Bioefficacy of *Oscheius nadarajni* against *Helicoverpa armigera* in *Vigna mungo* field

IRAM KHAN TAHIR
Research Scholar, Department of Zoology
Mohammad Ali Jauhar University
Rampur (U.P.) India

Dr. AZRA SHAHEEN
Assistant professor of Zoology
Mohammed Ali Jauhar University
Rampur (U.P.) India

Abstract

The objective of this paper is to improve the EPN formulation process based on the scientific and technological research developed so far. Entomopathogenic nematode formulation technology has made significant progress in past 15 years. The results show great progress in the EPN survival time, the mortality of insect pest, decrease in pod damage and the increase in crop yield. Also, EPNs formulated and applied as insect cadavers showed higher efficacy for the pest control than the EPNs applied in an aqueous solution. Major challenges have included the development of room-temperature shelf stability, ease of use, and contamination control.

Keywords: Adjuvants, *Vigna mugna*, formulation, *Oscheius nadarajni.*

Introduction

The nematode species which are endoparasitic on insect pests are broadly termed as **Entomopathogenic nematodes (EPN).** Entomopathogenic nematodes possess many attributes of an excellent biological control agent. They have a broad host range and are virulent, killing the host rapidly (within 24-72 h). They can be produced in large quantities on artificial media and are easily applied with standard spraying equipments or irrigation systems (Shapiro-Ilan & Gaugler 2002). These nematodes can also provide effective control of some agriculturally important lepidopteran, coleopteran and dipteran pests (Ghode et al. 1998; Karunakar *et al.*1999b; Banu & Rajendran 2002; Shapiro-Ilan & Gaugler 2002; Vyas *et al*. 2002). However, there is scant research work on pod borer control by the use of Entomopathogenic nematodes. Selection of an EPN for control of a particular pest insect is based on several factors that include the nematodes' host range, host finding or foraging strategy, tolerance of environmental factors and their effects on survival and efficacy (temperature, moisture, soil type, exposure to ultraviolet light, salinity and organic content of soil, means of application, agrochemicals, and others). The 4 most critical factors are moisture, temperature, pathogenicity for the targeted insect, and foraging strategy (Kung *et al.,* 1991; Kaya and Gaugler, 1993; Campbell et al., 2003; Grewal *et al.,* 2005a). Within a favourable range of temperatures, adequate moisture and a susceptible host, those EPNs with a mobile foraging strategy (cruisers and intermediate foraging strategies) could be considered for use in subterranean and certain above-ground habitats (foliar, epigeal and cryptic habitats). Those with a sit and wait foraging strategy (ambushers) will be most effective in cryptic and soil surface habitats. (Lacey; Georgis 2012). As compare to chemical control, biological control using EPN has several positive attributes in agriculture environmentally and nutritionally. Biological control program can be effectively implemented. Application of chemical pesticides/insecticides, fungicides etc. have health concern issues which eventually diminish as more bio-control agents are identified and applied. This could also be helpful in non-resistance development and effective to suppress pests of agricultural problems (Georgis et al., 2006).

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)| 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) technique. The infective juveniles were collected using White trap method (White, 1927) 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

Mass production of EPN

Entomopathogenic nematodes (EPN) were baited out and multiplied on host insects. Three host species, *viz Galleria mellonela, Corcyra cephalonica and Helicoverpa armigera* have been found to be good hosts for *in vivo* production of EPN. These host insects turn are multiplied on semi synthetic diet. The method of preparation of diet and mass multiplication of host insects were:

Galleria mellonela: Mass culture of *G. mellonela* was done and maintained in laboratory. Eggs and larvae of *G. mellonela* were purchased from NBAIR bangaluru and reared in laboratory. The ingredients used for the artificial diet are as follows:

Table 1

Part A

Corn flour	150g
Nestle Milk powder	100g
Gram flour Part B	30g
Glycerin	80 ml
honey	50 ml

Part A is mixed separately and part B is mixed separately and finally part A and part B mixed together thoroughly and a homogenous mixture was prepared. The content of artificial diet was distributed in plastic containers. 1st and 2nd instar *G. mellonella* larvae were released in each container at 35 ° C. The larvae were ready for use within three weeks. If the temperature was < 35 ° C, development of larvae was slow. After 2-3 weeks, the larvae were drawn for the multiplication of EPN. Some of the *G. mellonella* larvae were left to complete their life cycle and emergence of moth. These moths were collected and put in separate jar in which several small pieces of honey comb were provided with folded paper strips which were hanged serving as dark area for hiding and substrate for egg laying. If the temperature was> 30 ° C, then changes of drying of eggs or delay in egg hatching was expected. The neonate larvae of honey comb or eggs collected from paper strips were released in artificial diet. About 500 larvae of *G. mellonella* were produced during year.

Corcyra Cephalonica: Culture of this insect was maintained on sorghum grains. The sorghum grain was

heat sterilized in oven at 80° C for 30 minutes and then crushed into pieces. About 1 kg broke grain was put in plastic containers and treated with 0.1% formaline to prevent the growth of mould. About 1.0 cc Corcyra eggs were mixed with this perforated lid was secured and kept for about a month at a temperature of 28-30° C. the fully grown larvae were utilized for the multiplication of EPN. Some of them were left for emergence of moth which were collected and transferred to a specially designed oviposition plastic container. The eggs were collected and put in fresh broken grains for further culture about 5000 larvae of *Corcyra cephalonica* were produced during year.

Helicoverpa Armigera: The ingredients for artificial diet of *H. armigera* were:

Table 2

Ingredients	Quantity
Chick pea flour	80g
Yeast	10g
Sorbic acid tablets	3
Multi Vitamin	5ml
Ascorbic acid	3g
Vegetable oil	5g
Distilled Water	500ml

Sorbic acid tablets were grinded into fine powder ad mixed with chickpea flour, yeast, ascorbic acid. Then multi vitamin, vegetable oil and distilled water were thoroughly mixed in a grinder. Thereafter, the ingredient was poured in petri plates and kept at room temperature to cool down. The diet was ready to use. *H. armigera* larvae were collected from field of chickpea or sweet pea and maintained on artificial or natural diet under laboratory conditions. Moths emerging from pupa were kept in egg laying jar and eggs were left for hatching. Freshly hatched *H. armigera* larvae were transferred with the help of horse hair brush to the diet and sealed properly with cotton wool if kept in vial or covered with lid of Petri plates sealed with parafilm. After 7-8 days, the larvae was removed for trial or reared individually to prevent cannibalism about 3000 larvae are produced during year.

Maintenance of Entomopathogenic Nematodes

Cleaning and surface sterilization of instruments like Forceps, needles, scalpels and glassware like pipettes and petri dishes, watch glasses were done before use. Lab cultures of all isolates of EPN were maintained on final instar larvae of *G. mellonella, H. armigera and C. cephalonica.* Nematodes were multiplied using the methods of woodring and kaya (1988). Modified white trap was prepared and emerging infective juveniles (1Js) from dead larvae of host insect were extracted and stored at 5° C in distilled water for future use.

Methods for transforming the EPN into various Formulations

The fresh EPN was used for every new formulation in all carriers. EPNs were harvested in culture flask. 250g each of carrier material were taken in 500ml flask containing 25ml distilled water & 3 flasks were maintained for further use. These flasks were inoculated with EPN suspensions. The flasks content were then transformed for the incubation at room temperature. The experiments were carried out with 3 treatments & 3 replications. Treatment was done by EPN along 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 require, an active ingredient and an additive or adjuvant. An active ingredient is EPN & additive or adjuvants are the material used to enhance the work of EPN, they can be anti desiccant, phagostimulant & UV protectant etc. Different adjuvants were mixed in adequate amount in the liquid culture of EPN & formulations were made. These formulations then were tested for their effect on the survival & infectivity. 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 3: Adjuvants used in the formulation

S. No	Adjuvants	Utility
1	Glycerin	Anti desiccant
2	Sugar solution	Phagostimulant
3	Robin blue	UV protectant
4	Sodium bicarbonate	pH regulator

Various adjuvants at different concentration were combined with EPN & check for their effect. The adjuvants at different concentration (0.1 to 1.5) 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 & 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 were 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.

Field Efficacy EPN against *Helicoverpa Armigera* Infesting *Vigna Mungo* (urd)

Vigna mungo is one of the most widely used pulse crop of India. It is commonly called as urd. It is a fast growing herbaceous plant. It is one of the most highly priced pulses of India. Dried seeds of urd contain 23.4% protein, 57.3% carbohydrates, 1.0% fats, 3.8% fibres & 4.8% minerals. They are especially rich in phosphoric acid.

Entomopathogenic nematodes (EPN) of the family Steinernematidae and some nematode of family Rhabditidae like species from genus *Oscheius* recently *Oscheius nadarajni* is recognized as potential bio-control agents against some of the agriculturally important insect pests (Kaya & Gaugler 1993; Ali et al., 2005a, 2005b). An attempt was made to study the survival of *Oscheius nadarajni* on urd after foliar spray at fruiting stage through introducing adjuvant at high temperature regimes.

Experimental Details:

Location	:	Gymnasium, M.A.J.U Rampur
Year	:	2019
Number of replication	:	Three
Number of treatments	:	3
Sowing date	:	2 -6 -2019
Date of Drenching	:	10- 8- 2019
Crop	:	urd
Variety	:	Shimla
Plot size	:	4m x 4m

Formulation Applied to the Field

To test the bioefficacy of *Oschieus nadarajni* against *H. armigera* in urd bean field, foliar application was made on urd bean variety, shimla at MAJU, Rampur during july august 2018-19. Liquid suspension contained *Oscheius nadarajni* IJs, 1% glycerin, 0.01% robin blue, sugar solution and 0.5% sodium bicarbonate. Three nematode applications were made at 10-days interval (8, 20 and 30 july 2019) during fruiting and podding stage using hand sprayer in urd field. There were three treatments (1, 2 or 3 × 10^3 IJs/plot) having 3 replications in each. In the control set, only distilled water was sprayed.

Table 4

Treatment	Concentration	Mortality
O.nadarajni +glycerin+ robin blue + sugar sol+ sodium bicarbonate	1 × 10^3 IJs/ 100ml	75%
O.nadarajni +glycerin+ robin blue + sugar sol+ sodium bicarbonate	2 × 10^3/ 100ml	86%
O.nadarajni + glycerin+ robin blue + sugar sol +sodium bicarbonate	3 × 10^3/ 100ml	94%
Control	-	-

Observation Recorded

In these experiments the treatment with *O. nadarajni*, glycerin, sugar solution, robin blue & sodium bicarbonate with the concentration of 1 × 10^3 IJs/ 100ml the mortality was 75% recorded. The treatment with *O. nadarajni*, glycerin, sugar solution , robin blue & sodium bicarbonate with the concentration of 2 × 10^3 IJs/ 100ml, 86% mortality. The treatment of *O. nadarajni* with glycerin, robin blue, sugar solution & sodium bicarbonate with the concentration of 3 × 10^3 IJs/ 100ml marked the highest mortality with 94%.

Table 5

Treatment	Pod damage %	Yield kg/plot
1x10^3 + glycerin + robin blue +sugar sol+ sodium bicarbonate	22%	22.24 kg/plot
2x10^3 + glycerin + robin blue+ sugar sol	12%	33.12 kg/plot

+sodium bicarbonate		
$3x10^3$ + glycerin + robin blue+ sugar sol +sodium bicarbonate	10%	36.55 kg/ plot
Control	28%	15.88 kg/plot

Observation Recorded

Highly significant results were obtained with respect to % pod damage and grain yield. The lowest pod damage (10%) was recorded by the foliar application of *O.nadarajni* at 3 × 10^3 IJs/plot + antidesiccant + UV retardant + sugar solution followed by 12, 22 and 28% pod damage by treatment of 2 and 1 × 10^3 IJs/plot, and control, respectively. With the application of 3 × 10^3 IJs/plot, urd bean yield of 36.55 kg/plot was obtained resulting in 43.46% increased yield over the untreated control.

Conclusions

In the present programme utilizing EPNs as a component, some special considerations are needed. Overall objective of the present investigation was to evaluate the effectiveness of locally isolated nematodes in controlling *H. armigera* in the field. *O. nadarajni* ($3x10^3$ IJs) along with the glycerin, robin blue, sugar solution & sodium bicarbonate found much more effective than the *O. nadarajni* at $2x10^3$ IJs & *O. nadarajni* at $1x10^3$ IJs but all the formulations were effective and help in increased yield and decreased pod damage. *O. nadarajni* proved effective against *H. armigera.* So it can be used in future to regulate the effect of the insect pests on the crop.

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