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Infectivity of entomopathogenic nematode, *Steinernema glaseri* on fall army worm (FAW), *Spodoptera frugiperda* (Smith, 1797) in Maize (*Zea Mays*)

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Abstract
Fall army worm, *Spodoptera frugiperda* is a major invasive pest of maize crop from Brazil. Entomopathogenic nematode, *Steinernema* sp. is widely used as a biological control agent against many insect pests. A leaf disc bioassay experiment was conducted to evaluate the efficacy of *Steinernema glaseri* against all the six instars of fall army worm. Maize leaf discs of approximately 3cm diameter were placed on sterilized Petri plates and sprinkled with a nematode suspension containing infective juveniles of *S.glaseri* and later fall army worm (FAW) larvae were released. A total of 20 larvae were released per plate and various concentrations of entomopathogenic nematodes *viz*, 0, 250, 500, 1000 and 2000 infective juveniles per plate were evaluated in five replications for its efficacy against FAW. Insect mortality rate was observed at periodic intervals *viz*, 24, 48, 72 and 96 hours after nematode inoculation. After 24 hours of inoculation, higher mortality was observed in 1st, 2nd and 3rd instars when compared to 4th, 5th and 6th instar larvae. But uniform mortality was observed after 48 hours of treatment. The highest insect mortality of 100% was observed at 2000 IJ/ plate, followed by 95% mortality at 1000 IJ/ plate after 96 hours of nematode inoculation. With increase in the exposure time of FAW larva to *S. glaseri*, 100% insect mortality was observed in all concentrations evaluated. The infected insect cadavers were then set up in Modified White's trap to extract freshly multiplied infective juveniles in order to confirm the infectivity of *S. glaseri* on fall army worm.

Keywords: *Steinernema glaseri*, *Spodoptera frugiperda*, insect mortality, leaf disc bioassay, infective juveniles

1. Introduction
The fall army worm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a principal pest of maize in Brazil. They are highly polyphagous migratory species that can colonize over 80 different plant species of which maize is not an exception. The insect pest also cause damages to other grass crops like rice, sorghum, sugarcane and wheat, and other vegetable crops like tomato and cotton [6]. *S. frugiperda* can cause corn yield loss as much as 70 % of a whole production. The caterpillar feeds on all stages of the corn plant by consuming the foliage and mostly prefer the young plants [6]. Chemical pesticides provide only short term solution for pest control. Moreover, the random use of insecticides has posed many problems such as increased resistance in this insect against all groups of pesticides, insect resurgence, bio accumulation and health hazards [7]. Excessive use of pesticides also has a negative impact in the environment and agriculture sustainability [10]. The use of various biological organisms may lead a great path for fall army worm control. Several entomopathogenic organisms such as fungi, viruses and bacteria are used in pest control [3]. Among those organisms studied for *S. frugiperda* population control, entomopathogenic nematodes present a great potential [4]. The planting system and no tillage system facilitate the maintenance of EPN in the area and keep them viable and pathogenic. Before evaluating in the field condition, the nematode populations should be evaluated in relation to their virulence against *Spodoptera frugiperda*, since there is evidence that the nematode populations have different virulence from the hosts. The ideal concentration of EPN that causes pest mortality also changes [1]. Therefore, it is aimed to standardize a suitable concentration of the entomopathogenic nematodes based on the mortality of *S. frugiperda* in laboratory condition as a preliminary study.

2. Materials and Methods

2.1 Multiplication and maintenance of entomopathogenic nematodes

The nematodes used for the experiment were those that are maintained in the Department of Nematology, TNAU, Coimbatore. The nematode culture of *Steinernema glaseri* was stored as aqueous suspension with 500 to 1000 infective juveniles per ml and maintained in BOD at 15 to 20°C in canted tissue culture flasks. The nematodes were cultured using *Corcyra cephalonica* larvae. The eggs for the *Corcyra* larval culture was obtained from the Department of Agricultural Entomology, TNAU, Coimbatore. The larvae were grown and used for culturing nematodes according to the methodology described by Askary and Ahmad (2017) [2] with the following few steps.

Inoculation of nematodes onto the *Corcyra* larvae

Corcyra eggs were introduced into an artificial diet containing pearl millet grains and groundnut mixture. The grown up larvae were used for culturing nematodes. Approximately 10 *Corcyra* larvae were taken in a Petri plate floored with Whatman filter paper. Nematode suspension of 1 ml containing 500 to 1000 infective juveniles were sprayed on to the larvae and left undisturbed for 2 to 4 days for the nematodes to penetrate the larval body and multiply in it, ultimately causing death of the insect.

Harvesting the multiplied nematodes from the larval cadaver

After 4 days of inoculation, larval death was observed that was identified with its change in colour. The dead larvae turned blackish in colour due to infection by *Steinernema*. The nematodes were extracted using Modified White's trap. This is a set up in which a small Petri plate containing Plaster of Paris is placed into a large sized Petri plate containing distilled water. The larval cadavers were placed over the small Petri plate and then sealed. After 2 to 3 days nematodes were collected in water and used for further studies.

Concentration of nematode suspension and storage

The collected water was kept in a beaker in order to concentrate the nematode extraction. After few minutes the active nematodes settle down at the bottom. Hence the top empty portion was discarded and the concentrated suspension was stored in a canted tissue culture flask in BOD at 15 to 20° C.

2.2 Evaluation of *Steinernema glaseri* against *Spodoptera frugiperda*

Collection of fall army worm (FAW)

In order to evaluate the efficacy of nematodes against *S. frugiperda*, various instars of FAW larvae were collected from maize fields untreated with any chemical pesticides. Larvae of 1st instar to 6th instar were collected by hand picking method of collection and brought to the laboratory for evaluation [5].

Leaf disc bioassay method

The various instars of larvae collected were washed in distilled water to remove dust particles and to clean the excreta on its body. These larvae were separated instar wise based on its size and body length from 1 to 6. Maize leaves that are succulent and tender were collected from the same field and chopped to discs of 3 cm diameter [11]. These leaf discs were then placed on sterilized Petri plates. Five treatments of various nematode concentrations *viz*, 0, 250, 500, 1000 and 2000 *S.glaseri* infective juveniles per ml per plate were maintained with five replications.

Various concentrations of nematode suspension were sprayed on to the maize leaf discs on the plate before releasing the larvae according to the treatments. A total of 20 *S. frugiperda* larvae were released per plate for all treatments. The Petri plates were then sealed tightly with the klin film and kept undisturbed. The methodology followed was done in reference with the work of Sunanda *et al*., (2014) who evaluated the various concentrations of entomopathogenic nematodes *Steinernema* sp. against *Plutella xylostella*. The nematode inoculated on to the leaf discs were consumed by the FAW larvae [11].

Fig 1: Leaf disc bioassay

The nematodes after being introduced inside the larval body releases its associated bacteria *Xenorhabdus* spp. into the larval body which multiplies throughout the body of FAW and finally causes mortality. Insect mortality was observed 24, 48, 72 and 96 hours after larval release. *S. glaseri* nematodes were then extracted from the dead FAW larvae by Modified White's trap method of nematode extraction in order to confirm that the larval death was due to the nematode *S. glaseri*.

Fig 2: Healthy FAW

Fig 3: Nematode infected FAW

3. Statistical Analysis

The observations recorded were analyzed statistically and the significance of the results was tested. For the above laboratory experiment, Completely Randomized Design (CRD) was followed. The means of all the values in the experiment was used to compare the efficacy of the treatments. With the resulted mean values, percent insect mortality for all the

treatments was analyzed. Each value in the table is the mean of five replications.

4. Results and Discussions

The nematode culture of *Steinernema glaseri,* exhibited significant effectiveness against *S. frugiperda.* All the concentrations evaluated were effective and resulted in mortality of fall army worm larvae at various intervals. The mortality percentage of fall army worm larvae was calculated at periodical intervals *viz*, 24, 48, 72 and 96 hours after nematode inoculation and statistically analysed in similarity with the work done by Andalo *et al.* (2010).

A maximum of 100% mortality was observed in fourth and sixth instar larvae of FAW at a concentration 2000 IJ's/plate, followed by 99% mortality in second instar larvae at 2000 IJ's/plate, 98% mortality in third and fifth instar larvae at 2000 IJ's/plate after 96 hrs of application. No mortality (0%) was observed in the control treatments *ie*., 0 IJ's/plate. The mortality of FAW larvae due to *S. glaseri* was confirmed by Modified White's trap method. There was a significant difference in the mortality percentage between the treatments. This may be due to the relation between the increased number of infective juveniles and the duration taken by the nematodes to penetrate the larval body and infect it. Similar results have been observed in the study of Riga *et al*., (2001) who evaluated the potential of controlling insect pests of corn using entomopathogenic nematodes [9].

The ability of *Steinernema* sp. to infect and cause mortality in *Spodoptera frugiperda* was also observed by Raulston *et al*., (1992). In this study the suppression of *Helicoverpa zea* and *Spodoptera frugiperda* suggested *Steinernema* sp. as a novel bio pesticide [8]. The present study is also supported by the work of Andalo *et al*., (2010) [1] in which *Steinernema* sp. showed 100% mortality against *Spodoptera frugiperda* at the rate of 200 IJ's per larvae at laboratory conditions [1].

In this work, the optimal concentration of the entomopathogenic nematode *Steinernema glaseri* has been observed and also proved its efficiency on the destructive pest FAW. Insect mortality was observed within 24 hours of treatment at all concentrations varying with the fatal counts due to increased number of infective juveniles. With increased time of exposure of *S. frugiperda* to *Steinernema glaseri*, the mortality percentage increases.

Table 1: Mean mortality and mortality percentage of Second instar FAW larvae at different time intervals

Treatments (IJ's / plate)	Mean mortality and mortality percentage of Second instar FAW larvae at different time intervals			
	24 hours after treatment	48 hours after treatment	72 hours after treatment	96 hours after treatment
T1 (Control)	0	0	0.2 (1%)	0.2 (1%)
T2 (250 IJ's /plate)	5.6 (28%)	8.4 (42%)	11.4 (57%)	15.4 (77%)
T3 (500 IJ's /plate)	5.6 (28%)	8.0 (40%)	12.8 (64%)	16.2 (81%)
T4 (1000 IJ's /plate)	7.2 (36%)	10.6 (53%)	14.2 (71%)	16.6 (83%)
T5 (2000 IJ's /plate)	10.2 (51%)	13.0 (65%)	16.0 (80%)	19.8 (99%)
S Ed	0.3110	0.2230	0.2275	0.1640
CD (0.05)	0.6488	0.4651	0.4746	0.3420

*Each value in this table are the mean value of five replications.

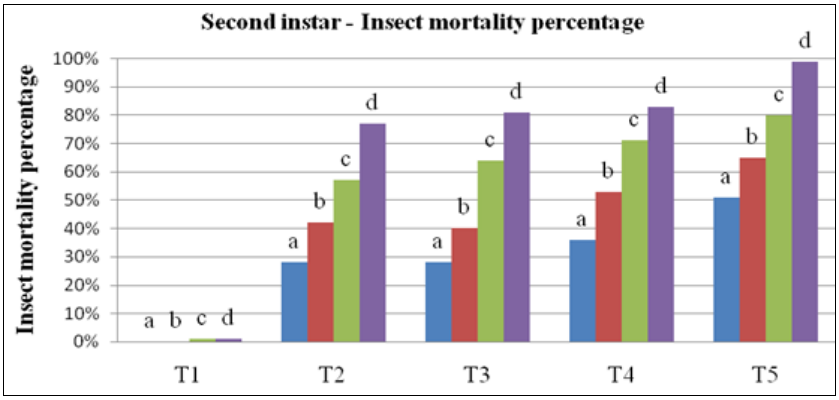

Fig 1: T1 - Control (0 IJ's/plate); T2 - 250 IJ's/plate; T3 - 500 IJ's/plate; T4 - 1000 IJ's/plate; T5 - 2000 IJ's/plate; The four bars in each treatment represent the time interval at which the observations were taken *viz*, a - 24 hours, b - 48 hours, c - 72 hours and d - 96 hours after the treatment

Table 2: Mean mortality and mortality percentage of Third instar FAW larvae at different time intervals

Treatments (IJ's / plate)	Mean mortality and mortality percentage of Third instar FAW larvae at different time intervals			
	24 hours after treatment	48 hours after treatment	72 hours after treatment	96 hours after treatment
T1 (Control)	0	0	0	0
T2 (250 IJ's /plate)	2.2 (11%)	4.8 (24%)	7.6 (38%)	12.0 (60%)
T3 (500 IJ's /plate)	4.6 (23%)	7.2 (36%)	12.4 (62%)	15.8 (79%)
T4 (1000 IJ's /plate)	7.6 (38%)	9.2 (46%)	14.6 (73%)	17.6 (88%)
T5 (2000 IJ's /plate)	8.6 (43%)	13.0 (65%)	17.4 (87%)	19.6 (98%)
S Ed	0.2648	0.2444	0.1620	0.1164
CD (0.05)	0.5524	0.5099	0.3380	0.2427

*Each values in this table are the mean value of five replications.

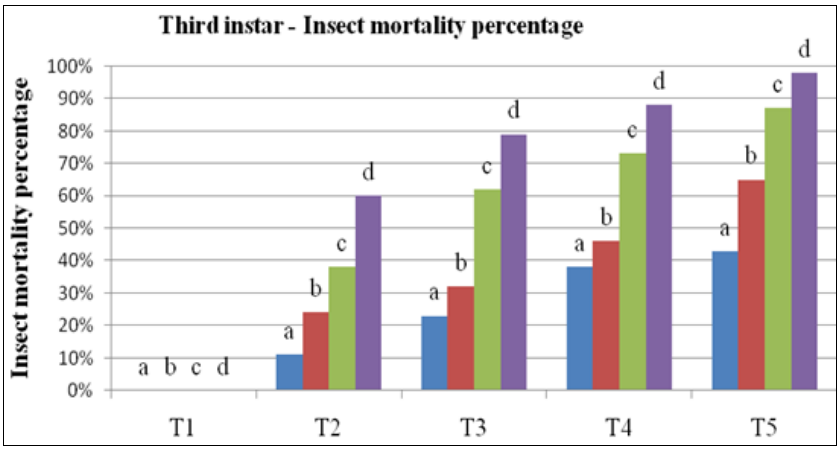

Fig 2: T1 - Control (0 IJ's/plate); T2 - 250 IJ's/plate; T3 - 500 IJ's/plate; T4 - 1000 IJ's/plate; T5 - 2000 IJ's/plate; The four bars in each treatment represent the time interval at which the observations were taken *viz*, a - 24 hours, b - 48 hours, c - 72 hours and d - 96 hours after the treatment.

Table 3: Mean mortality and mortality percentage of Fourth instar FAW larvae at different time intervals

Treatments (IJ's / plate)	Mean mortality and mortality percentage of Fourth instar FAW larvae at different time intervals			
	24 hours after treatment	48 hours after treatment	72 hours after treatment	96 hours after treatment
T1 (Control)	0	0	0	0
T2 (250 IJ's /plate)	1.4 (7%)	4.2 (21%)	6.8 (34%)	10.2 (51%)
T3 (500 IJ's /plate)	7.2 (36%)	11.6 (58%)	14.4 (72%)	17.2 (86%)
T4 (1000 IJ's /plate)	7.0 (35%)	9.8 (49%)	16.2 (81%)	19.0 (95%)
T5 (2000 IJ's /plate)	10.4 (52%)	14.0 (70%)	18.2 (91%)	20.0(100%)
S Ed	0.2301	0.1875	0.1558	0.1093
CD (0.05)	0.4800	0.3910	0.3249	0.2279

*Each values in this table are the mean value of five replications.

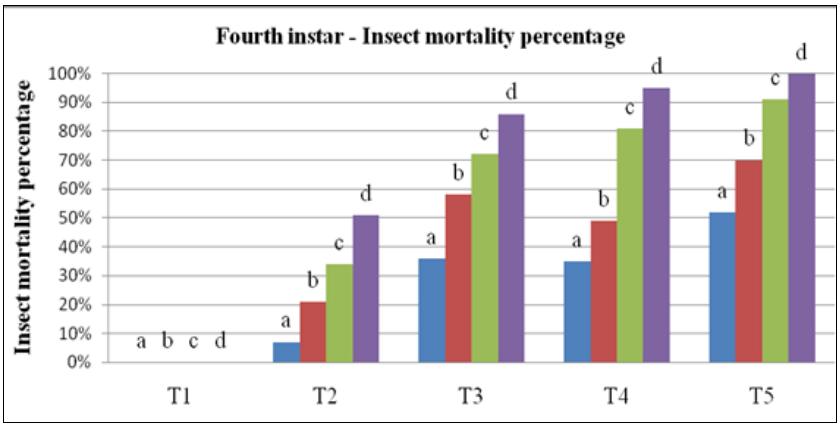

Fig 3: T1 - Control (0 IJ's/plate); T2 - 250 IJ's/plate; T3 - 500 IJ's/plate; T4 - 1000 IJ's/plate; T5 - 2000 IJ's/plate; The four bars in each treatment represent the time interval at which the observations were taken *viz*, a - 24 hours, b - 48 hours, c - 72 hours and d - 96 hours after the treatment.

Table 4: Mean mortality and mortality percentage of Fifth instar FAW larvae at different time intervals

Treatments (IJ's / plate)	Mean mortality and mortality percentage of Fifth instar FAW larvae at different time intervals			
	24 hours after treatment	48 hours after treatment	72 hours after treatment	96 hours after treatment
T1 (Control)	0	0	0	0
T2 (250 IJ's /plate)	3.6 (18%)	6.8 (34%)	10.4 (52%)	14.4 (72%)
T3 (500 IJ's /plate)	2.4 (12%)	6.0 (30%)	11.0 (55%)	16.4 (82%)
T4 (1000 IJ's /plate)	7.2 (36%)	10.8 (54%)	15.6 (78%)	18.4 (92%)
T5 (2000 IJ's /plate)	9.6 (48%)	13.6 (68%)	16.6 (83%)	19.6 (98%)
S Ed	0.2739	0.1639	0.1601	0.0924
CD (0.05)	0.5713	0.3419	0.3340	0.1927

*Each values in this table are the mean value of five replications.

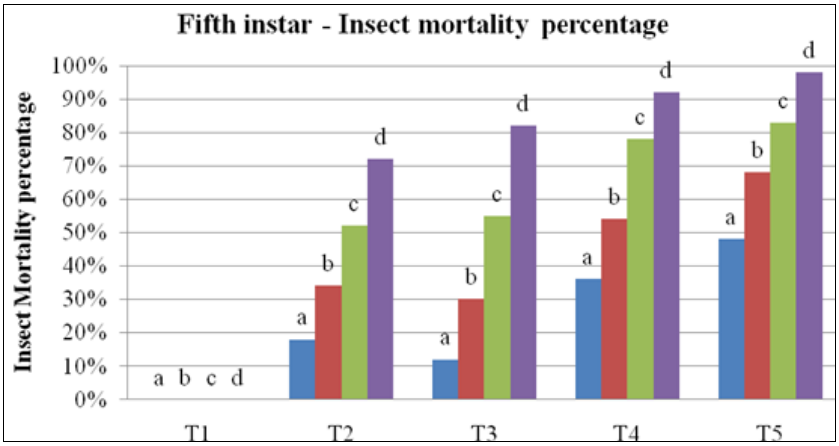

Fig 4: T1 - Control (0 IJ's/plate); T2 - 250 IJ's/plate; T3 - 500 IJ's/plate; T4 - 1000 IJ's/plate; T5 - 2000 IJ's/plate; The four bars in each treatment represent the time interval at which the observations were taken *viz*, a - 24 hours, b - 48 hours, c - 72 hours and d - 96 hours after the treatment.

Table 5: Mean mortality and mortality percentage of Sixth instar FAW larvae at different time intervals

Treatments (IJ's / plate)	Mean mortality and mortality percentage of Sixth instar FAW larvae at different time intervals			
	24 hours after treatment	48 hours after treatment	72 hours after treatment	96 hours after treatment
T1 (Control)	0	0	0	0
T2 (250 IJ's /plate)	3.4 (17%)	6.6 (33%)	11.4 (57%)	16.4 (82%)
T3 (500 IJ's /plate)	6.2 (31%)	11.4 (57%)	14.8 (74%)	17.8 (89%)
T4 (1000 IJ's /plate)	7.4 (37%)	11.8 (59%)	15.6 (78%)	19.0 (95%)
T5 (2000 IJ's /plate)	10.6 (53%)	14.0 (70%)	18.8 (94%)	20.0 (100%)
S Ed	0.1575	0.1392	0.1091	0.0703
CD (0.05)	0.3284	0.2904	0.2276	0.1465

*Each values in this table are the mean value of five replications.

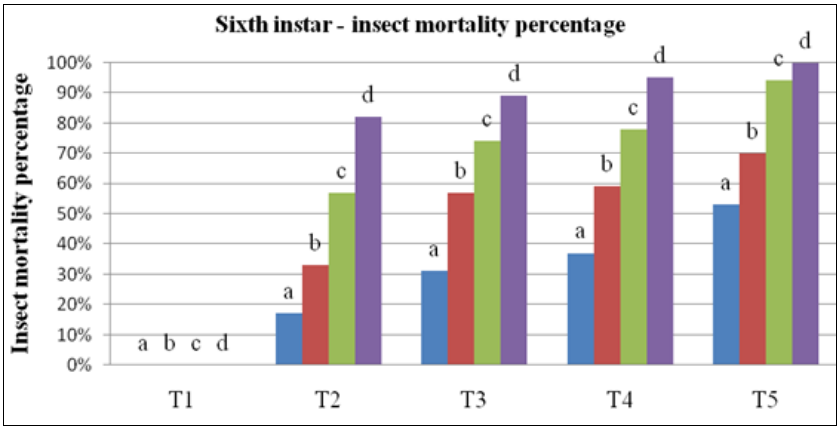

Fig 5: T1 - Control (0 IJ's/plate); T2 - 250 IJ's/plate; T3 - 500 IJ's/plate; T4 - 1000 IJ's/plate; T5 - 2000 IJ's/plate; The four bars in each treatment represent the time interval at which the observations were taken *viz*, a - 24 hours, b - 48 hours, c - 72 hours and d - 96 hours after the treatment.

5. Conclusion

Fall Army Worm is the most destructive invasive pest infecting maize. From the experiment conducted, it is concluded that the entomopathogenic nematode *Steinernema glaseri* is an effective biocontrol agent against *Spodoptera frugiperda*, which causes 100% mortality in fourth and sixth instar FAW larvae at the concentration of 100 IJ's per larvae. As an alternative to chemical control of fall army worm in maize, this preliminary study indicated the use of entomopathogenic nematode *Steinernema glaseri* as a potential biocontrol agent.

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