ABSTRACT

Of the total, only 25 soils samples were found positive for nematodes, of which all were identified on the basis of the morphological and molecular characterization by using the different tools and techniques. On the basis of morphological and molecular characters the nematodes species were identified to the generic level *Heterorhabditis, Steinernema, Oscheius* and *Acrobeloides* followed by species level. The data showed that among *Heterorhabditis* 14 isolates belong to the genus *H. indica*, only 2 isolates were belong to *H. bacteriophora* whereas in case of *Steinernema* only 3 isolates belong to *S. abbasi* and 3 isolates belong to *S. siamkayai*. However, the isolates recovered from white grub which was identified as a new species *Oscheius endotokus* and two isolates of the genus *Acrobeloides* which was identified as *A. saeedi*. All the nematodes were subjected for the testing of their virulence against different lepidopteran pests (*G. mellonella, H. armigera* and *S. litura*) reared in laboratory except *Acrobeloides* (due to its entomophilic nature). Further the isolates selected isolates showing pathogenicity DH2, DH7, and DS6 were recovered from the Meerut, DD7 and DS7,were tested against coleopteran pest White grub in the laboratory condition. These were the most efficacious isolates were further selected (DS6 isolate of *S. abbasi*) for the field trials against *Pieris brassicae* infesting Cabbage. Finally isolate DD7 was selected to test its efficacy against white grub (*Holotrichia seerata*) in field conditions.

DH7 and DH8 nematodes killed 50% of white grublarvae (LT50) in 5.0 and 5.2 days, respectively at lowest dose of 500 IJs/larva. LC50 values were calculated at day 2 to day 7 post infection periods, where the LC50 at day 3 was calculated in DH8 with 6401 IJs/larva and least in DH7 with 7581 IJs/larva.During the surveys of agriculture of Sugarcane field of Chota Mawana, Uttar Pradesh, India. Isolate WGN was isolated from the dead cadaver of white grub. This isolate were separated by white trap method. The nematodes were identified using morphology, morphometry and molecular studies. The isolate WGN recovered from the cadaver of white grub (*Holotrichia* sp.) and identified as *Oscheius endotoxus* sp. n. However, *O. endotokus* isolate WGN showed few morphological similarities with *O. indicus, O. carolenensis, O. microvilli* and *O. oniciri* but few divergence were also present in morphometrical obsevvations.

Liquid formulations of 1, 2, 3, 4 and 8 months old culture of three most efficacious isolates of EPNs; *H. indica* (DD7), *H. bacteriophora* (DH7) and *S. abbasi* (DS6) were tested for their efficacy against *G. mellonella* in lab conditions. All the doses (10, 20, 40, 80, 100, 150, 200 and 250 IJs/larva) were found efficacious against *G. mellonella*. One month old culture showed the highest efficacy against *G. mellonella* because they cause complete mortality within 60 hours after infection. Here isolate DS6 of *S. abbasi* was found most virulent at 36 hours infection and showed LD50 value of 13.91 IJ/Larva where it was 36.87 and 39.98 in the case of DH7 and DD7, respectively. DS6 caused complete mortality within 48 hours, where DD7 and DH7 caused this within 60 hours. At 48 hours DD7 LD50 value was 4.54 in case of DD7 however it was 7.75 IJ/Larva in DH7. Two month old culture efficacy tests showed approximately same results, however complete mortality was observed at 72 hours. LD50 value at 36 hours showed DD7 was the most efficacious isolate and required 10.35 IJ/Larva and 11.61 and 18.24 IJ/L by in case of DS6 and DH7 respectively. 4 month old culture showed slight decreased mortality in all the three isolates and required 84 hour to cause complete mortality. LD 50 value were 131;, 180.82 and 361 IJ/L in DD7,; DS6 and DH7 respectively. LD50 value in case DD7 was 54.0 IJ/Larva at 36 hours whereas in case of DS6 and DH7 it was 81.7 and 291.5 LD50 IJ/Larva respectively. However, in all the isolates there was a decreased in their pathogenicity with increase the time period. Comparing with each other, *S. abbasi* DS6 isolates has longer survival period and may be preserved for a long time in liquid state. The results obtained from the extensive experiments revealed that the prodigious progress in the EPN pathogenicity and survival time, from 1 month to 8 months of storage period. However, Heterorhabditids, they were not able to kill the pest at low doses whereas Steinernematids can survive for a longer duration as compared to Heterorhabditids. There is a need to find out the specific factors the efficacy and applications technologies are required which may increase the efficacy of in the field conditions.

The efficacy of *S. abbasi* DS6 was tested under field conditions against *Pieris brassicae*. In the field experiment, liquid treatment of *S. abbasi* isolate DS6 treatments caused significant reductions in insect survival relative to the control, and no mortality were detected in the control treatments. The mean value of the insects infesting on each plant was 26 (20-33).

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The larval mortality was significantly affected by the *S. abbasi* DS6. On day 2nd LC50 value was 1039 IJ/L where as it was 352.4 IJ/L on 5th day of PIP. The Mean of Mortality on day 2nd was 13.3 (11-15) whereas it was 17.3 (14-20) on day 5th. The T test value 0.0039 was significant and confirmed that the *S. abbasi* could be implemented as a biological control agent in field conditions in India.

H. indica DD7, the most pathogenic and reproductive isolate of the study were tested for their efficacy in field of against *Holotrichia seerata* infesting sugarcane. After 10th day of their introduction in the soil, mortality was found 57%. Grubs have immune barrio to the nematodes. A single cadaver ball was found to produce 4020000 IJs/Cadaver balls on white trap. Plant growth of the EPN formulated cadaver (*H. indica* DD7) treated differed significantly ($F = 13.3$; $df = 19, 48$; $P < 0.005$). The number of IJs recovered from formulated cadavers-balls (3100000-4900000) was significantly less when compared with non-formulated cadavers (6700000-9500000) after 1 week storage.

In summary, we have developed a novel formulation and EPN production system for entomopathogenic nematode, and have demonstrated potential for using this new formulation machine in pest control applications. In prior research, we demonstrated a protective mechanism to enhance storage, cost effective, easy to handle, safe for human and easy to applicable and can be made by the small farmer or stakeholder at small scale. The formulation machine was subjected to EPN production in liquid media this study was different from other investigations that here we place an EPN production machine. This method had an advantage that it was made by the waste material and facilitated by an automatic production machine of the EPNs. This formulation creates an option for hand application home-owners or small farmers use that avoids touching a dead insect. However in the present study, we did not demonstrate the use of the novel formulation against insect pest and we did not tested pest control efficacy and IJ yield under laboratory conditions. Research is required to determine if the formulated tape can be stored prior to application (e.g., under refrigeration or partial desiccation) without loss of IJ yield or efficacy. Furthermore, to determine their efficacy under field conditions studies are required.

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