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Bio-efficacy of *Steinernema carpocapsae* against *Spodoptera litura* under Laboratory Condition

Saroj Yadav*, Jaydeep Patil and H.K. Sharma

Department of Nematology, Rajasthan College of Agriculture,

Maharana Pratap University of Agriculture and Technology, Udaipur-31300, India

*Corresponding Author E-mail: sarojhau29@gmail.com

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ABSTRACT

Tobacco caterpillar; Spodoptera litura (Fab.) (Lepidoptera: Noctuidae), has been recognised as most devastating pest of several cultivated crops, including vegetables throughout the world. Several bio-pesticides have been tested for the management of tobacco caterpillar in different parts of the country, but only few have shown promising results. Among different bio-agents, entomopathogenic nematodes were found comparatively better over others in suppressing the population of tobacco caterpillar. Laboratory studies was carried out to evaluate the efficacy of an indigenous population of Steinernema carpocapsae against fourth instar larvae of tobacco caterpillar. Mass multiplication of S. carpocapsae on different artificial media i.e., Nutrient broth, Wheat flour, Maize flour, Lipid and Modified Wout's media. Different population of S. carpocapsae recovered from different artificial media of different dosage i.e., 100, 200, 300 and 400 IJs/Petri plate tested against S. litura. The per cent mortality of S. litura larvae after 96 hours was recorded, maximum 100 per cent mortality of S. litura was recorded at an inoculum level of 400 IJs/Petri plate recovered from Nutrient broth media. While, minimum 55 per cent mortality of S. litura was observed after 96 hrs at 100 IJs of S. carpocapsae produced on Wheat flour and Maize flour media respectively.

Key words: *Entomopathogenic nematode, Steinernema carpocapsae, In vitro, Bio-efficacy, Inoculum level, Infective juveniles, Spodoptera litura.*

INTRODUCTION

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae), the common cutworm, is an economically serious and polyphytophagous pest in India. *S. litura* is also known as the common or tobacco cutworm, or the cluster or tobacco caterpillar. This pest attacks a wide range of food plants belonging to diverse botanical origins (112 cultivated food plants belonging to 44 families all over the world; 60 plants known from India)[5,10,12,16].

The losses caused by *S. litura* on tomato are much more severe as this pest has been reported to cause skelatalization of leaves in early stage and severe defoliation in later stage thus reducing the photosynthetic capacity of plants.

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Realizing the role of these pests as limiting factors in agricultural productivity, several methods were developed and incorporated in to management programme of economically important pests. Out of these insecticides could initially catch up to the growers because of their ready availability, quick ability to suppress pest's population and increasing productivity. Widespread development of resistance to chemical insecticides including the widely used pyrethroids has been reported in *S. litura*[1]. There are many disadvantages in their use. Firstly, the pests are widely spread and the large quantities of toxic chemical put into the environment leads to environmental pollution. Secondly, the use of insecticides protects the crops in the treated field only and does not curb the pest population in the surrounding areas. Besides, indiscriminate use of insecticide proved to be hazardous to livestock, plants, beneficial insects, human health and also to the beneficial fauna and flora. Since then search for some other environmentally safer method of pest management has started. Efforts were made to identify and develop effective non-chemical methods with the aim to reduce pest population by using naturally occurring macro and microorganism, capable of suppressing pest population.

Pest infestation normally leads to reduction in market value and in some cases total crop failure. Farmers in India have been applying various synthetic pesticides to reduce damage caused by these pests, at different growth stages of the crop. These pesticides have been reported to cause toxicological and environmental problems (toxic residues in food, soil, water bodies and elimination of non-target organisms) as well as the development of resistant strains of pests[13]. The improper use of pesticides is an issue of much concern.

Entomopathogenic nematodes (EPNs) are beneficial nematodes parasiting insect pests and are being effectively used as a bio pesticide against a wide variety of insect pests. The impressive attributes of EPN have stimulated strong commercial interest in nematodes as biological insecticides and are perceived as viable alternative to chemicals in integrated pest management (IPM) programme. EPN have many attributes, which make them a good and promising bio control agent[2]. They often behave like insecticide or other plant protection chemicals and they can be easily incorporated as a component of IPM programme. The EPNs can easily be cultured and applied using standard defined methods and application equipment's. They are compatible with many chemical pesticides and can be incorporated as an important component in the Integrated Pest Management (IPM).

Entomopathogenic nematodes (EPN's) of the families Steinernematidae and Heterorhabditidae are lethal to insects. These pathogens contribute to the regulation of natural populations of insects, but the main interest in them is an inundatively applied biocontrol agent[9]. Because of their biocontrol potential, considerable attention has been directed over the past few decades to genus *Heterorhabditis* and *Steinernema* and their respective bacterial partners, *Photorhabdus* and *Xenorhabdus*[7].

Biological control of pests using entomopathogenic nematodes (EPNs) may be one such method which may prove to be an ideal alternative to chemicals as it has long term effect, without any harmful effect on environment and also non-target organisms[3]. The infective juveniles (IJs) of these nematodes are free living, non-feeding and have the ability to search out their hosts. These are potential agents as they serve as vectors of bacteria, achieve a quick kill of target insect pests, have broader host range, highly virulent, possess chemoreceptor and can be cultured easily *in vitro* and *in vivo.* They can be easily applied using standard application equipment's and are found compatible with many chemical pesticides[6]. They have potential for long term establishment in soil through recycling on infected insect's larvae. EPN based pesticides are now largely marketed in the western countries in comparison to other organism.

MATERIAL AND METHODS

The efficacy of entomopathogenic nematodes, *Steinernema carpocapsae* Steiner was tested against fourth instar larvae of *Spodoptera litura* (Fab.) at the four nematode concentrations i.e., 100, 200, 300 and 400 infective juveniles/Petri plate and control (water without IJs).. Filter paper is chosen to test nematode's efficacy against the fourth instar larvae of *S. litura.*

Nematode cultures

Steinernema carpocapsae was reared on the fifth instar larvae of greater wax moth, *Galleria mellonella* (L) under laboratory conditions. This population was further multiplied on different artificial diet i.e., Nutrient broth, Wheat flour, Maize flour, Lipid and Modified Wout's media under laboratory conditions at 25±1°C in BOD. A stock suspension of infective juveniles was made in sterile distilled water and used against *S. litura*.

Insects

The original population of *S. litura*, collected from cotton field in MPUAT, Udaipur. *Spodoptera litura* was reared on the leaves of castor, *Ricinus communis* (L.) in the laboratory under hygienic conditions at room temperature (27±1^{o}C) and fourth instar larvae were used for treatments. Prior to the experiment, the insect larvae were washed three times with distilled water to remove soil and organic particles.

Bioassay

The sterilized glass plates (9 cm size) were kept in sequence according to treatments and replication. The plates were used for the bio-efficacy test of *S. carpocapsae* produced on different artificial media against *S. litura*. The EPNs suspension consisting of IJs stored in sterile distilled water was first examined under stereoscopic microscope to check the activity of the juveniles and diluted with known quantity of sterile distilled water for making the suspension according to required number of IJs. The larvae of tobacco caterpillar (*S. litura*) ten in numbers were placed on Whatman filter paper No. 1 in glass Petri plates and IJs of *Steinernema carpocapsae* multiplied on different artificial media (Nutrient broth, Wheat flour, Maize flour, Lipid and Modified Wout's Media) were released @ 100, 200, 300 and 400 IJs/Petri plate respectively and kept at 25°C in a BOD

incubator. The observations were taken on the mortality of host larvae after every 12 hrs up to 168 hrs from the time of inoculation of IJs.

Statistical analysis

The observations recorded were statistically analysed and significance of results was tested. For above experiments, completely randomized design was followed. Means of all experiments were used to compare the efficacy of treatments. Per cent insect mortality data were analysed by multifactor ANOVA followed by Duncan's multiple range tests ($P < 0.05$) for separation of means. Figures in parentheses are arc sin transformed values.

RESULT AND DISCUSSION

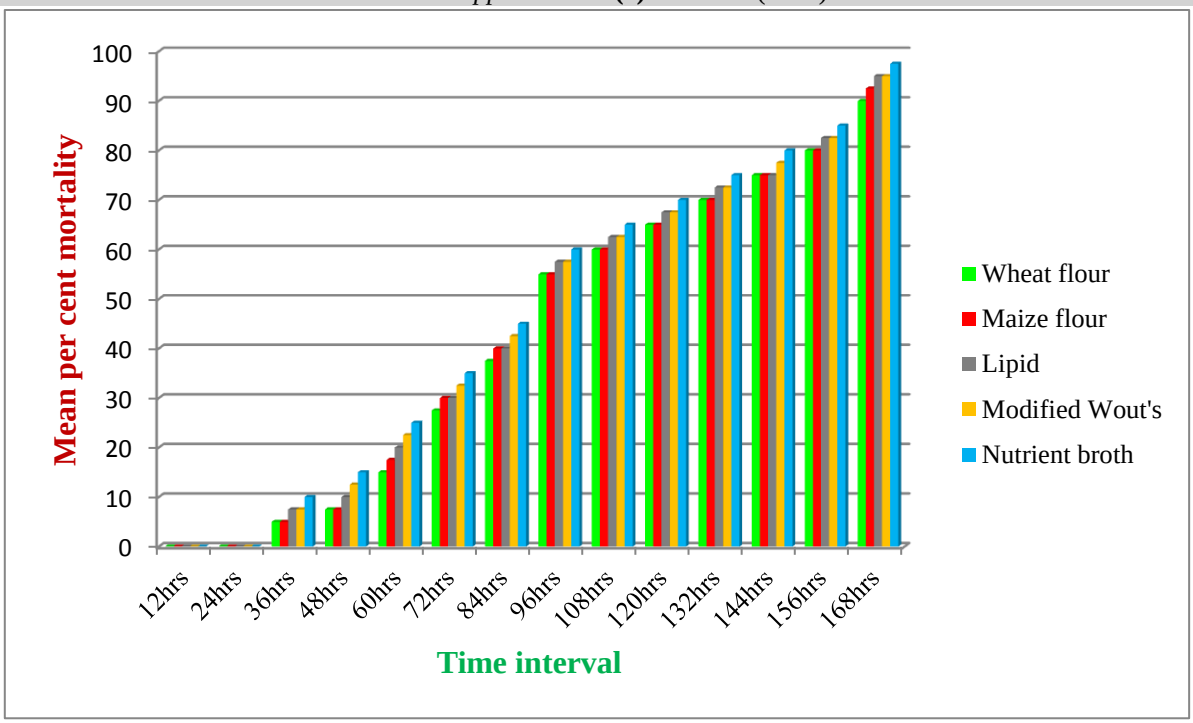
The experimental results revealed that maximum (100.00) per cent mortality of *S. litura* recorded within 96 hrs of exposure at an inoculum levels 400 IJs recovered from Nutrient broth media followed by 97.5 per cent mortality obtained from Modified Wout's media and 95 per cent at 400 IJs produced on Lipid and Maize flour media respectively. While, minimum (55) per cent mortality was observed at 100 IJs produced on Wheat flour as well as Maize flour media. Similar studies in this regard were conducted by Rajkumar *et al*[14]., conducted bioassay tests of *Heterorhabditis* sp. (HUDP-1 strain) using sand column assay against *S. litura*. The dosages of entomopathogenic nematode were 25, 50, 75, 100, 125 and 150 IJs/caterpillar. The caterpillar mortality ranged between 16.66 to 88.88 per cent under different inoculum levels and time of exposure i.e. caterpillar mortality increased with an increase in the inoculum levels and period of exposure. Similar studies were conducted by Karunakar *et al*[8]., who found that lepidopteran insect hosts, *Galleria mellonella, Corcyra cephalonica, Helicoverpa armigera, Spodoptera litura* and *Sesamia inferens* were highly susceptible to all the three species (*H. indicus, S. feltiae* and *S. glaseri*). The least susceptible host was *Sesamia inferens, C. cephalonica* required significantly less time of 37.6 hrs for its mortality, while *H. armigera* was found to be alive for prolonged duration of 52.4 hrs after infection.

Mortality of insect larvae increased with an increase in the inoculum levels and period of exposure. These findings of experiments performed under Indian conditions andthe results of the experiment are in agreement with results obtained by other workers[4,11,15] who have reported the efficacy of different *Steinernema* spp. against DBM. A linear increase in the percentage mortality of DBM was observed withan increase in exposure time at 24 hrs, the percentage mortality of the DBM larvae was lowest for all the EPN inoculum levels and highest at 72hrs. This may be because of time required for entry of IJs in to host insect and bacterial infection starting after the penetration of the nematodes in to the body cavity of the insects. Therefore, infective nematode juveniles must locate the insect host and gain entry into the haemocoel.

Therefore, it was concluded that the mortality of insect larvae increased with an increase in the inoculum levels and period of exposure. The IJs produced on animal protein based media had higher infectivity against *S. litura* under laboratory conditions compared to plant protein based media.

Table 3: Bio-efficacy of *S. carpocapsae* recovered from different artificial media against *S. litura* in laboratory condition

Population	Treatments	Mean per cent mortality at different time intervals (hrs)													
		12hrs	24hrs	36hrs	48hrs	60hrs	72hrs	84hrs	96hrs	108hrs	120hrs	132hrs	144hrs	156hrs	168hrs
Nutrient broth Media	T_1	0.00 (0.00)	0.00 (0.00)	10.00 (18.43)	15.00 (22.50)	25.00 (29.89)	35.00 (36.22)	45.00 (42.12)	60.00 (50.77)	65.00 (53.78)	70.00 (56.79)	75.00 (60.11)	80.00 (63.43)	85.00 (67.50)	97.50 (85.39)
	T_2	0.00 (0.00)	5.00 (9.22)	15.00 (22.50)	22.50 (28.23)	35.00 (36.22)	50.00 (45.00)	65.00 (53.78)	75.00 (60.11)	80.00 (63.43)	85.00 (67.50)	90.00 (71.57)	95.00 (80.78)	100.00 (90.00)	100.00 (90.00)
	T_3	0.00 (0.00)	12.50 (20.47)	25.00 (29.89)	42.50 (40.67)	50.00 (45.00)	67.50 (55.28)	77.50 (61.77)	85.00 (67.50)	90.00 (71.57)	95.00 (80.78)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	T_4	0.00 (0.00)	27.50 (31.55)	42.50 (40.67)	57.50 (49.33)	70.00 (56.79)	80.00 (63.43)	90.00 (71.57)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Wheat flour media	T_1	0.00 (0.00)	0.00 (0.00)	5.00 (9.22)	7.50 (13.83)	15.00 (22.50)	27.50 (31.55)	37.50 (37.73)	55.00 (47.88)	60.00 (50.77)	65.00 (53.78)	70.00 (56.79)	75.00 (60.11)	80.00 (63.43)	90.00 (71.57)
	T_2	0.00 (0.00)	0.00 (0.00)	10.00 (18.43)	17.50 (24.53)	30.00 (32.21)	42.50 (40.67)	60.00 (50.77)	70.00 (56.79)	75.00 (60.11)	80.00 (63.43)	85.00 (67.50)	90.00 (71.57)	95.00 (80.78)	100.00 (90.00)
	T_3	0.00 (0.00)	10.00 (18.43)	20.00 (26.57)	35.00 (36.22)	42.50 (40.67)	62.50 (52.27)	72.50 (58.45)	80.00 (63.43)	85.00 (67.50)	90.00 (71.57)	97.50 (85.39)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	T_4	0.00 (0.00)	20.00 (26.57)	35.00 (36.22)	47.50 (43.56)	62.50 (52.27)	72.50 (58.45)	82.50 (65.47)	92.50 (76.17)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Maize flour media	T_1	0.00 (0.00)	0.00 (0.00)	5.00 (9.22)	7.50 (13.83)	17.50 (24.53)	30.00 (33.21)	40.00 (39.23)	55.00 (47.88)	60.00 (50.77)	65.00 (53.78)	70.00 (56.79)	75.00 (60.11)	80.00 (63.43)	92.50 (76.17)
	T_2	0.00 (0.00)	0.00 (0.00)	10.00 (18.43)	17.50 (24.53)	30.00 (33.21)	45.00 (42.12)	60.00 (50.77)	70.00 (56.79)	75.00 (60.11)	80.00 (63.43)	85.00 (67.50)	90.00 (71.57)	97.50 (85.39)	100.00 (90.00)
	T_3	0.00 (0.00)	10.00 (18.43)	20.00 (26.57)	37.50 (37.73)	45.00 (42.12)	62.50 (52.27)	72.50 (58.45)	80.00 (63.43)	85.00 (67.50)	90.00 (71.57)	97.50 (85.39)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	T_4	0.00 (0.00)	20.00 (26.57)	35.00 (36.22)	47.50 (43.56)	65.00 (53.78)	72.50 (58.45)	85.00 (67.50)	95.00 (80.78)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Lipid Media	T_1	0.00 (0.00)	0.00 (0.00)	7.50 (13.83)	10.00 (18.43)	20.00 (26.57)	30.00 (33.21)	40.00 (39.23)	57.50 (49.33)	62.50 (52.27)	67.50 (55.28)	72.50 (58.45)	77.50 (61.77)	82.50 (65.47)	95.00 (80.78)
	T_2	0.00 (0.00)	2.50 (4.61)	10.00 (18.43)	20.00 (26.57)	30.00 (33.12)	47.50 (43.56)	60.00 (50.77)	72.50 (58.45)	77.50 (61.77)	82.50 (65.47)	87.50 (69.53)	92.50 (76.17)	100.00 (90.00)	100.00 (90.00)
	T_3	0.00 (0.00)	10.00 (18.43)	22.50 (28.23)	37.50 (37.73)	45.00 (42.12)	65.00 (53.78)	72.50 (58.45)	80.00 (63.43)	87.50 (69.53)	92.50 (76.17)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	T_4	0.00 (0.00)	22.50 (28.23)	37.50 (37.73)	50.00 (45.00)	65.00 (53.78)	75.00 (60.11)	85.00 (67.50)	95.00 (80.78)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Modified Wout's media	T_1	0.00 (0.00)	0.00 (0.00)	7.50 (13.83)	12.50 (20.47)	22.50 (28.23)	32.50 (34.72)	42.50 (40.67)	57.50 (49.33)	62.50 (52.27)	67.50 (55.28)	72.50 (58.45)	77.50 (61.77)	82.50 (65.47)	95.00 (80.78)
	T_2	0.00 (0.00)	5.00 (9.22)	15.00 (22.50)	20.00 (26.57)	32.50 (34.72)	47.50 (43.56)	62.50 (52.27)	72.50 (58.45)	77.50 (61.77)	82.50 (65.47)	87.50 (69.53)	92.50 (76.17)	100.00 (90.00)	100.00 (90.00)
	T_3	0.00 (0.00)	12.50 (20.47)	25.00 (29.89)	40.00 (39.23)	47.50 (43.26)	65.00 (53.78)	75.00 (60.11)	82.50 (65.47)	87.50 (69.53)	92.50 (76.17)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	T_4	0.00 (0.00)	25.00 (29.89)	40.00 (39.23)	55.00 (47.88)	67.50 (55.28)	77.50 (61.77)	87.50 (69.53)	97.50 (85.39)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SEm±	0.00	0.927	1.151	0.943	0.597	0.600	0.638	1.023	0.554	0.910	0.786	0.861	0.771	0.881
	CD 5%	0.00	2.621	3.254	2.639	1.686	1.696	1.803	2.891	1.566	2.572	2.222	2.434	2.178	2.491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84hrs
96hrs
108hrs
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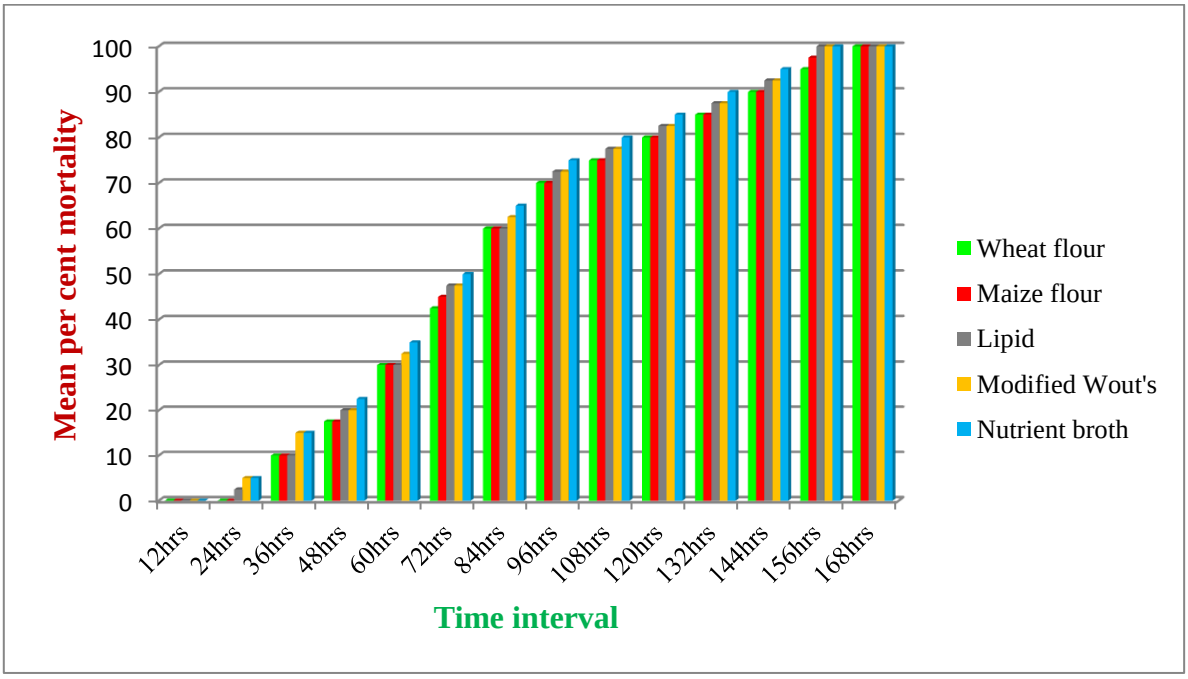
156hrs
168hrs
Time interval
Wheat flour
Maize flour
Lipid
Modified Wout's
Nutrient broth

A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Time interval
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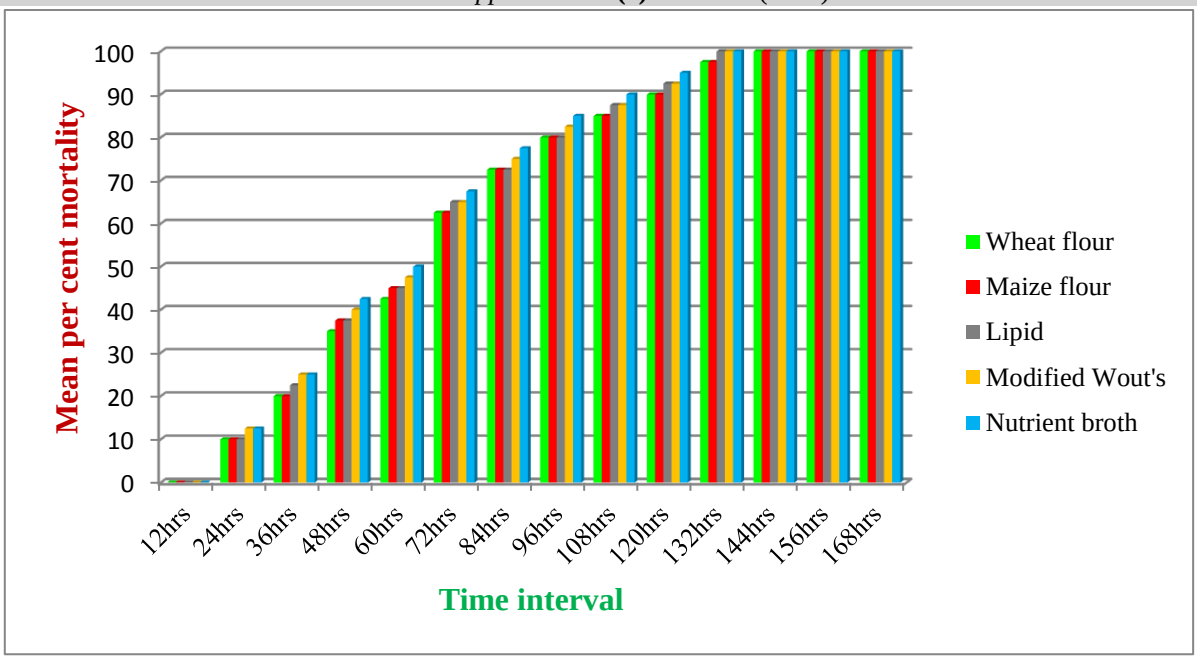
Maize flour
Lipid
Modified Wout's
Nutrient broth

B



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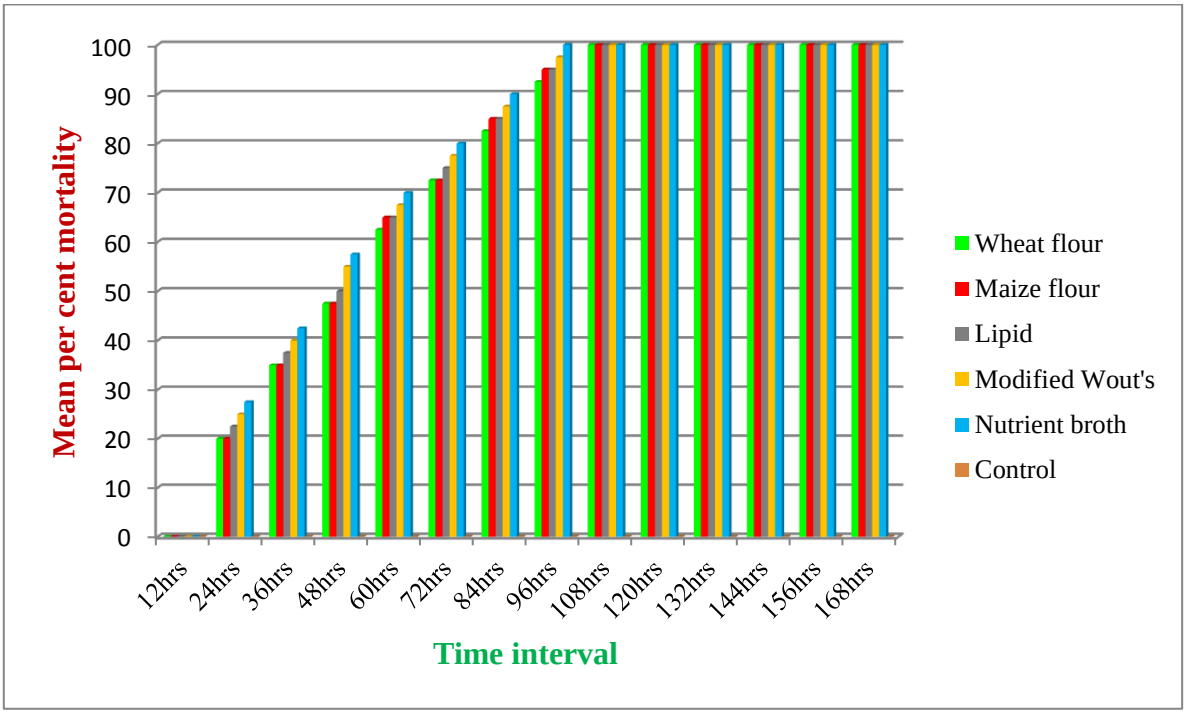

D

Fig. 3: Bio-efficacy of *S. carpocapsae* recovered from different artificial media at different inoculum levels (A. 100, B. 200, C. 300 and D. 400 IJs/petri plate) against *S. litura* under laboratory condition

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