appear (Hazir *et al*., 2001). In *Galleria mellonella*, it typically takes 6–18 days at a temperature of 18–28 ºC (Nguyen and Smart, 1992).

Over the past few decades, the use of nematodes as biological pest control agents has grown considerably due to their excellent virulence, high reproduction rate, and long-lasting benefits without destroying non-targets organisms (Georgis *et al*., 1991). EPNs are effective and favored biological control agents because of their wide

range of insect hosts, superior seeking capabilities, ease of production in large quantities and application, quick host death, and safety for plants, animals, and other morphological, physiological, and behavioral abnormalities, (Gaugler, 1988; Laznik and Trdan, 2012). When it comes to controlling soil-dwelling insects and plant-boring insects, EPNs are not in conflict with other biocontrol agents. Because they are suited to the local soil and atmospheric conditions, native nematodes are thought to be superior for local biological control efforts. It is crucial to look for regional strains of EPNs, and scientists from different nations are still identifying regional strains from national surveys (Hazir *et al.*, 2003; Malan *et al.*, 2006; Noosidum *et al.*, 2010). It is suggested that novel biological control agents be created using these native nematodes (Ehlers, 2005; Lewis *et al.*, 2006).

II. MATERIALS AND METHODOLOGY

Culture of EPNs: *Metarhabditis amsactae* strain HAR-St-II and HAR-Ht-III were mass cultured under laboratory conditions and pure culture of individual strain was maintained on late instar larvae of *Galleria mellonella* (Plate 1-a and b). These strains were multiplied using the method (Plate 1-c) of Woodring and Kaya (1988). Nematodes were extracted with white traps and stored in a thin layer of distilled water in tissue culture flasks (Plate 1-d) which were kept in BOD at 16 ºC that served as inoculum for further experiments.

Rearing of insects: First and second instar larvae and eggmasses of Tobacco caterpillar, *S. litura* alongwith leaves were collected from the castor crop, Department of Genetics and Plant Breeding. Larvae of semilooper, *P. orichalcea* and cabbage butterfly, *P. brassicae* were collected from the cucumber and cauliflower crop, Department of Nematology. These larvae were reared on cucumber and caster leaves in glass jars under laboratory at room temperature in month of August-September at Department of Nematology (Plate 1-e). Fresh leaves of cucumber and castor were provided to larvae daily in hygienic conditions. The glass jars were covered with muslin cloth and larvae were carefully handled. Sufficient larvae (fourth instar) of cabbage butterfly, *P. brassicae* were available in field area, Department of Nematology, were collected. These larvae were used for check the pathogenecity of both strains of *M. amsactae*.

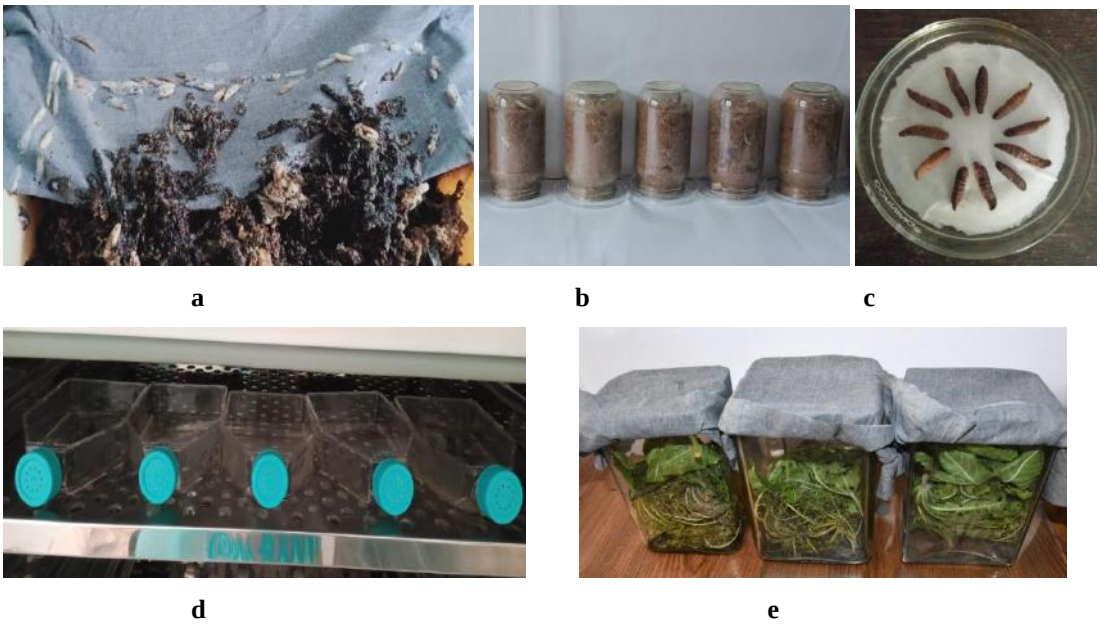


Plate 1. Larvae of Galleria mellonella cultured in wax comb (a), white trap method (b), soil baiting technique (c), EPNs stored in tissue culture flask (d) and rearing of Spodoptera litura larvae (e).

Efficacy of EPNs against insect- pests of cauliflower: The present study was conducted in Petri plates under laboratory condition. The isolated strains of EPNs were tested for virulence on *S. litura*, *P. orichalcea* and *P. brassicae*. To test the pathogenicity of *M. amsactae* on these insects, filter paper was spread into Petri plates and 10 fourth instar larvae of the test insect per Petri plate were released. The EPN suspension consisting of IJs, stored in tissue culture flasks was diluted with a known quantity of sterile distilled water for making the suspension to get the required number of IJs. Insect larva, inoculated with inoculums levels @ 5, 10, 20 and 40 IJs per larvae in 5 ml distilled water. Distilled water without nematodes were taken as untreated check. Each treatment was replicated

four times and kept at room temperature. Observations on the mortality of larvae were recorded at 24 hours interval up to 4 days after inoculation and the per cent mortality was calculated.

Mortality (%) = Number of dead larvae x 100 / Total number of larvae

The recovery of EPNs were recorded from the dead larvae of test insects. EPNs infected dead larvae of *P. brassicae*, *P. orichalcea* and *S. litura* were removed from the Petri plate and kept on white trap for the emergence of EPNs from the body of insect larvae. IJs were collected at three days intervals, up to 21 days, till the emergence of IJs were stopped. From this collection, the total emerged populations of EPNs were counted thrice under a microscope and mean values were worked out.

Statistical Analysis

The statistical analysis of data obtained in experiments was done based on completely randomized design using OPSTAT software available online at CCS HAU website (www.hau.ernet.in). Comparison of treatments was made at 5 % level of significance. Necessary transformation of data was done as per requirement.

III. RESULTS AND DISCUSSION

Two selected isolates of *Metarhabditis amsactae viz.*, HAR-St-II and HAR-Ht-III were tested in lab for their virulence in causing larval mortality of *P. brassicae*, *P. orichalcea* and *S. litura*. Maximum and significantly higher mean mortality of *P. brassicae* was observed at 40 IJs of strain HAR-Ht-III followed by 20, 10 and 5 IJs resulting in a mortality of insect larvae i.e. 89.2, 81.1, 69.9 and 38.1 per cent, respectively (Table -1). Irrespective of inoculums level, maximum mean mortality of *P. brassicae* larvae was 69.9 per cent on 4th day which was 37.0 per cent on 1st day. Nearly 90.0 per cent mortality was caused by 10 IJs on 4th day and by 40 IJs on 2nd day. On 4th day, 20 and 40 IJs caused 97.3 and 99.7 per cent death of *P. brassicae* larvae which were statistically at par. Per cent mortality on 4th day at 10 IJs were significantly higher than at 5 IJs but significantly lower than 20 and 40 IJs.

Table 1. In vitro per cent larval mortality of Pieris brassicae caused by Metarhabditis amsactae

Treatments (IJs/Petri plate)	Strain HAR-Ht-III at different time intervals					Strain HAR-St-II at different time intervals				
	1st Day	2nd Day	3rd Day	4th Day	Mean	1st Day	2nd Day	3rd Day	4th Day	Mean
5 IJs	12.5 (20.4)	30.0 (33.0)	47.5 (43.5)	62.5 (52.3)	38.1 (37.3)	27.5 (31.5)	35 (36.2)	52.5 (46.4)	72.5 (58.6)	46.8 (43.2)
10 IJs	42.5 (40.5)	62.5 (52.2)	85.0 (67.4)	89.9 (73.3)	69.9 (58.4)	37.5 (37.7)	52.5 (46.4)	77.5 (62.1)	87.4 (71.4)	63.7 (54.4)
20 IJs	57.5 (49.3)	77.5 (61.7)	92.3 (77.2)	97.3 (83.2)	81.1 (67.9)	62.5 (52.3)	77.5 (62.1)	89.9 (73.4)	97.3 (83.2)	81.8 (67.7)
40 IJs	72.5 (58.4)	89.9 (73.3)	94.8 (79.3)	99.7 (87.0)	89.2 (74.5)	72.5 (58.4)	85 (67.5)	94.8 (79.3)	99.7 (87.1)	88.0 (73.1)
Untreated check	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
Mean	37.0 (34.3)	52.0 (44.6)	64.0 (54.0)	69.9 (59.7)	-	40.0 (36.6)	50.0 (43.0)	63.0 (52.8)	71.4 (60.6)	-
C. D. at 5 %	treatment:(4.1), time: (3.7), treatment x time: (8.4)					treatment: (3.9), time: (3.4), treatment x time: (7.8)				

Values in parentheses are angular transformations

As the observation time and level of IJs increased in both strain of *M. amsactae*, HAR-Ht-III and HAR-St-II, there was a significant increase in per cent mortality of *P. brassicae* larvae. In strain HAR-St-II, at minimum inoculum level of 5 IJs/insect larva, mean mortality of 46.8 per cent was recorded followed by 63.7 per cent at 10 IJs, 81.8 per cent at 20 IJs and 88.0 per cent mortality at 40 IJs per larva of *P. brassicae*. Highest inoculum level of 40 IJs per larva resulted in 72.5 per cent death of *P. brassicae* larvae on 1st day. Similarly, at the lowestinoculum level of 5 IJs, a maximum of 72.5 per cent mortality were recorded on 4th day of inoculation. At inoculum level of 20 and 40 IJs/insect larva could result in causing highest mortality upto an extent of 97.3 to 99.7 per cent on 4th day, which

were statistically at par. Larval mortality recorded on 2nd day, at 10 and 20 IJs /insect larva differed significantly from each other. Yadav and Lalramliana (2012) showed that susceptibility of *Athalia lugens proxima* varied in response to the infection caused by *Steinernema glaseri, S. thermophilum* and *Heterorhabditis indica*. The difference in the pathogenicity level may be due to different insect pest species. Differences in the susceptibility among insect life-cycle stages have also been observed in the family Pyralidae, with the pupae being less susceptible than the larvae. Similar study was conducted by Walia *et al.* (2006) and reported that *S. pakistanense* @ 50 IJs per larva caused 100 per cent mortality of *Agrotis ipsilon* and *P. brassicae* after 48 h while it was only 75 per cent in *Helicoverpa armigera* even after 72 h.

Data in Table 2 show that maximum (97.3 %) larval mortality of *P. orichalcea* was recorded on 4th day, at 40 IJs of strain HAR-Ht-III. It was followed by 20, 10 and 5 IJs resulting in mortality of insect larvae of 97.3, 89.9, 82.5 and 72.5 per cent, respectively. On 4th day, 72.5 per cent larval mortality of *P. orichalcea* was obtained at 5 IJs. Similarily, on 2nd day, 72.5 per cent mortality was observed at inoculum level of 40 IJs/ insect larva. As the period of observation increased in both strains, larval mortality increased significantly. When comparing effect of observation timing irrespective of IJs levels, mean mortality of *P. orichalcea* larvae was maximum (68.5 %) on 4th day and minimum (36.5 %) on 1st day.

Table 2. In vitro per cent larval mortality of Plusia orichalcea caused by Metarhabditis amsactae

Treatments (IJs/Petri plate)	**Strain HAR-Ht-III at different time intervals**					**Strain HAR-St-II at different time intervals**				
	1st Day	**2nd Day**	**3rd Day**	**4th Day**	**Mean**	**1st Day**	**2nd Day**	**3rd Day**	**4th Day**	**Mean**
5 IJs	22.5 (27.7)	42.5 (40.7)	60.0 (50.8)	72.5 (58.4)	49.3 (44.4)	17.6 (22.2)	32.5 (34.5)	47.5 (43.5)	60.0 (50.8)	39.4 (37.7)
10 IJs	45.0 (42.1)	55.0 (47.9)	70.0 (56.8)	82.5 (65.4)	63.1 (53.0)	35.0 (36.2)	52.5 (46.4)	72.5 (58.9)	82.5 (65.4)	60.6 (51.7)
20 IJs	52.5 (46.4)	67.5 (55.3)	77.5 (61.7)	89.9 (73.4)	71.8 (59.2)	47.5 (43.5)	67.5 (55.4)	82.5 (65.4)	94.9 (79.3)	73.1 (60.9)
40 IJs	62.5 (52.3)	72.5 (58.4)	85.0 (67.5)	97.3 (83.2)	79.3 (65.3)	62.5 (52.2)	75.0 (60.0)	89.9 (73.3)	97.3 (83.2)	81.2 (67.2)
Untreated check	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
Mean	36.5 (34.3)	47.5 (41.0)	58.5 (47.9)	68.5 (56.7)	-	32.6 (31.4)	45.6 (39.8)	58.5 (48.8)	67.0 (56.3)	-
C. D. at 5 %	treatment: (3.1), time: (2.8), treatment x time: (6.2)					treatment: (4.1), time: (3.8), treatment x time: (8.3)				

Values in parentheses are angular transformations

Results on effect of strain HAR-St-II showed that as the level of IJs increased, there was significant increase in mean per cent mortality of *P. orichalcea*. It was 39.4, 60.6, 73.1 and 81.2 per cent at 05, 10, 20 and 40 IJs, respectively. At inoculum level of 5 IJs per insect larva, 47.5 per cent mortality was recorded on 3rd day whereas same larval mortality was obtained on 1st day at 20 inoculum level. On 4th day, 20 and 40 IJs caused 94.9 and 97.3 per cent mortality of *P. orichalcea* larvae which were statistically at par. Mean larval mortality was significantly increased with time. Maximum mean per cent mortality of *P. orichalcea* larvae was 67.0 per cent on 4th day which was 32.6 per cent on 1st day. Gulcu *et al.* (2014) found that five native EPN strains caused 100 per cent mortality of *Spodoptera cilium* larvae. Abbas *et al.* (2021) were also revealed that *H. bacteriophora* and *S. glaseri* at 1500 IJ/ml concentration resulted 100 per cent mortality of all larval instars of *P. brassicae* after 48 h, under *in vitro* conditions. Kalia *et al*. (2014) who reported that after 36 h 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 IJ/larva). In addition to virulence to the larval stages, ovicidal activity up to 84 per cent was observed at 200 IJ/50 and 100 eggs of *H. armigera* and *S. litura*, respectively.

Table 3. In vitro per cent larval mortality of Spodoptera litura caused by Metarhabditis amsactae

Treatments (IJs/Petri plate)	Strain HAR-Ht-III at different time intervals					Strain HAR-St-II at different time intervals				
	1st Day	2nd Day	3rd Day	4th Day	Mean	1st Day	2nd Day	3rd Day	4th Day	Mean
5 IJs	10.1 (16.6)	32.5 (34.7)	55.0 (47.9)	75.0 (60.1)	43.1 (39.8)	17.6 (22.3)	27.5 (31.0)	50.0 (44.9)	62.5 (52.5)	39.4 (37.7)
10 IJs	42.5 (40.6)	72.5 (58.4)	80.0 (63.8)	87.4 (71.4)	70.6 (58.5)	52.5 (46.4)	62.5 (52.3)	84.9 (69.3)	87.4 (71.4)	71.8 (59.8)
20 IJs	57.5 (49.3)	75.0 (60.1)	89.9 (73.4)	94.9 (79.3)	79.3 (65.5)	67.5 (55.3)	82.5 (65.4)	92.4 (75.4)	94.9 (79.3)	84.3 (68.9)
40 IJs	77.5 (61.7)	89.9 (73.4)	97.3 (83.2)	99.8 (87.1)	91.1 (76.4)	77.5 (61.7)	92.4 (77.3)	97.3 (83.2)	99.8 (87.1)	91.7 (77.3)
Untreated check	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
Mean	37.6 (34.2)	54.0 (45.9)	64.5 (54.2)	71.5 (60.1)	-	43.1 (37.7)	53.0 (45.8)	65.0 (55.2)	69.0 (58.6)	-
C.D. at 5 %	treatment:(4.2), time: (3.8), treatment x time: (8.4)					treatment: (5.2), time: (4.4), treatment x time : (10.4)				

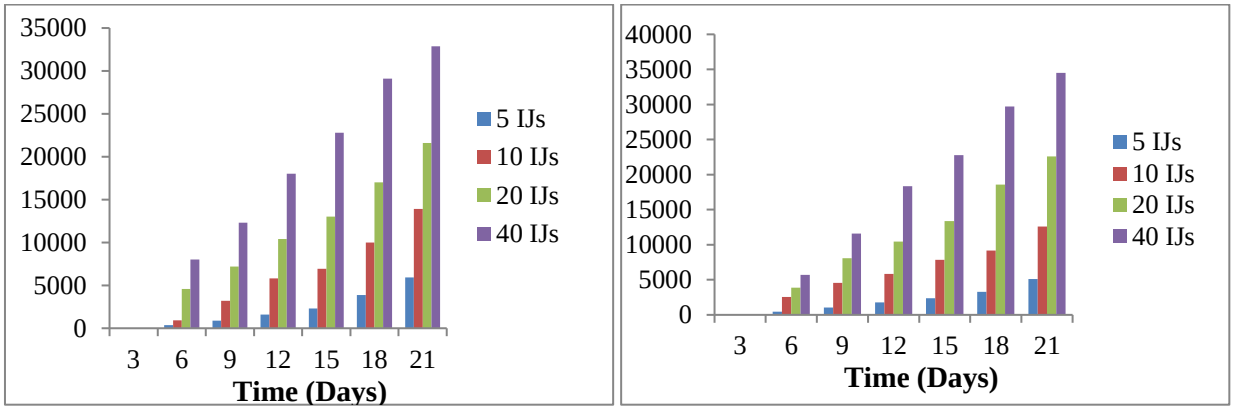
Values in parentheses are angular transformations

It can be inferred from data in Table 3 that significant higher mean per cent mortality (91.1 %) of *S. litura* larvae at 40 inoculum level of strain HAR-Ht-III, followed by 20, 10 and 05 inoculum level, respectively. As the period of observation increased, mean per cent mortality of *S. litura*, 37.6 per cent on 1st day significantly increased to 71.5 per cent on 4th day. Nearly 92.0 per cent mortality of *S. litura* larvae was achieved on 3rd day at 20 inoculum level which was similar to mortality obtained on 2nd day at 40 inoculum level. At inoculum level of 40 IJs, mortality of *S. litura* larvae was 97.3 and 99.8 per cent which were statistically at par. There was no mortality of *S. litura* larvae in untreated check. Per cent mortality on 4th day at 10 IJs were significantly higher than at 5 IJs but statistically at par with inoculum level of 20 IJs. Maximum and significantly higher mean per cent mortality was observed at 40 IJs followed by 20, 10 and 05 IJs resulted in mortality of *S. litura* larvae *i.e.* 91.7, 84.3, 71.8 and 39.4 per cent, respectively.

Irrespective of inoculum level of strain HAR-St-II, maximum mean per cent mortality of *S. litura* was 69.0 per cent on 4th day which was 43.1 per cent on 1st day. On 2nd day, 92.5 per cent mortality of *S. litura* larvae was observed at an inoculum level of 40 IJs per larva whereas same larval mortality was obtained on 3rd day at 20 inoculum level. On 3rd and 4th day, 10 IJs caused 84.9 and 87.4 per cent death of *S. litura* larvae which were statistically at par. On 4th day, 10, 20 and 40 IJs per insect larva, it resulted in 87.4, 94.9 and 99.8 per cent mortality of *S. litura* larvae which were also statistically at par. Sooraj *et al.* (2019) revealed among the three strains used, strain 2nd of *Metarhabditis rainai* at inoculum level of 300 IJs showed highest mortality of *S. litura* which was 29.99 per cent and maximum emergence of IJs ($3.5x10^5$) at 24 h after treatment. This strain at 200 inoculum level caused 80.52 and 99.35 per cent at 60 and 72 h after treatment. Results of Thakur *et al*. (2022) showed that LC 50 values of 3rd and 4th instar of *H. armigera* were 60.14 and 57.90 IJs of *H. bacteriophora*. *S. litura* were 59.95 and 50.91 IJs/larvae and *A. segetum* were 54.86 and 57.90 IJs/larvae at inoculum level of 50, 100, 150, and 200 IJs, after 120 hours.

The data in Figure 1 (a) show that highest population of *M. amsactae* strain HAR-St-II (32852

IJs/cadaver) was recovered at 40 inoculum level on 21st day followed by 18th, 15th, 12th, 9th and 6th day which were 29118, 22808, 18000, 12306 and 8000 IJs /cadaver, respectively. Minimum population of IJs was recovered at 5 inoculum level, on 6th day which was 364 IJs/cadaver of *P. brassicae* followed by 878, 1600, 2312, 3874 and 5920 IJs/ cadaver up to 9th, 12th, 15th, 18th and 21 day, respectively (Plate-2, left).



(a) *Metarhabditis amsactae* strain HAR-St-II (b) *Metarhabditis amsactae* strain HAR-Ht-III

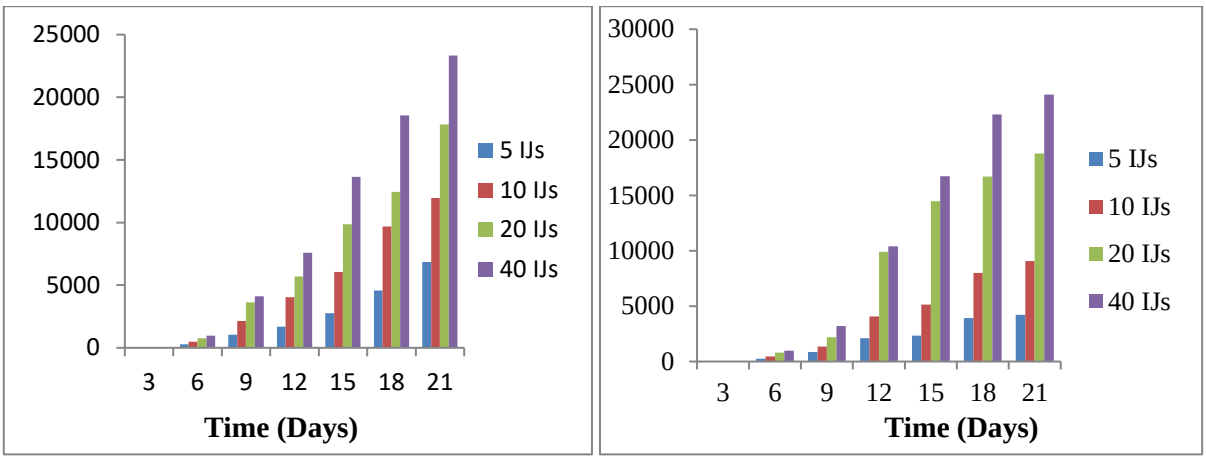
Fig.1. Recovery of IJs of Metarhabditis amsactae strain HAR-St-II (a) and Metarhabditis amsactae strain HAR-Ht-III (b) from dead larvae of Pieris brassicae

Plate 2. Recovery of Metarhabditis amsactae strains HAR-St-II (left) and HAR-Ht-III (right) on Pieris brassicae

Maximum IJs of *M. amsactae*, strain HAR-Ht-III emerged from cadavers of *P. brassicae* (Plate-2, right) on 21st day which was 34516 IJs/cadaver, at 40 IJs per insect larva followed by 20, 10 and 05 inoculum levels showed in Figure 1 (b). For recovery of EPNs, (Figure-1-3), almost all *Metarhabditis amsactae* strains multiplied on the tested insects, but multiplication level varied within *M. amsactae* strains. Population of IJs increased with increase of time at all inoculum level *i.e.* 05, 10, 20 and 40 IJs/ insect larva. No recovery of EPNs were recorded on 3rd day in all strains. The present studies, revealed that recovery of IJs in both strains at 40 inoculum level was observed maximum i.e. 44615 IJs/cadaver in strain HAR-Ht-III and 38710 IJs/cadaver in strain HAR-St-II from dead larvae of *S. litura.* In *P. brassicae*, recovery obtained 34516 IJs/cadaver in strain HAR-Ht-III and 32852 IJs/cadaver in strain HAR-St-II up to 21st day at 40 inoculum level. In *P. orichalcea*, recovery was 23330 IJs/cadaver in strain HAR-Ht-III and 22308 IJs/cadaver in strain HAR-St-II up to 21st day at 40 inoculum level. Pervez (2017) found that eight native EPNs were virulent on semi-looper and caused 100 per cent mortality within 72 h. Maximum multiplication of 9,324 IJs per larva was observed with *O. gingeri* (IISR-EPN 07) within 15 days, followed by 8,638 and 8,236 IJs per larva with *Oscheius* sp. (IISR-EPN 04) and *Oscheius* sp. (IISR-EPN 08) respectively.

In Figure 2 (a), as is evident less recovery of IJs (268) was observed on 6th day at 05 inoculum level. Highest yield of *M. amsactae* HAR-St- II, which was 23330 IJs/cadaver was obtained on 21st day, emerged from the body of the *P. orichalcea* at 40 inoculum level followed by 18562, 13655, 7585,4126 and 977 IJs/ cadaver of *P. orichalcea* on 18th, 15th, 12th, 9th and 6th day, respectively. Recovery of IJs of *M. amsactae* strain HAR-

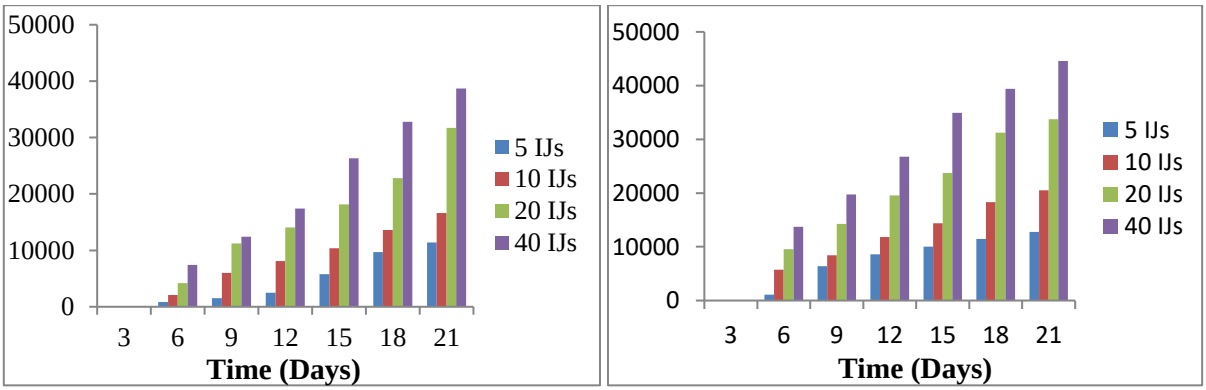
Ht-III on 6th day at 5, 10, 20 and 40 inoculum level which was 265, 470, 795 and 982 IJs/cadaver showed in Figure 2 (b). Maximum population of IJs was recovered on 21st day at 40 inoculum level which was 24098 IJs/cadaver of *P. orichalcea* followed by 22308, 16732, 10401, 3200 and 982 IJs/cadaver on 18th, 15th, 12th, 9th and 6th day, respectively.



(a) *Metarhabditis amsactae* strain HAR-St- II (b) *Metarhabditis amsactae* strain HAR-Ht-III

Fig.2. Recovery of IJs of Metarhabditis amsactae strain HAR-St- II (a) and HAR-Ht-III (b) from dead larva of Plusia orichalcea

Data presented in Figure 3 (a) clearly show that highest recovery (38,710 IJs/ cadaver) obtained from cadavers of *S. litura*, at 40 IJs inoculum level of *M. amsactae* strain HAR-St-II /larva were used. At inoculum level of 5 IJs /larva, yield of IJs was very low i.e. 818 on 6th day followed by 1514, 2478, 5798, 9716 and 11412 IJs/larva of *S. litura* upto 9th, 12th 15th, 18th and 21 day, respectively (Plate-3, left).



(a) *Metarhabditis amsactae* strain HAR-St-II (b) *Metarhabditis amsactae* strain HAR-Ht-III

Fig.3. Recovery of IJs of Metarhabditis amsactae strain HAR-St-II (a) and Metarhabditis amsactae strain HAR-Ht-III (b) from dead larvae of Spodoptera litura

Highest population of *M. amsactae* strain HAR-Ht-III (44615 IJs/cadaver) was recorded at 40 inoculum level, on 21st day followed by 18th, 15th, 12th, 9th, and 6th day which were 39408, 34960, 26800, 19765 and 13695 IJs/cadaver (Figure 3.b). Less recovery obtained at 5 inoculum level, on 6th day which was 1092 IJs/cadaver of *S. litura* followed by 6412, 8602, 10036, 11446 and 12796 IJs/cadaver upto 9th, 12th, 15th, 18th and 21th day, respectively (Plate-3, right).

Plate. 3 Recovery of Metarhabditis amsactae strains HAR-St-II (left) and HAR-Ht-III (right) on Spodoptera litura

Similarily, Banu *et al.* (2003) suggested that EPNs multiplied well on the adult of red weevil, *Rhynchophorus ferrugineus* and highest nematode multiplication was observed in *Steinernema* sp. (12.01 x 10^3 IJs/weevil) followed by *H. indica* (8.99 x 10^3 IJs/weevil) and *S. glaseri* (2.4 x 10^3 IJs/weevil). Results of Lalramliana and Yadav (2010) showed that the progeny production of EPNs by larvae of *P. brassicae* was noted to be highest in case of *H. indica*. It was considerably low in *S. thermophilum* and *S. glaseri*. The production increased along the concentrations till the highest concentration for both *H. indica* and *S. thermophilum* but declined from 50 IJs/larva onwards in case of *S. glaseri.* In my study, *Metarhabditis* strains were found more virulent against *S. litura*, *Pieris brassicae* and *Plusia orichalcea.* Similar results were reported by Gaugler and Kaya (1990) and Ali *et al*. (2008), which stated that EPNs were considered potential biopesticides and used on IPM field of these insect pests under field conditions. The larvae of *S. litura* were more suitable host for multiplication of IJs and these insects could be selected as the alternate host for production of IJs of EPN under laboratory conditions.

IV. CONCLUSION

The efficacy of these local strains, HAR-St-II and HAR-Ht-III of EPNs were tested against *Pieris brassicae*, *Spodoptera litura* and *Plusia orichalcea* under laboratory condition, at four inoculum levels i.e. 05, 10, 20 and 40 IJs /insect larva. In both strains, as the observation time and level of IJs increased, there was a significant increase in per cent mortality in all the three insects. In *P. brassicae*, mean mortality at 10 IJ/larva in HAR-St-II and HAR-Ht-III was 63.7 and 69.9 per cent, respectively. In *P. orichalcea*, mean mortality at 10 IJ/larva in HAR-St-II and HAR-Ht-III was 60.6 and 63.1 per cent, respectively. In *S. litura*, mean mortality at 10 IJ/larva in HAR-St-II and HAR-Ht-III was 71.8 and 70.6 per cent, respectively. Larvae of *S. litura* and *P. brassicae*, were found to be more susceptible to EPNs than *P. orichalcea*. Although all the *Metarhabditis amsactae* strains tested caused 100 per cent mortality at 40 inoculum level on 4th day, their infectivity levels varied at different IJs in different insect species. For recovery of EPNs, both strains of *Metarhabditis amsactae* multiplied on the tested insects, but multiplication level varied within strains. Further this experiment was continued at field level, no doubt there was comparative difference in result of laboratory experiment and on field application which was due to several environmental and edaphic factors. From this experiment we have concluded that application of EPNs is as much effective method of control of insect pest as chemical control.

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