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Entomopathogenic nematode as biopesticides on *Cicer arietinum* field

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Abstract
The gram pod borer, *Helicoverpa armigera* is a widespread polyphagous pest species of many agricultural and horticultural crops. As this pest has been recorded to be a serious pest, damage almost every vegetation like cotton, chickpea, pea, pigeon pea etc, Economic loss of near about 4500 crore. It has developed resistance to almost all conventional insecticides including synthetic pyrethroids. Therefore, to control this pest and the loss this investigation has been done to check the infectivity of Entomopathogenic nematode *Oscheius ciceri* on *Helicoverpa armigera*.

Keywords: *Oscheius cicero*, *Helicoverpa armigera*, Agro plus, Desiccated cadavers, *Cicer arietinum*

Introduction
Nematodes are found in almost all types of ecosystems and occur in unimaginable numbers in wide variety of shapes and sizes. They are termed based on habitat in which they are found like free living marine and freshwater, soil, saprophytes, parasitizing plant, microphagous or animals. Those nematodes which parasitize insects are regarded as Entomopathogenic nematodes (EPN). They are lethal obligatory parasites of insects; yet pose no threat to plants. The discovery of Entomopathogenic nematode species and the rate at which they have been described is correlated with the historical need for the biological alternatives to manage insect pests. After the initial discovery and subsequent development of *Steinernema glaseri* and *Oscheius shamimi* as a biological control agent in the early 20th century, research on Entomopathogenic nematodes remained somewhat dormant as chemical based pest control measures remained cheap, effective and relatively unregulated. (R. Gaugler 2002) [3]. As pesticides gave some negative effects on environment, they gradually became more and more restricted, less effective and much more costly. Entomopathogenic nematodes (EPN) of the genus *Steinernema*, *Heterorhabditis & Oscheius* (Rhabditida: Steinernematidae, Heterorhabditidae) are symbiotically associated with bacteria of the genus *Xenorhabdus*, *Photorhabdus & Alcaligenes faecalis* (Enterobacteriacae), respectively.

Materials and Methods
To extract nematodes, first we took soil samples from different localities. We kept them separately in perforated plastics boxes. All the boxes were labeled with locality and date. Insect larvae of same size and age were picked from insect culture. We use *Helicoverpa armigera* (Hubner), *Corcyra cephalonica* or *Galleria mellonela* larvae for this purpose.
The samples were processed by Cobb's (1918) [1] sieving and decantation technique. About 500 cc soil was placed in a bucket and thoroughly mixed with a small amount of water. The debris and stones were removed and soil lumps, if present, were broken by hand. The bucket was then filled with water to about 3/4th of its volume and then the suspension was stirred to make it homogeneous. The bucket was left undisturbed for about ½ a minute to allow the heavy soil particles to settle at the bottom. The muddy suspension was then poured in to another bucket through a coarse sieve (2mm pore size) which retained debris, roots and leaves. The suspension in the second bucket was then poured through a 300 mesh sieve (pore size 53 µm). The nematodes and find soil particles were retained on this sieve. The process was repeated thrice for better recovery of nematodes.

Isolation
The residue on the sieve on the sieve was collected into a beaker and poured on a small coarse sieve lined with tissue paper. The sieve was then placed on a Bearmann's funnel containing water sufficient to touch the bottom of the sieve and water level. The stem of the funnel was fitted with rubber tubing provided with a stopper. The nematodes migrated from the sieve into the clear water of the funnel and settled at the bottom. After about 24 hours a small amount of water was drawn from the funnel through the rubber tubing into a cavity block. The nematodes isolated as above were fixed and processed for mounting on slide.

Nematode culture: The four potential strains of *Oscheius nadarajni* were cultured in the fifth instar larvae of *G. mellonella* following the Dutky *et al*., (1964) [2] technique. The infective juveniles were collected using White trap method (White, 1927) [4] and were stored at 15ºC in BOD incubator for further analysis. The EPN 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 a known quantity of sterile distilled water for making the suspension according to the required number of IJs.

Results and Discussion
Treatment was done by EPN alone, along with carriers & also with different adjuvants. All the treatments were used and the effect was seen & calculated and then it was also showed that which treatment was more effective and can be use in future. The formulation made with the adjuvants

requires three things, an active ingredient, a carrier and an additive or adjuvant. An active ingredient is EPN, carriers are dust, coir, talc etc & additive or adjuvants are the material used to enhance the work of EPN, they can be anti desiccant, humectant, & UV protectant etc. Different adjuvants were mixed in adequate amount in the liquid culture of EPN & 10 formulations were made. These formulations then were tested for their effect on the survival & infectivity. The experiment was carried out with 3 replications. Survival of IJs of EPN was recorded at every 24 hours at 28- 29°C. The infectivity was tested against 3rd instar larvae of *H. armigera* by mixing adjuvants in distilled water 100 IJs / ml.

Table 1: Adjuvants used in the formulation

S. No	Adjuvants	Utility
1	Glycerin	Anti desiccant
2	Sugar solution	Phagostimulant
3	Robin blue	UV protectant
4	Agro plus	Humectants

Various adjuvants at different concentration were combined with given EPN & check for their effect. The adjuvants at different concentration (0.1 to 1.5) Agro plus (non toxic easily available humectants which increase the effect of EPN), glycerin, Robin blue & sugar solution (phagostimulant which allow formulation to spray evenly on the foliage) were taken in optimum concentration of aqueous solution of EPN & four formulations were made. Then these were tested for their effect. The formulations of EPN were tested against 3rd instar larvae of *Helicoverpa armigera.* Lab experiments was carried with 3 replications. 1 g of each preparation was mixed with 99ml of water & sprayed on 3rd instar larvae of *Helicoverpa armigera.* In laboratory in the UV sterilized vial larvae were placed to avoid the growth of microorganisms and each vial contains formulation treated filter paper & the larval mortality was then recorded after every 24 hours.

EPN formulations against *Helicoverpa armigera* in chickpea

Chickpea or chana is important pulse crop that grows as a seed of a plant named *Cicer arietinum* in the Leguminosae family. This light brown colored pulse is considered to be a good source of protein for human and also used as fodder for cattles. Chickpea is a highly nutritious pulse and places third in the importance list of the food legumes that are cultivated throughout the world. It contains 25% proteins, which is the maximum provided by any pulse and 60% carbohydrates. Entomopathogenic nematodes (EPN) of the family Steinernematidae and some nematode of family Rhabditidae like species from genus *Oscheius* recently *Oscheius ciceri* is recognized as potential bio-control agents against some of the agriculturally important insect pests. An attempt was made to study the survival of *Oscheius ciceri* on chickpea after foliar spray at fruiting stage through introducing adjuvant at high temperature regimes.

The experiment was conducted at the fields of Gymnasium M.A.J.U Rampur during 2018-19 to check the efficacy of EPN formulations against insect infesting chick pea. The variety G 10 was sown at a spacing of 5cm between plants & 10cm between the rows. The crop was raised following agricultural practices but no insecticide was used. The formulations applied at the interval of 8 days by spraying along with mixed adjuvants and sodium bicarbonate was added to nullify the effect of pH persuade on chick pea leafage. The experimental details are as follows

Experimental details

Location	:	Gymnasium, M.A.J. U Rampur
Year	:	2018
Sowing date	:	18 – 10- 2018
Date of spraying	:	15 -12- 2018
Crop	:	Chick pea
Variety	:	G 10 & abrodha 256
Plot size	:	4x4 m^2

The effect of EPN with carriers & adjuvants on *H. armigera* larvae in different concentration is presented in given table.

Experiment

The experiments are done with the formulation consisting a carrier & an additive along with the EPN. In first treatment EPN with dust (carrier) & agro plus, sugar solution, robin plus & glycerin (adjuvants or additive) concentration of 205.62ml/ 16m^2, the second treatment was done with the same EPN & adjuvants but different carrier at the same concentration, in all the further treatments only carrier was different or no carrier, the concentration, adjuvants & EPN used was same. Then the mortality was calculated as shown in the table below.

Table 2

T. No	Treatment	Concentration	Mortalilty %
1	*O.ciceri*+ dust+agro plus+ sugar solution+ robin plus+ glycerin	205.62ml/ 16m^2	46%
2	*O.ciceri*+ coir +agro plus+ sugar solution+ robin plus+ glycerin	205.62ml/ 16m^2	67%
3	*O.ciceri*+ talc+agro plus+ sugar solution+ robin plus+ glycerin	205.62ml/ 16m^2	45%
4	*O.ciceri*+ desiccated cadavers+agro plus+ sugar solution+ robin plus+ glycerin	205.62ml/ 16m^2	78%
5	*O.ciceri*+ soil +agro plus+ sugar solution+ robin plus+ glycerin	205.62ml/ 16m^2	54%
6	*O.ciceri* + agro plus+ sugar solution+ robin plus+ glycerin	205.62ml/ 16m^2	95%

Observation recorded

After the treatment with formulations mentioned above the mortality with the EPN mixed with agro plus, sugar solution, robin plus & glycerin was seen the highest among all other formulations. The treatment with dust & all other adjuvants mortality was 46%, with coir & adjuvants 67%, with talc & adjuvants it was 45% recorded, with desiccated cadavers & adjuvants 78% mortality, with soil 54% mortality along with the adjuvants, with the EPN alone & adjuvants the mortality was 95% recorded. Thus it was proved that the improved formulation by the addition of Agro plus as humectants, glycerin as anti desiccant, robin blue as UV protectant & sugar solution as phagostimulant aimed at prolonging IJs survival & worked so well.

Table 3

Treatment	Pod damage kg	Yield per plot
O.ciceri+ dust+agro plus+ sugar solution+ robin plus+ glycerin	22.33kg	28.22 kg/plot
O.ciceri+ coir +agro plus+ sugar solution+ robin plus+ glycerin	13.00kg	67.99 kg/plot
O.ciceri+ talc+agro plus+ sugar solution+ robin plus+ glycerin	20.72kg	43.78 kg/plot
O.ciceri+ desiccated cadavers+agro plus+ sugar solution+ robin plus+ glycerin	11.11kg	78.67 kg/plot
O.ciceri+ soil +agro plus+ sugar solution+ robin plus+ glycerin	18.77kg	45.5 kg/plot
O.ciceri alone+ agro plus+ sugar solution+ robin plus+ glycerin	7.33 kg	91.58 kg/plot
Control	55.25kg	18.44 kg/plot

Conclusions

The yield after the formulation spray was seen much higher than the yield before the spray that is it has increased from 18.44 kg to 78.67kg, the pod damage decreases from 55.25g to 7.33g which is a big margin. The formulation worked very well as calculated in table above. These formulations were highly effective in management of *Helicoverpa armigera* & thus create a new opportunity for its utilization as biocontrol agent against the same.

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