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Efficacy of entomopathogenic nematodes *Steinernema kraussei* to control the fruit fly larvae

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Abstract

Entomopathogenic nematodes (EPN's) belongs to family Steinernematidae and Heterorhabditidae which are obligate parasites of various species of insect pests and used as a bio-control agent. Nematode juvenile enter through the natural opening of insect larvae's and release the bacteria into the body of insect which release the toxic substances into the insect body and kill the insect larvae's in 24-72 hours depends on the species of insect. The objective of current research was to check out the reproductive potential of EPN's (*Steinernema kraussei*) on fruit fly larvae's at different levels. The reproductive potential was recorded at different days (1, 2, 3, 4 and 5), at different concentrations (100IJs, 200IJs, 300IJs and 500IJs) and different storage time (2, 4, 6 and 8 weeks) of culture. The maximum progeny was recorded 29367 at day 5 and at 500IJs concentration the maximum reproductive potential was recorded 30733 and the maximum population 40933 of nematode was recorded at 2 week old culture. So the results are clearly indicated that reproductive potential of entomopathogenic nematodes (*Steinernema kraussei*) was increased day by day, with the increased in concentration of nematode the population of nematodes (EPN's) also increased but with the increased in storage time of EPN's cultures the reproductive potential was decreased.

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Introduction

In order Lepidoptera, many species of insect pests present which are the main pest in agriculture. The larvae's of different insect pests like fruit fly, army warms, pink bollworm etc. cause the large number of losses. Females produced the large number of eggs; sometimes it may produce up to the 30,000 eggs in 24 hours. After hatching the large number of larvae's, caterpillars are come out from eggs and cause the great losses in agriculture (Resh *et al*., 2009). It cause up to the 5-37% losses under different environmental conditions (De Groote, 2002).

Entomopathogenic nematodes were used as a bio control agent is the successful and safe method to control the insect pests (Ehlers, 2001). Entomopathogenic nematodes (EPN's) were first time discovered in 1923 and Steiner described the species *Aplectana kraussei,* then Glaser also discover a nematode from Japanese beetle *Popillia japonica* USA and this nematode were defined by Steiner as *Steinernema glaseri* (Steiner, 1929). The efficacy of this nematode was checked in field trial and applies this species in 73 different pots to resist *Popillia japonica* (Glaser, 1932). The different genus of *Heterorhabditis* was explained in family Heterorhditidae (Poinar, 1976). Hominick, (2002) was discovered the 48 species of entomopathogenic nematodes all over the world.

Steinernematidae and Heterorhabditidae families of EPN's were used as a bio control agent against various species of insects. These EPN's are obligate parasites of various types of insects and beneficial to human beings, plants, animals which attack on various types of insect pest. Insect pests cannot be controlled only the use of insecticides, biological control is essential for the successful management of insect pest. Biological control is the successful management of insect pest and safe for the human health and environment. Insects are mostly present deep in the soil, to control these insects the chemical compounds must apply deep in the soil. But the main drawback of chemical control is that it causes the pollution of underground water and atmospheres as well. Many insecticides also kill the beneficial insects. These are the drawback of chemical control. Therefor biological control is used to manage the insect pests (Ehlers, 1996).

Entomopathogenic nematodes belong to genera Heterorhabditis and Steinernema. It has four developmental stages (J1, J2, J3, and J4). The first 2 stages J1 and J2 are completed into the egg and in J3 stage the nematode juvenile come out from the egg. The third stage of EPN's are J3 stage which is free living and also called as infective stage or infective juvenile (IJ) stage. This stage is also called the dauer stage. The J3 stage (infective juvenile) does not feed on host when it is present outside of the insect body. Infective juvenile enter into insect larvae's through natural opening and release the bacteria which is present into the gut of the juvenile, these bacteria release the toxic substances into the body of the insect and kill the insect larvae. Then these juveniles feed on dead insect body and multiply into the dead insect cavity and brust the insect body and come out the cavity of insect after this it take the bacteria in gut and start to find the next host (Ehlers, 2001).

The life cycle of nematode are completed in specific days, after completing life cycle thousands of infective juveniles produced and start searching for the new host. The life cycle of entomopathogenic nematode (EPN's) from entrance of infective juvenile into the host and till emergence of new juveniles, a specific temperature is required for completing the life cycle. If temperature range is 18-28 ^{o}C then the life cycle is complete usually 6 to 8 days in wax moth (*G. mellonella*) (Nguyen and Smart, 1992). Host penetration of specie *S. carpocapsae* in peat substrate is slow (Kruitbos *et al*. 2010).

Abiotic factors are affected on the life cycle and pathogenicity level of entomopathogenic nematodes (EPN's). Most of the species of entomopathogenic nematodes are survive at 30°C. Optimum temperature of different species of entomopathogenic nematodes (EPN's) is correlate to each other, which are present on different geographic locations (Grewal *et al.,* 1994). Some species like *S. kraussei* survive at low temperature and infect their insect host below 10°C

(Willmott *et al.*, 2002), similarly some species like *H. megidis, S. carpocapsae* and *S. feltiae* can infect their host at 20 to 25°C (Dunphy and Webster, 1986). The life cycle of nematode is disturbed (not complete) when temperature is unfavorable or low humidity due to this the emergence of juveniles disturbed (Brown and Gaugler, 1997). Keeping all above mention facts, the main objective of present study was to determine the reproductive potential and the efficacy of EPN's (*Steinernema kraussei*) on fruit fly larvae's at different levels.

Materials and methods

Insect's culture

The fruit fly larvae's infected guava fruits were collected from guava orchards (horticulture research area 9 scale) and brought into the Nematology lab, cut the guava fruits and larvae's were separated from guava fruits with the help of forceps (Armes *et al.,* 1992).

Larvae were surface sterilized in 0.1% formalin solution or/and 70% alcohol for 3 minutes to avoid surface contamination and then take the petri plates, cut the filter paper with the size of petri plate bottom and place the blotter paper into the petri plate. After this wet the blotter paper with water and transferred the fruit fly larvae's in petri plates. Then the culture of EPN's (*S. kraussei*) was poured @ 300 juveniles in each petri plate and sealed with Nescofilm and incubated at 27°C in the incubator for 2-3 days.

Isolation of nematodes (infective juveniles) from dead fruit fly larvae's

White trap methods is used for the isolation of nematodes from dead insect larvae's (White, 1927). White trap method consisting of plastic container with 1cm depth, small petri plate, and sheet of filter paper (9cm in diameter). Take the plastic container and filled with distill water, an inverted small petri plate was placed in the bottom of container then cut the filter paper about 9cm in diameter and placed on small petri plate, the edges of filter paper was touch the distill water. After this the dead fruit fly larvae's were placed on filter paper and closed the plastic container with lid. These plastic containers were further incubated at 27°C until the emergence of nematode. After some time nematodes (infective juvenile) start to leave the dead larvae's and passed through the filter paper in to the distill water which is present in to the plastic container. Then water is collected from the container, nematodes were isolated and further studied under microscope. These nematodes juveniles were stored at 8-10°C for further use (Dutky *et al.,* 1964).

Counting and storage of entomopathogenic nematodes

Counting of nematodes were done under microscope, nematodes were collected from white trap method and take a nematode suspension in a petri plate where boxes (rectangular boxes were draw on back side of petri plate for counting purpose) were present allow the nematodes settling down. After some time (2-3 hours) when nematodes were settled down then it was counted under stereomicroscope and repeated three times. The average of this repetition was determined and multiplies with total volume. Nematodes were stored in refrigerator at 10°C in small plastic cups.

Identification of entomopathogenic nematodes

The identification of entomopathogenic nematodes were done on the basis of dead larvae's color. The dead/ infected larvae's of genus *Steinernema* were showed grey color and the larvae's of genus *Heterorhabditis* showed red color. Due to this reason, only entomopathogenic nematodes (EPNs) have ability to penetrate and cause infection in insect pest while other species of nematodes cannot able to penetrate in insect pests, cause infection and mortality in insect pests.

Determination the efficacy of EPN's Steinernema kraussei on fruit fly larvae's

Entomopathogenic nematodes (*Steinernema kraussei*) suspension was made and stored for 2 weeks at 9°C to check out efficacy against fruit fly larvae's. 300 IJs were taken from 2 weeks old nematode suspension that was stored at 9°C and allowed to attack on fruit fly larvae's (which were collected from guava orchard field UAF) to checked the potential of nematodes. Each treatment was three times replicated and dead insect larvae's were shifted

to white trap to check the potential of EPNs. After 7-10 days nematodes were emerged and come out the dead insect body into the water, by this way nematodes were isolated from the insect body then it was counted under stereomicroscope and check the multiplication potential of *Steinernema kraussei* on fruit fly larvae's at same concentration of nematodes (300 IJ's).

Effect of different concentrations of Steinernema kraussei (EPN's) on fruit fly larvae's

Entomopathogenic nematodes (*Steinernema kraussei*) suspension was made and stored for 2 weeks at 9°C to check out efficacy against fruit fly larvae's. 300 IJs were taken from 2 weeks old nematode suspension that was stored at 9°C and allowed to attack on fruit fly larvae's (which were collected from guava orchard field UAF) to checked the potential of nematodes. Each treatment was three times replicated and dead insect larvae's were shifted to white trap to check the potential of EPNs. After 7-10 days nematodes were emerged and come out the dead insect body into the water, by this way nematodes were isolated from the insect body then it was counted under stereomicroscope and made the different concentrations (100IJs, 200IJs, 300IJs and 500IJs) of nematodes (*Steinernema kraussei*) to check out the efficacy of different concentrations of nematodes on fruit fly larvae's. Each concentration of nematode was applied on fruit fly larvae's which were present in petri plates and checked the efficacy/ reproductive potential of nematodes. J3 of EPNs (*Steinernema kraussei*) were applied on fruit fly larvae's as following treatments.

Treatments:

T1: 100IJs (infective juveniles) / 5 insect
T2: 200IJs (infective juveniles) / 5 insect
T3: 300IJs (infective juveniles) / 5 insect
T4: 500IJs (infective juveniles) / 5 insect

Each treatment was replicated three times and to check out the potential of (EPNs) *Steinernema kraussei,* the dead fruit fly larvae's were shifted to white trap. After 7 days the progeny of *Steinernema kraussei* were emerged. Data was collected after the progeny emerged.

Determination the efficacy on the basis of storage time of EPN's (Steinernema kraussei)

Entomopathogenic nematodes (*Steinernema kraussei*) suspension was made and stored for 2 weeks at 9°C to check out efficacy against fruit fly larvae's. 300 IJs were taken from 2 weeks old nematode suspension that was stored at 9°C and allowed to attack on fruit fly larvae's (which were collected from guava orchard field UAF) to checked the potential of nematodes. Each treatment was three times replicated and dead insect larvae's were shifted to white trap to check the potential of EPNs. After 7-10 days nematodes were emerged and come out the dead insect body into the water, by this way nematodes were isolated from the insect body and stored for 2, 4 and 6 weeks at 9°C to check out the efficacy of EPN's. 300 infective juveniles (IJs) were taken from 2 week old culture of EPN's (*Steinernema kraussei*) and applied on fruit fly larvae's present in petri plates to check the reproductive potential of nematodes. After this, 300 infective juveniles (IJs) were taken from 4 week and 6 week old cultures of EPN's (*Steinernema kraussei*) and applied on fruit fly larvae's present in petri plates to check the reproductive potential of nematodes.

Statistical Analysis

The data was analyzed statistically by using completely randomized design (CRD) for laboratory studies according to Fisher's analysis of variance (Steel *et al.*, 1997). Means were compared by least Significant Difference (LSD) test at 0.05 probability test.

Results

Determination the efficacy of EPNs (Steinernema kraussei) on fruit fly larvae's

Maximum production/ progeny of *Steinernema kraussei* was observed at day 5 and minimum production of nematodes (*Steinernema kraussei*) recorded at day 2 (Table 1). The results clearly showed that maximum nematodes (*Steinernema kraussei*) concentration on five days. Day 1 the nematode concentration was 15567, day 2, day 3, day 4 and day 5 the nematodes concentration was 18567, 22500, 25867 and 29367. These mean values are clearly indicated that on day 1 the nematode concentration is minimum and nematode concentration/ progeny was increased with the passage of time and the maximum concentration of nematodes (*Steinernema kraussei*) was on day 5 (Fig. 1).

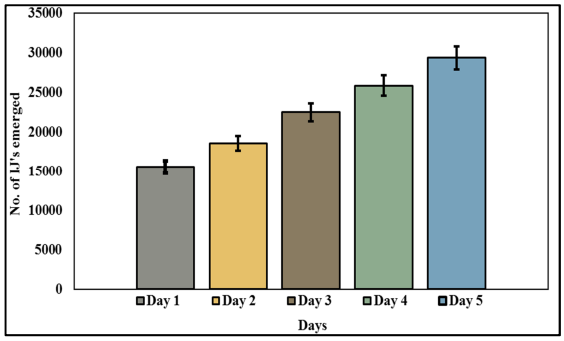

Fig 1. Effect of EPNs (*Steinernema kraussei*) on fruit fly larvae's.

Table 1. All-Pairwise Comparisons Test of Juveniles by Days.

Days	Mean
5	29367^{A}
4	25867^{B}
3	22500^{C}
2	18567^{D}
1	15567^{E}

Effect of different concentrations of EPNs (Steinernema kraussei) on fruit fly larvae's

Results indicated that at concentration 100IJs the nematode progeny was maximum i.e. 8666.7. Similarly, at concentrations 200IJs, 300IJs and 500IJs the nematode progeny was 15167, 21600 and 30733 respectively. These mean values were clearly indicated that the minimum progeny (reproductive potential) at 100IJs concentration, reproductive potential was increased with the increase in concentration of nematodes, and the maximum reproductive potential was on 500IJs concentration nematodes (*Steinernema kraussei*) (Table 2). Results clearly demonstrated that the progeny/ population of nematodes (*Steinernema kraussei*) were increased or decreased with the increase or decrease in concentration of nematode (Fig. 2).

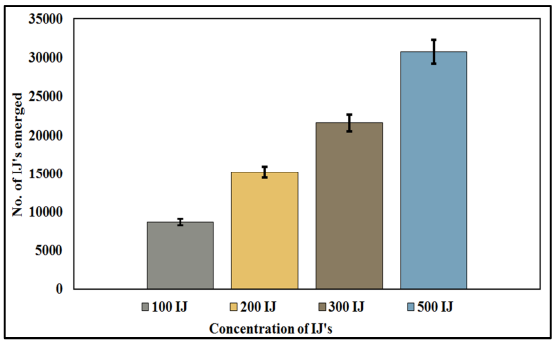

Fig 2. Effect of different concentrations of EPNs (*Steinernema kraussei*) on fruit fly larvae's.

Table 2. LSD All-Pairwise Comparisons Test of Juvenile by Concentration.

Concentration	Mean
500	30733^{A}
300	21600^{B}
200	15167^{C}
100	8666.7^{D}

Determination the efficacy on the basis of storage time of EPNs (Steinernema kraussei)

Results indicated that the different storage times (2, 4, 6 and 8 weeks) of nematodes were effected on the reproductive potential of nematode that showed the significant relationship between them. The nematode population was decrease with the increase in storage time, at the lowest storage time (2 weeks) the nematode population was maximum and nematode population was started to decrease with the increase in storage time.

The minimum population of nematodes was recorded after 8 weeks (Table 3). These results clearly showed that the progeny/ population of nematodes (*Steinernema kraussei*) were depending on the storage time of nematode culture.

After 2 weeks the nematode progeny was 40933, after 4 weeks 32733, after 6 weeks 20333 and after 8 weeks the population was 10233 respectively. These results were clearly showed that the nematode population was decrease with the increase of storage time of nematode culture.

The maximum population of nematode was after 2 weeks, with the increase in storage time the nematode population was decrease and the minimum population after 8 week (Fig. 3).

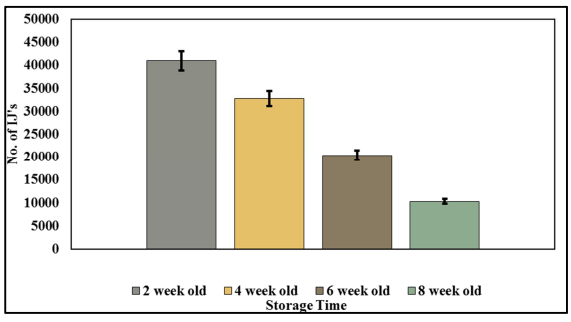

Fig 3. Effect of *Steinernema kraussei* reproduction and the efficacy on the basis of storage time of EPNs.

Table 3. LSD All-Pairwise Comparisons Test of Juveniles by Weeks.

Weeks	Mean
2	40933^{A}
4	32733^{B}
6	20333^{C}
8	10233^{D}

Discussion

Several studies have shown the efficacy of Entomopathogenic nematodes (*Steinernema kraussei*) as a bio control agent against many species of insect pest. Many authors reported the mode of action and virulence of EPN's against different species of insect pest (Kung *et al.,* 1990; Poinar, 1979).

Our results were showed that the maximum progeny of *Steinernema kraussei* was on day 5 as compared to the 1, 2, 3 and 4 days because entomopathogenic nematodes enter the insect body through natural opening at juvenile (j2) stage and feed the insect body and kill the insect at the end, day 1 multiplication of nematodes is less and with the passage of time multiplication of nematodes was increased so at day 5 maximum concentration of nematodes were recorded. Kung *et al.,* (1990) also described the mass production and field application of EPN's.

In second experiment different concentrations of infective juveniles (IJs) were prepared to check efficacy of entomopathogenic nematodes. At 500 IJs concentration insect larvae dead more rapidly as compared to the other concentrations and at the end maximum progeny was recorded those concentration because high concentration of nematodes were enter into the insect larvae which kill the insect larvae more rapidly and maximum progeny was produced as compared to low concentrations. So the increase in concentration of nematodes the progeny was also increased. Salam and Sahina (2012) were conduct the experiment on eight EPNs species of the two genera *Steinernema* and *Heterorhabditis* named *S. pakistanense, S. carpocapsae, S. feltiae, S. asiaticum, S. abbasi, S. siamkayai, H. indica* and *H. bacteriophora* and described the production potential of different species of nematodes.

In third experiment the entomopathogenic nematodes (*Steinernema kraussei*) suspension were prepared and stored for 2, 4, 6 and 8 weeks at 9ºC to check out efficacy against fruit fly larvae's. After this 2, 4, 6 and 8 week cultures were applied on fruit fly larvae's.

The maximum progeny was recorded 2 weeks old culture and minimum progeny was recorded 8 weeks old culture, so results were clearly indicated that with the increase in storage time of culture progeny was decreased because after the specific time of culture nematodes start to die, this was the reason that after 2 weeks old culture death ratio of nematodes was less and progeny ratio was high as compared to other cultures (4, 6, and 8 weeks old) so the results were cleared that with the increase in storage time progeny ratio was decreased. Van Driesche *et al.* (2008) described various species of *Steinernema* and *Heterorhabditis* as bio pesticide and check the efficacy of different species on the basis of storage time.

Conclusion

The reproductive potential and efficacy of EPN's (*Steinernema kraussei*) are affected by time, concentration of EPN's and storage time of culture. The reproductive potential of Entomopathogenic nematodes (*Steinernema kraussei*) was increased day by day, with the increased in concentration of nematode the population of nematodes also increased but with the increased in storage time of EPN's cultures the reproductive potential was decreased.

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