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FORMULATION TECHNOLOGY OF ENTOMOPATHOGENIC NEMATODE FOR THE CONTROL OF THE COTTON BOLLWORM, *HELICOVERPA ARMIGERA*

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ABSTRACT

Entomopathogenic nematode formulation technology has made significant progress in the past few years. Successful control of insect pests through the application of entomopathogenic nematode, *Heterorhabditis indica* can only achieved when the nematode reaches the end user in good condition. Storage and formulation techniques must provide optimum conditions to guarantee a maximum survival and infectivity of the nematodes. Hence, six innovative nematode formulations developed in the laboratory (sawdust, hydrogel, coirdust, talc, sponge and water) were evaluated its survival at 27 ± 2 °C and their pathogenicity assessed against cotton bollworm, *Helicoverpa armigera*. Sawdust (95%) and hydrogel (85%) formulations were enhanced highest survival than the other formulations (coirdust (80%), talc (75%), water (70%) and sponge (65%)) till 5th week period. At the end, a maximum shelf-life of more than 11 week periods (75 days) achieved in a water dispersible hydrogel formulation with 65% of highest survival than sawdust (15%) formulation. Accomplishment of *Heterorhabditis indica* survival and virulence under formulation was lead to cause 85% and 70% pathogenicity on *Helicoverpa armigera* in hydrogel and sawdust respectively, exposed for 48 h treated 100 infectives /larva.

KEYWORDS Biocontrol, formulation, *Heterorhabditis indica,* cotton bollworm, *Helicoverpa armigera*

INTRODUCTION

The current millennium demands that the pest management studies should be bio-intensive. One of the methods in recent years that have gained increased attention is the use of biopesticides in order to develop environment friendly, safe and compatible approaches and tactics for pest management. Most of these attributes do not harm the natural enemies and play a significant role in pest management systems that seek to reduce pesticide inputs and conserve natural fauna. The genus *Helicoverpa* contains the most damaging insects to destroy agriculture crop worldwide. In the India, *Helicoverpa* (= *Heliothis) armigera* (Hub.) attacks a wide variety of cultivated crops and this insect is known by different common names such as; cotton bollworm, tomato and brinjal fruit worm, pigeonpea and chickpea pod borer, world old worm, and soybean pod worm. Corn is its preferred host in which *H. armigera* larvae feed in the pod, leaf, flower, the tassel, silk, and grain, which often results in the introduction of secondary pests and/ or molds. The primary control strategy for *H. armigera* is the application of insecticides that result in egg and larval mortality. Because of development of insect resistance to several insecticides and concern about environmental damage resulting from chemical pesticides there is an increased interest in biological control by the researchers and scientists. Entomopathogenic nematodes of the genera steinernema and heterorhabditis possess most of the characteristics of an ideal biological control agent for insect control. The pathogenicity of entomopathogenic nematodes to *Helicoverpa sp.* has been demonstrated previously (Armes *et al.* 1992; Bong 1986). However, their infectivity is quite different depending on entomopathogenic nematode species and developmental stage of the insects (Bong *et al.*1983; Divya *et al.* 2010). At present, the utility of these entomopathogenic nematodes against larvae, pre-pupae or pupae stages of insect pest has been limited by the farmers due to the lack of awareness, non-availability of suitable formulation and application technologies. The purposes of this study were to determine the suitable formulation technologies developed for *Heterorhabditis indica* in the laboratory to be used as soil and foliar applications and to estimate the survivability of infective juveniles (IJs) and its lethal effect on *Helicoverpa* larvae.

MATERIALS AND METHODS

Nematode culture

Commercially available entomopathogenic nematode (EPN), *Heterorhabditis indica* "Soldier" was procured from Multiplex Biotech Pvt. Ltd., Bangalore (INDIA) and cultured them on final instar larvae of *Galleria mellonella* (Elanchezhyan 2006). Emerged infective juveniles (IJs) were harvested through modified White trap's method (Glazer and Navon 1990) and stored them in tissue culture flasks (250 ml) at of micro-filtration assembly for 2 days at 27 ± 2°C in the laboratory before use in the experiments.

Insects' culture

The test insect, cotton bollworm, *Helicoverpa armigera* larvae was initially collected from pigeonpea/ chickpea fields in and around Andarasanahalli village, Tumkur Dt. (Karnataka-India) reared them individually on chickpea based semi-synthetic diet in a cylindrical transparent plastic cup (15 cm ht. x 2.4 cm dia.) capped with flexible, polyethylene lids with small slits for aeration as described at room temperature (27 ± 2 °C and 65 + 2% RH) (Grewal 1998). Final instar larvae used in the study were collected 30 to 35 days post eclosion and weighed an average of 425 mg.

PREPARING ENTOMOPATHOGENIC NEMATODE FORMULATIONS

a) Talc formulation

A new wettable powder formulation was prepared by using talc powder purchased from local market at Bangalore (Karnataka). Twenty five millilitre of distilled water added in 250 g of talc powder in a 500 ml beaker, they were mixed thoroughly with help of glass rod. Fifty ml of freshly harvested IJs of *H. indica* @ 1 lakh/ml were added in the moisten talc then the contents were thoroughly mixed systematically till the nematodes suspension spread over evenly into the talc. Such kind of 10 replications were prepared in a polythene envelop and sealed them individually for further survival and pathogenicity observation.

b) Coir pith and saw dust formulation

A readymade coconut coir brick was purchased from a coir industry at Erode (Tamil Nadu) and saw dust/ powder was obtained from local saw industry at Tumkur (Karnataka). Both the raw material was grinded separately to get fine dust with help of domestic mixer and they were sieved with fine mesh, sterilized under sun light (1 hrs.. Hundred grams of each was moistened adding 50 ml of distilled water separately. IJs suspension of 50 ml @ 1 lakh/ml were added evenly and mixed them gently till nematodes spread over into the coir and the same procedure was followed to saw dust powder too and sealed them individually in a plastic container, stored for further observation.

c) Sponge formulation

Two gram sponge pieces (4 cm. length X 2 cm. height X 2 cm. width) were washed thoroughly in tap water and autoclaved for 10 min. Infective juveniles of *H. indica* impregnated by squeezing in 50 ml nematode suspension (1 lakh/ml) and they packed in a suitable polythene cover, sealed them smoothly.

d) Gel formulation

Hydrogel granules obtained from Hyderabad (Andhra Pradesh) local market. One gram of hydrogel granules were directly mixed by adding 50 ml IJs suspensions (@ 1 lakh/ml) and distribution of IJs evenly in hydrogel content were done with help of a glass rod and sealed them finally in a polythene paper.

e) Water

Freshly harvested IJs were washed twice in distilled water and 50 ml @ 1 lakh /ml were stored in 250 ml conical flask. Flask was closed with non absorbent cotton which was used in the experiment as control treatment.

BIOASSAY STUDY

Survival of Heterorhabditis indica

Survival infective juveniles in various formulations were evaluated by a weekly interval diluting 0.5 g of formulated IJs in 5 ml distilled water from each and the percent IJs survival were counted by a *Syracuse* counting dish method by repeating eight times under Nikon SMZ 800 (Japan) stereozoom binocular microscope and the percent mean data of survived IJs were worked to draw a conclusion from each formulation.

Pathogenicity test

The Petri plate bioassay procedure was used to determine the pathogenicity of this nematode against *Helicoverpa armigera* larvae. Survived IJs of entomopathogenic nematode, *Heterorhabditis indica* from each formulation was tested their pathogenicity on final instar larva of *H. armigera* inoculating 100 active IJs/ larva releasing on a Whatman's No-1 filter paper lined Petri plate (4.5 dia.). Bioassay test was done weekly interval followed by survivability of nematode IJs. A set of freshly harvested infective juveniles were tested as control treatment at an every week. The Petri dishes were subsequently placed in a plastic bag and incubated in the dark at room temperature (27 ± 2°C) for 5 days. There were 25 replicates were prepared for each formulations and this experiment was repeated twice under the same condition. After five days, all dead larvae were individually transferred onto White trap dishes (White 1929) and held an additional 7 days for nematode multiplication. The insects were then examined for the presence of nematode progeny and estimates of the number of infective juveniles further. The percent helicoverpa larval mortality data was worked out from the mean 25 larval replications of each treatment at an every test and the entire experiment was repeated twice to confirm the results. Analysis of variances (ANOVA) was carried out using SAS software version 6.12 and means were separated by Duncan's Multiple Range Test (DMRT). Data obtained in a percent survival of IJs were

transformed to arcsine and the data from percent larval mortality induced by *H. indica* nematodes was subjected to Square root transformation and then analysed.

RESULTS AND DISCUSSION

Survival of H. indica in various formulations

Among the formulations, there was no significant difference in percent survival of IJs upto 5 week periods of test at room temperature (27°C ± 2). The results of percent IJs survival data from the six various formulations are illustrated (Figure. 1) and no IJs mortality was recorded in the first two week periods in all the formulations. Among the six formulations sawdust and hydrogel showed significantly highest percent IJs survival (95% and 85% respectively) than the other formulated IJs *viz.*, coir dust (80%), talc (75%), water (70%) and sponge (65%) in the 5th week of test. The percent IJs survival in six formulations were resulted showing decreasing trend and it was negatively correlated with an increasing time. However, more than 50% of IJs survival was recorded in all the formulations on 6th week however, it was recorded only 35% in distilled water (control). A maximum room temperature shelf-life of more than 11 weeks (75 days) has been achieved for only *Heterorhabditis indica* in a water dispersible hydrogel formulation showing 65% highest survival than sawdust (15%) formulation. Though, the IJs formulated in talc and sponge was lead to survive 12% and 6% respectively on 10th week, however, the IJs formulated in coir and water (control) ceased their survival on day 9th and 8th week respectively (Table-1).

Pathogenicity of EPN, Heterorhabditis indica to Helicoverpa armigera larvae

Among the treatments, the percent mortality on helicoverpa larvae caused by *H. indica* from various formulations showed significantly different however, it was invariably recorded 100% larval infectivity during the 1st two week periods. The highest percent larval mortality was recorded by the IJs formulated in hydrogel and sawdust and there were no significant differences among them till 7th week (varied from 75% and 70% respectively). Thereafter, the IJs had sudden changes in causing helicoverpa larval mortality and it was reduced to 65% and 25% respectively, on 11th week of pathogenic test (Figure. 2). However, IJs formulated in sponge, talc and coir dust showed better infectivity till 8th week under formulation and the larval mortality was recorded 80%, 70% and 50% respectively, thereafter, it was declined to 75% by sponge and 70% by talc formulated IJs during 9th week and 40% and 35% respectively on 10th week, later periods of test the IJs ceased to kill the host larva even they survive. The IJs formulated in coir and water (control) lost their infectivity during 8th and 6th week itself and it was recorded only 50% and 45% respectively, on helicoverpa (Table-2). *H. indica* infected cadavers of *Helicoverpa armigera* turned red and brick colours and its symbiotic associated bacteria, *Photorhabdus luminescence* growth was observed and progeny produced by nematodes on each cadaver was recorded for further conclusion.

EPN are very effective in controlling soil inhabiting-stages of various insect pests. At present, many agricultural pests such as lepidopteran borers, mole crickets, banana weevils and citrus weevils are successfully controlled by various species of insect parasitic nematodes in India, Florida and other states. The most important discovery in this study is that the EPN *H. indica* tested against the cotton bollworm, *Helicoverpa armigera* larvae and pupae were successfully controlled in laboratory and field experiments by an indigenous specie *H. indica* IJs and no subsequent aerial or foliar applications applied (PDBC 2005; Singh 1994). In the present study, IJs of *H. indica* stored in various formulations was not affected its survival much and it was recorded 50% during the end of 6th week. Among the formulations tested for nematodes survival and its pathogenicity on helicoverpa, hydrogel media provided better support to survive (65%) upto 11 week periods under storage and it pathogenicity on helicoverpa larvae was recorded maximum of 65 percent within 48 h after treatment. It is in close agreement with the latest study conducted with the infectives of *H. indica* killed successfully 90% mortality on helicoverpa larvae when they applied in combination with entomopathogenic fungi, *Metarhizium anisopliae* (Bong 1986; Howell 1979). It may be take place that the combined pathogenic effects induced by both the entomopathogens (*Heterorhabditis indica + Metarhizium anisopliae*) to bring larval mortality upto 90% on helicoverpa (Sankar *et al.* 2009). However, IJs of *H. Indica* formulated in sawdust, talc and sponge materials lead to survive upto 10 weeks periods with 25%, 12% and 6% respectively, which could able to cause only 25%, 10% and 5% mortality respectively, on helicoverpa. In our studies clearly demonstrate that IJs of *H. indica* survived in various formulations for prolong periods could declined their survival even-though they lead to cause pathogenicity to *Helicoverpa* larva equally since from beginning, and that given a positive correlation with the pathogen-host matching.

Pathogenicity of *H. indica* to bollworm, *Helicoverpa armigera* presented in this paper is a novelty to use in the field for further exploitation. In the scientific literature no data on efficacy of this biological agent on the tested formulations were found. A reason for this is probably a limited applicability of the results of such

Table 1: Survival of Infective juveniles of *Heterorhabditis indica* in various formulations.

Formulations	Percent survival of *H. indica* IJs in various formulations (%)#										
	week*										
	1	2	3	4	5	6	7	8	9	10	11
Talc	100 (90)^{a}	100 (90)^{a}	95 (80)^{b}	80 (70)^{c}	75 (60)^{c}	65 (60)^{c}	50 (45)^{d}	50 (45)^{d}	20 (25)^{e}	12 (20)^{e}	0 (0)^{e}
Coir dust	100 (90)^{a}	100 (90)^{a}	95 (70)^{b}	95 (70)^{b}	80 (70)^{b}	50 (45)^{c}	30 (35)^{c}	10 (15)^{d}	0 (00)^{e}	0 (00)^{e}	0 (00)^{e}
Saw dust	100 (90)^{a}	100 (90)^{a}	95 (85)^{a}	95 (85)^{a}	95 (80)^{a}	80 (60)^{b}	70 (60)^{b}	50 (45)^{c}	30 (30)^{d}	25 (28)^{d}	15 (20)^{e}
Sponge	100 (90)^{a}	100 (90)^{a}	95 (70)^{a}	85 (70)^{a}	65 (50)^{a}	55 (45)^{b}	45 (40)^{b}	25 (30)^{b}	15 (20)^{c}	6 (12)^{c}	0 (00)^{d}
Hydrogel	100 (90)^{a}	100 (90)^{a}	95 (85)^{b}	90 (75)^{c}	85 (70)^{d}	75 (60)^{e}	75 (60)^{e}	75 (60)^{e}	65 (55)^{f}	65 (50)^{g}	65 (05)^{h}
Water	100 (90)^{a}	95 (90)^{a}	90 (75)^{b}	85 (65)^{b}	70 (55)^{c}	35 (35)^{d}	10 (20)^{e}	0 (0)^{f}	0 (00)^{f}	0 (00)^{f}	0 (00)^{f}

*Figures in parentheses are arc sine transformed values, in a column, means followed by same letter(s) are not significantly different (P=0.05 DMRT).

* Percent IJs survival data were analyzed by multifactor ANOVA followed by Duncan's multiple range tests ($P < 0.05$) for separation of means.

#Percent mean and standard error for the number of survived infective juveniles of *Heterorhabditis indica* in various formulations with moist condition at 27 °C upto 11 week periods.

Table 2: Pathogenicity of *Helicoverpa armigera* by *Heterorhabditis indica* in various formulations.

Formulations	Percent larval mortality of *Helicoverpa armigera* (%)#										
	Week (pathogenicity test period)										
	1	2	3	4	5	6	7	8	9	10	11
Talc	100	100	100	100	85	85	85	70	70	35	-
Coir	100	100	100	100	100	90	60	50	-	-	-
saw dust	100	100	100	90	90	85	80	80	75	70	70
Sponge	100	100	100	95	90	85	85	80	75	40	-
Hydro gel	100	100	100	100	100	100	100	100	90	85	85
Water	100	100	100	90	60	45	-	-	-	-	-

*Figures in parentheses are arc sine transformed values and data were analyzed by multifactor ANOVA followed by Duncan's multiple range test ($P < 0.05$) for separation of means.

#Percent mean of larvae of *Helicoverpa armigera* diseased by *H. indica* under various formulations with moist condition at 27 °C upto 11 week periods.

Fig. 1: Percent survival of *H. indica* IJs stored in different formulations

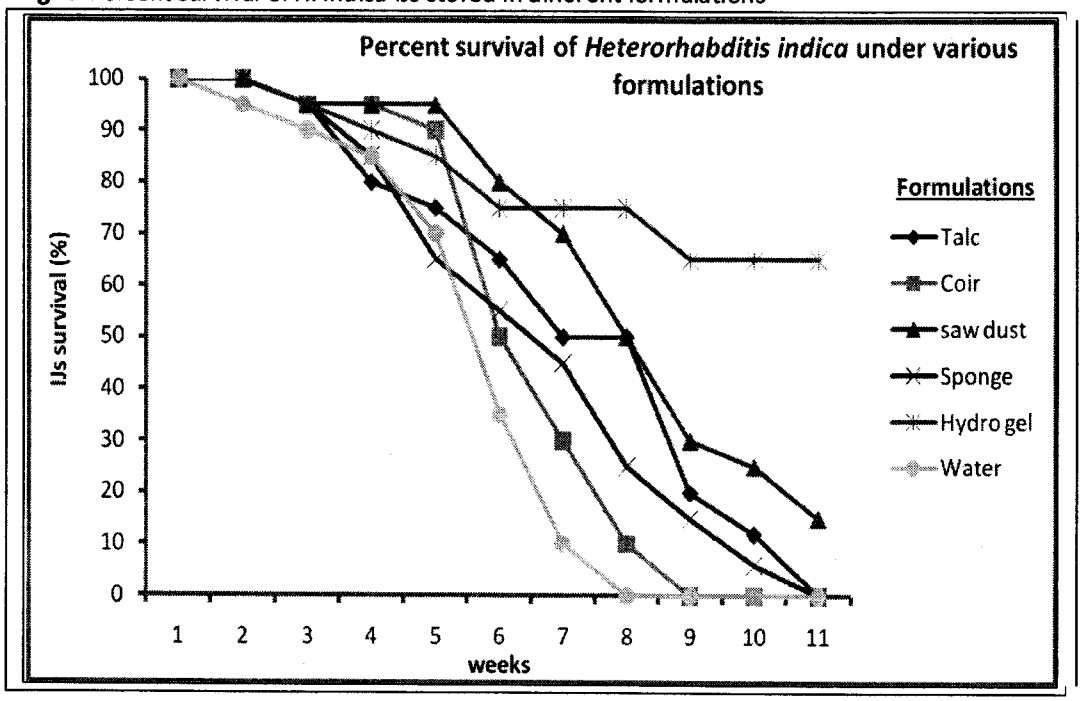
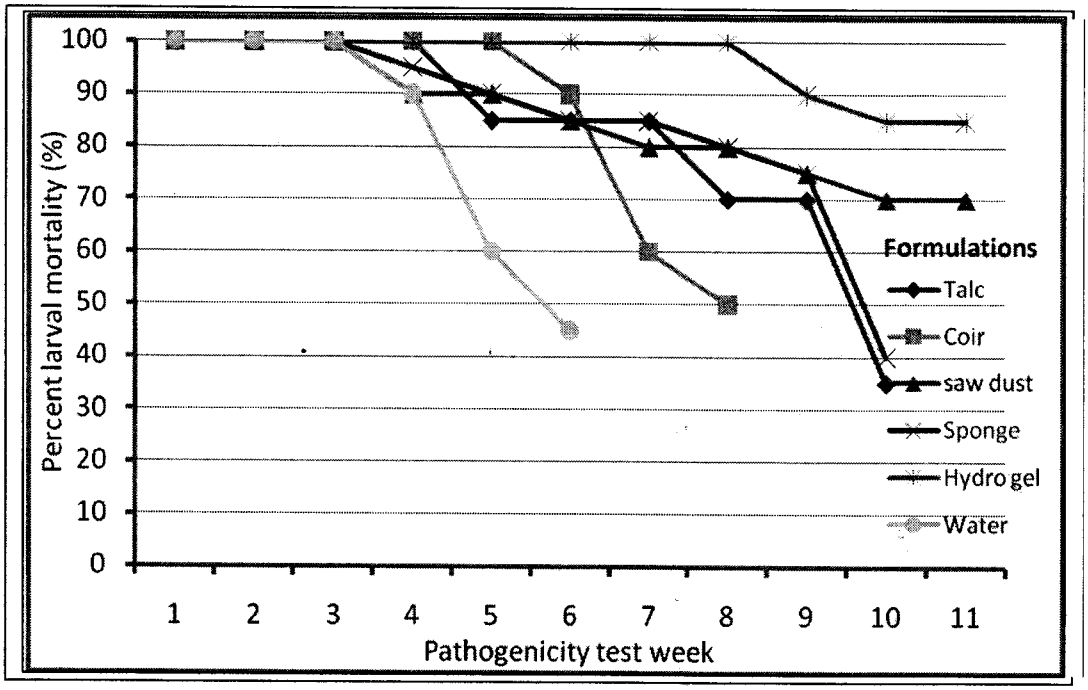


Fig. 2: Percent larval mortality of *H. armigera* by *H. indica* treated from different formulations.



IJs Inoculum: 100 active IJs/larva;
Each % value mean of 25 replicated count.

researches, though they have not done any experiments on formulation basis for the storage strategy of entomopathogenic nematodes. Results obtained in the study, showed that the longevity of nematodes and its pathogenicity to helicoverpa larva was encouraged when they formulated in hydro gel and sawdust. Our pathogenicity results on helicoverpa indicate that, an increasing the age of nematode in formulations may affect their pathogenicity and virulent on larva which was observed correlating negatively (Figure-2). This confirms the fact that pest susceptibility to EPN is complicated process when they formulated in different media for long periods and it depends not only on the current physical condition of the nematodes but also on percent survivability and hostility of the insect.

Virulence and physical condition of infective juveniles on the pest and aggressiveness of the invader depend mostly on abiotic factors. In the study, percent survival of EPN was achieved more than 77 days recording its pathogenicity upto 85% and 70% to *H. armigera* by hydrogel and sawdust formulations respectively. *Heterorhabditis indica* IJs stored in slurry formulation with antidessicants A.V. gel (1% and 10%), CMC 1%, Glycerine 1% was found to be enhanced IJs survivability upto 88- 90 days than they stored in suspension and granular formulation at room temperature (27 ± 2 °C) (Richter 1990) and similar results were reported by IJs of steinernema and heterorhabditis species nematodes which were survived upto 2-3 months (60-90 days) at room temperature in water dispersible granular formulation (WDG) (Samsook 1981).

CONCLUSION

Entomopathogenic nematode, *H. indica* was most virulent and provides an environmentally sound, easily applicable, and more effective in controlling all the stages of lepidopteran insect pests. Large quantity of inoculum of *H. indica* is required for field application against cotton bollworm *H. armigera*. Among the various non-synthetic, synthetic and semi-synthetic culture media evaluated in the laboratory, hydrogel and saw dust based formulations were the best as maximum numbers of *H. indica* were survived without cease their pathogenicity on host and these formulations may be useful for the biocontrol of insect pests in the soil. Formulations ranging from the impregnation of nematodes on hydrogel and sawdust is highly advanced, enhancing storage stability, reduction in the rate of stored energy consumption and nematode activity by either physically trapping them in gels or through reduced water activity of the substrates leading to partial anhydrobiosis. At present, a maximum room temperature shelf-life of more than 11 weeks (75 days) has been achieved for only *Heterorhabditis indica* in a water dispersible hydrogel formulation. Results of our study indicate that these formulated nematodes will kill larvae, pre-pupae and pupae of *H. armigera* thus, utilization of EPN against this pest in the soil and foliar appears feasible for the control and further research is necessary to use *H. indica* more effectively as a potential biocontrol agent against cotton bollworm in field and other harmful crop insect pests.

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