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Formulation of entomopathogenic nematode

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Abstract
Entomopathogenic nematode used as biocontrol is formulated to be sprayed on the foliage and increased the yield and decrease the pod damage. In this paper the formulation is briefly described. The formulation of Entomopathogenic nematode is done in various methods like dust, talc, desiccated cadavers, liquid formulation etc. Among all the formulation liquid formulation was turn out the best. Many formulations are still being prepared for further use and Ph.D. Programme.

Keywords: desiccation, entomopathogenic nematode, formulation, *Oscheius* species*, WDG*

Introduction
The nematodes are highly diversified, perhaps most numerous multicellular animals on earth. They are either free living or parasitic species are of considerable agricultural, clinical and veterinary importance as pests of plants and parasites of human and livestocks. 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). EPN on the other hand are beneficial nematodes parasitizing crop insect pests, and are used as a bio pesticide agents a wide variety of insect pests.

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 mellonella* 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 Baermann'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* sps. were cultured in the fifth instar larvae of *G. mellonella* following the Dutky *et al*., (1964) [3] technique. The infective juveniles were collected using White trap method (White, 1927) [5] 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 Discussions
Methods for transforming the EPN into various Formulations
Soil
EPN mixed with the soil can also be stored in sealed polythene bags at normal condition for a pretty long time. This type of storage requires less space and bags can be stacked over one another. There was no loss of moisture in the formulated product. However, due to depletion of oxygen, the infectivity of EPN declines fast as compared to open container like earthen pot, etc. Concentrated EPN were mixed with soil and put either in earthen pot and covered with muslin cloth are in polythene bags. Normal soil has been found better than sterilized soil and their infectivity declined gradually but was of tolerable level up to 15-18 weeks in polythene bags where as in earthen pots were need

based moisture can be added the population remained active up to 36 weeks and still the trail is containing.

Desiccated cadavers

Desiccated cadaver coated with clay was developed that allow storage without rupturing cadavers or adhering together. Such desiccated cadavers are put in water overnight before spray and EPN comes out of it and used as sprays, mixed with irrigation water or dispersed in soil. Cadavers of *Corcyra cephalonica, Helicoverpa armigera* were used for cadaver formulation. There is ease of handling, storage, transport etc. In this type of EPN storage. However, there was gradual depletion of moisture from the cadaver and they are only good for storage for a period of 4-12 weeks only.

Liquid formulation

EPN culture obtained *in vivo* and *in vitro* multiplication were kept aerated water solution and at the time of spray, sugar solution as phagostimulant 0.5%, robin blue as ultraviolet protectant 0.01% were added in water containing EPN. Liquid formulation so prepared can be sprayed with hand sprayer. Aerated water solution can be kept for 24- 30 weeks and viability will be 80- 90%.

Dust

Dust formulation based on inert carrier materials talc and bentonites were tried out. So far good viability was recorded after storage of 12 weeks in both the carrier formulations.

Talc formulation

100 ml of freshly harvested IJs of *Oscheius* species (2000 IJs/ ml) were added in the Talc powder (500 g) was added to 50 ml of distilled water in a 1000 ml beaker and mixed thoroughly and then the contents were thoroughly mixed till the nematode suspension spread over evenly into the talc.

Water dispersible granules (WDG) formulation

A water dispersible granules formulation (WDG) has been developed in which 1Js were enclosed in 10-20mm diameter granular matrix. This allows access of oxygen to nematodes, which enter into partial anhydrobiotic static due to the slow removal of body water by substrate. The shelf life studies are in progress and results are encouraging.

Mass production of EPN

Entomopathogenic nematodes (EPN) were baited out and multiplied on host insects. Three host species, *viz Galleria mellonella, 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. It has been discussed before..

Standardization of density of *Oscheius* sp. as bio ingredient in the different carriers at 27 ℃

For the preparation of formulation in different carriers all the time the Fresh EPN culture was used. In sterilized tissue culture flask the EPN was harvested and 100 ml each stored as sample in 250 ml capacity conical flasks which are sterilized. In 500 ml capacity flasks 250 g materials were taken and 25 ml distilled water is added and three such flasks were taken each for nematode population replication. These flasks were sterilized in an autoclave for 25 minutes and then left to cool. In flasks different densities of EPN suspension were inoculated from 1x 10^1 to 1x 10^3 at 50 ml and mixed well. Content in the flasks were then transferred to the bags for checking viability test for 30 days.

Viability test

The viability of EPN in different formulations was tested for 30 days before it is applied to the crop. 1 gram each EPN formulation was taken into 50 ml capacity flasks with 8 ml distilled water mixed well for uniform distribution of nematode. The mean data on % survival IJs was worked out. Immovable and straight nematodes are considered as dead whereas movable and round or curls are considered to be alive. In this programme experiments are taken out in 5 treatments.

Table 1

Sr. No	Formulation	Nematode density/5g
1	Dust	$1x10^1$,$1x10^2 1x10^3$
2	Desiccated cadavers	$1x10^1 1x10^2 1x10^3$
3	Soil	$1x10^1 1x10^2 1x10^3$
4	Liquid	$1x10^1 1x10^2 1x10^3$
5	Talc	$1x10^1 1x10^2 1x10^3$
6	Water dispersible granules(WDG	$1x10^1 1x10^2 1x10^3$

The carriers, dust, WDG, talc, desiccated cadavers, soil & liquid formulation were used with regard to storage & survival of the infective juveniles of different EPN (*O. ciceri & O. nadarajani*) at different period of intervals from 1st week to 5th week of storage. And then used on the field of *Pisum sativum, Vigna mugno & Cicer aritenum.*

Table 2

T. No	Treatment	Concentration	% Mortality
1	*O.sps*+ dust	100 IJs + 4g	23
2	*O. sps* + WDG	100 IJs+ 4g	28
3	*O. sps* + talc	100 IJs+ 4g	24
4	*O. sps* + desiccated cadavers	100 IJs+ 4g	37
5	*O. sps* + soil	100 IJs+ 4g	20
6	*O. .sps* alone	100 IJs	48

Observation recorded

Observations on larval population were recorded at 6 randomly selected plants. Counts were taken both days before & after the spray. Six treatments were done with different carriers & the mortality percent were then checked. EPN *O.sps* was taken with dust, WDG, talc, desiccated cadavers, soil & also used alone, *O.sps* used with dust at 100IJs +4g effect the survival at 23% mortality which is somewhat lesser than other treatment. *O.sps* used with WDG at the same concentration gives mortality percentage upto 28%. *O.sps* used with talc at the same concentration give 24% mortality, *O.sps* used with desiccated cadavers gives 37% mortality and with soil mortality decreases to 20% which is the least mortality but the *O.sps* alone was very much effective as it has 48% mortality. These results were the outcomes of EPN with carriers only. The increased mortality of insect pests result in the decreased pod damage. In further experiments the adjuvants were mixed with the same carriers & EPN.

Conclusions

In the present programme utilizing EPNs as a component, some special considerations are needed. Ultraviolet radiation and dehydration are considered prime mortality

factors resulting in 40-80% mortality or even more (Smits 1996) whereas relative humidity and temperature during and up to 8 h post-application were also predicted to influence rates of nematode infection obtained (Arthurs *et al*. 2004) [1]. In present study, some new molecules acting as phagostimulant and UV retardant has been incorporated, if used along with nematode, can extend their survival on leafage. Dust formulation was also tried out but no progress was reported.

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