Pathogenicity of Entomopathogenic Nematodes, *Steinernema feltiae* Against Different Life Stages of Mole Crickets

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Abstract-- Mole Cricket, *Scapteriscus sp*. (Orthoptera: Gryllotalpidae), is one of the known grass pests throughout the world. There are three stages in the life cycle of Mole Crickets - eggs, nymphs and adults. They molt many times as they grow and mature into adult caused damage to grass areas such as golf course, athletic field and home lawn. Mole Cricket damaged the turf grass by tunneling in the soil to expose the root and by direct feeding the root. Chemical control is generally used for Mole Cricket but can be expensive and having adverse effect on human and environment. Some sports complex managers and golf course have tried to reduce the use of insecticides because of the local limitations concern about the health of peoples. Entomopathogenic nematode (EPNs) may the replacement of chemical insecticides. These are elongated roundworms colorless and microscopic. These can be used as an alternative of chemical insecticides having no adverse effect on human as well as environment. The study was conducted to check the pathogenicity of EPNs (*Steinernema feltiae*) against different life stages of Mole Cricket. Different concentrations of *S. feltiae* 50, 100 and 200 1J/ml were used with three replications on different life stages of Mole Crickets. The mortality was recorded after 24, 48 and 72 h interval. The lower concentration 50 IJs/ml (T2) caused the less mortality of 20% at 24 h while the higher concentration 200 IJs/ml (T4) caused maximum mortality 100% at 72 h. The results suggested that *S. feltiae* was promising in the sustainable management of *Scapteriscus sp.* under laboratory conditions and provide the baseline for the management of Mole Cricket under natural conditions.

Keywords: Entomopathogenic nematode (EPNs), Pathogenicity, Roundworms, *Steinernema feltiae*, Turf grass

1 INTRODUCTION

Mole Crickets are insects that belong to the Gryllotalpidae family of Orthoptera order (grasshoppers, locusts, and crickets). Mole Crickets go through three stages in their life cycle eggs, nymphs, and adults. Majority of the Mole Crickets live under the ground and construct tunnels. The Mole Cricket's forelegs are expanded and flattened, allowing it to burrow rapidly in really dry soils and sandy. Mole Crickets are typically found in the maximum 20 to 25 centimeters of soil but have been observed tunnelling 75cm as deep [11]. Females mate for 24 hours, lay one or more egg clutches within 10-14 days of mating, and then die. A few weeks later, the eggs begin to hatch into nymphs. Although they are whitish, considerably smaller, and lack wings, they resemble adults. Nymphs start to hatch from eggs early in April, but they don't stop of hatching eggs until the end of June. Nymphs live for about 5 months before becoming adults in September. They undergo several molts [20]. Mole Crickets are omnivorous, feeding on a variability of plant species as well as other insects. Cabbage, beet, cantaloupe, cauliflower, collard, kale, onion, spinach, carrot, sugar cane, potato, eggplant, lettuce, pepper, collard, sweet potato, peanut, tomato strawberries, and tobacco are among the plants and crops that have been harmed. [6], [16], [17], [19], [22]. The damage of feed for animal graze on pastures, tunnelling and feeding of golf course, vegetable form, backyard lawns and sports fields.

Chemical controls for the Mole Crickets in the golf course, lawns and grass farms are available [5] but they can be costly and ineffective, as well as posing ecological hazards and provided that only short-term relief. The community is becoming increasingly concerned about the potential over usage and mis usage of chemicals on turf, as well as the potential for contamination of ground water[21]. Some sport complex managers and golf course have made an effort to use fewer insecticides because of local constraints and worries about the health of their members. As effect of Mole Cricket tunnelling and feeding, unacceptable levels of turf damage have occurred. The rate of chemical regulator in grasslands is excessively high [8]. As a result, golf course managers, turf producer cattlemen, and others have been looking for a cost-effective, ecologically harmless, and long-term resolution to Mole Cricket damage. Moreover, turf grass is chemically sensitive and some sports complex managers and sport course have tried to diminish the use of insecticides because of the local limitations. So, there must be alternative of chemicals insecticides such entomopathogenic nematodes (EPNs).

Entomopathogenic nematodes (EPNs), act as the biological agents of many insects, belongs to 23 different families [15]. EPNs belongs to families Steinernematidae and Heterorhabditidae have the characteristics of biocontrol agent of insects [1] [12] [15] and has been employed as protective, and supplemental biotic control managers. Mole Crickets and other insect pests of order lepidoptera can be controlled by EPNs [7], [18]. These infectious nematode stages immediately enter the insect's body cavity and release the bacteria from its intestinal lumen within 24-48 hours, the bacterium multiplies quickly in the host hemolymph and develops fatal bacteria. They feed on the microbial cells and dissolve host tissues. Throughout the world EPNs are widely dispersed in the soils [2], [10] . The present study aimed to evaluate the pathogenicity of entomopathogenic nematode, *Steinernema feltiae* was used against different life stages of Mole Crickets under lab condition and to evaluate the virulence of EPN *S. feltiae* as biotic control agent against Mole Cricket.

2. Material and method

2.1 Collection of test insect

Mole Cricket adults was collected by using pitfall traps from different locations of University of Agriculture, Faisalabad. Pakistan. Pitfall traps were installed on different locations of university premises with the surface of soil. Holes were drilled into the bottom of the plastic cups in order to allow the drainage of water. The traps were installed during the months of February and March 2023. The pitfall traps were monitor daily bases to check the presences of tested insects. After samples collection, specimens were brought to laboratory for species identification, and the tested insects were cultured in Insect Biodiversity and Biosystematics Laboratory (IBBL), Department of Entomology, University of Agriculture Faisalabad. Different life stages of Mole Cricket were subjected to pathogenicity of entomopathogenic nematodes.

2.2 Culture of entomopathogenic nematodes

The culture of *Steinernema feltiae* was increased by creating the new infection of EPNs on different life stage of wax worm larvae. The wax worm was inoculated with EPNs on petri plates layered with the piece of filter paper. Next 2-5 days the infected larvae of wax worm were checked under microscope and newly emerged juveniles were harvested by white tarp method (White, 1927). The visible contaminations were disposed off. The small petri plates containing the infected larvae of wax worms were placed into the large petri plates containing the distilled water upto 1cm depth. The newly emerged juveniles start to leave the cadavers and move to the larger petri plates. The newly emerged EPNs juveniles were collected in distilled water in plastic container daily bases. The container will be maintained at 25±2 ^{0}C. Different concentrations 50, 100 and 200 infectious juveniles per ml was obtained for bioassay.

2.3 Virulence of entomopathogenic nematodes (*Steinernema feltiae*) on Mole Crickets

The virulence of *Steinernema feltiae* was tested in Mole Cricket. Five nymphs and adult of different life stages were used for bioassay. Five different life stage nymph and adults were placed in petri plates layered with Whatman filter paper. In the distilled water different concentrations of EPNs (50, 100 and 200 IJs/ml) found was applied on tested insects as treatment while the untreated control was applied with distilled water. The treated and untreated petri plates were incubated in dark at room temperature (25±2 ^{0}C). After the application of treatment, the data about mortality was noted after 24, 48 and 72 hours of treatments application. The experiment was conducted under Completely Randomized Design (CRD) with three treatments and control with three replications. The data regarding the mortality was recorded after 24, 48 and 72 hours. The mortality data was calculated by using the Abbott formula (Abbott,1925). Analysis of variance (ANOVA) to check the significance of the treatments at 5% level of significant. Tukey's honestly significance difference (HSD) test was used to compare the treatment means.

3 RESULTS

3.1 Virulence of entomopathogenic nematodes (*Steinernema feltiae*) on Mole Crickets

The data represents the efficacy of Entomopathogenic nematodes (*Steinernema feltiae*) against Mole Crickets at different treatment levels (T1, T2, T3, T4) and various developmental stages (1st Instar, 2nd Instar, 3rd Instar, 4th Instar) of the Mole Crickets. The measurements are taken at three different time points (After 24 hours, After 48 hours, After 72 hours) to assess the impact of the treatment. It's likely that the values represent the percentage of Mole Cricket mortality or some other response to the nematode treatment. The maximum mortality of 1st instar of Mole Cricket has been caused by nematodes were 100% and minimum mortality was 46.7% at treatment (T4) after 24 to 72 hours, respectively. In case of 2nd and 3rd instars maximum mortality (100%) of Mole Cricket has been caused by treatment (T4) after 72 hours while

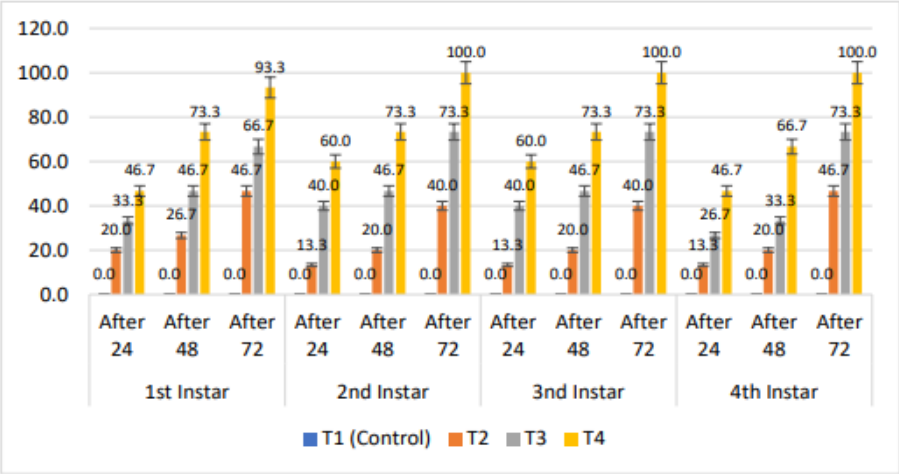
minimum mortality was 60% after 24. Maximum mortality of 4th instar of Mole Cricket has been caused by nematodes were 100% at treatment (T4) while minimum mortality was 46.7% after 72 and 24 hours, respectively that evaluated in the (table 1).

Table 1: Percentage mortality of 1st, 2nd, 3rd and 4th instar nymph of Mole Cricket After 24, 48 and 72 hours of treatments application

Treatment	1st Instar			2nd Instar			3nd Instar			4th Instar		
	After 24	After 48	After 72	After 24	After 48	After 72	After 24	After 48	After 72	After 24	After 48	After 72
T1 (Control)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T2	20.0	26.7	46.7	13.3	20.0	40.0	13.3	20.0	40.0	13.3	20.0	46.7
T3	33.3	46.7	66.7	40.0	46.7	73.3	40.0	46.7	73.3	26.7	33.3	73.3
T4	46.7	73.3	93.3	60.0	73.3	100.0	60.0	73.3	100.0	46.7	66.7	100.0

Mortality of different instar of Mole Crickets by using different concentration of infective juveniles

The graph shows the percentage mortality of 1st,2nd,3rdand 4th instar of Mole Cricket after 24,48 and 72 hours of application of entomopathogenic nematodes (*Steinernema feltiae*). The mortality of Mole Crickets were increased from 20 to 100 % when concentration of S*teinernema feltiae* has been increased from 50 to 200 infective juveniles. The highest mortality has been observed at 2nd,3rdand 4th instars treatment (T2, T3, T4) with the value of (100%) after 72 hours application. The lowest mortality has been observed at 1st instar treatment (T2) with the value of (20%) after 24 hours application and no mortality has been observed at control (T1) after 24,48 and 72 hours of application (Figure 1).



instars Mole Cricket after 24, 48 and 72 hours of treatment application

The data represents the efficacy of Entomopathogenic nematodes (*Steinernema feltiae*) against Mole Crickets at different treatment levels (T1, T2, T3, T4) and various developmental stages (5th Instar, 6th Instar, 7th Instar, adult) of the Mole Crickets. The measurements are taken at three different time points (After 24 ,48, 72 hours) to assess the impact of the treatment. It's likely that the values represent the percentage of Mole Cricket mortality or some other response to the nematode treatment. The maximum mortality of 5th instar of Mole Cricket has been caused by nematodes were 100% and minimum mortality was 46.7% at treatment (T4) after 24 to 72 hours, respectively. In case of 6th instars maximum mortality (100%) of Mole Cricket has been caused by treatment (T4) while minimum mortality was 46.7% after 24 and 72 hours. 7th instar of Mole Cricket has been caused by nematodes were 100% at treatment (T4) while minimum mortality was 40% at

treatment (T4) after 24 to 72 hours and the maximum mortality of adult of Mole Cricket has been caused by nematodes were 100% at treatment (T4) while minimum mortality was 40% at treatment (T4) after 24 to 72 hours that evaluated in the (table 2).

Table 2: Percentage mortality of 5th, 6th, 7th instar nymph and adult of Mole Cricket After 24, 48 and 72 hours of treatments application

Treatment	5th Instar			6th Instar			7th Instar			Adult		
	After 24	After 48	After 72	After 24	After 48	After 72	After 24	After 48	After 72	After 24	After 48	After 72
T1 (Control)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T2	13.3	33.3	66.7	13.3	33.3	66.7	20.0	400.0	66.7	20.0	46.7	73.3
T3	33.3	53.3	86.7	33.3	53.3	86.7	26.7	53.3	73.3	26.7	53.3	80.0
T4	46.7	73.3	100.0	46.7	73.3	100.0	40.0	66.7	100.0	40.0	60.0	100.0

The graph shows the percentage mortality of Mole Cricket at 5th,6th,7thand adult after 24,48 and 72 hours of application of entomopathogenic nematodes (*Steinernema feltiae*). The highest mortality has been observed at 5th,6th,7th and adult treatment (T) with the value of (100%) after 72 hours application. The lowest mortality has been observed at 5th instar treatment (T2) with the value of (13.3%) after 24 hours application and no mortality has been observed at control (T1) after 24,48 and 72 hours of application (Figure 2).

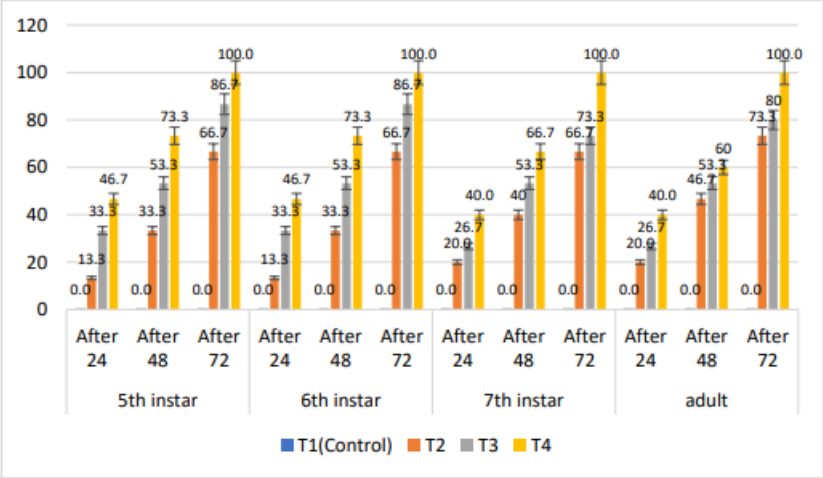


Figure 2 Graphical presentation of percentage mean mortality of 5th, 6th, 7th instars & Adult Mole Cricket after 24, 48 and 72 hours of treatment application

DISSCUSION

The EPNs are microscopic worms (less than 1 mm in length) very specific in their ability to target and infect pest species and show the virulence against Mole Crickets [9]. Additionally, EPNs can provide an efficient way to control Mole Crickets, while reducing the need for chemical or physical interventions and ensuring that the surrounding environment remains intact [4]. Research has shown that the EPNs *S. feltiae* and *H. bacteriophora* are the most effective in controlling Mole Cricket populations. These species have been experimentally tested against *Scapteriscus spp*. (Mole Crickets) indicated a higher percentage of mortality than other methods (Khan *et al.*, 2016). The nematodes are able to penetrate the soil and attach themselves to the insect's body, which then leads to death within 48-72 hours [13]. As such, this method of pest control has been found to be extremely effective in reducing Mole Cricket populations [13]. The data demonstrates a clear trend of increasing nematode efficacy as Mole Cricket instar stages progress from 1st to 7th instar nymph and adult. This observation aligns with the established understanding that EPNs are more effective against younger, smaller insect stages due to their ability to penetrate the insect host and establish infection more easily. The data also highlights the

importance of time in evaluating nematode efficacy. In all treatment groups (T2, T3, and T4), there is a noticeable increase in mortality rates from 24 to 72 hours after treatment. This trend suggests that EPNs require time to infect and kill The Mole Crickets. These findings are consistent with previous studies [12] demonstrated that EPNs typically take several days to kill their hosts.

The results were recorded on 24 h, 48 h and 72 h interval. At lower concentration of EPNs caused minimum mortality of 20% while the highest concentration of EPNs caused maximum mortality was 100% after 72 h of exposure. Same observations have been confirm by Lacey (2017) [14] studied the effectiveness of S. carpocapsae and H. bacteriophora against the white grubs. At lower concentration both nematodes caused minimum mortality of 18-22% after 7 days of exposure. At higher concentration both nematodes caused maximum mortality of 34-58%. In the present experiment the pathogenicity of entomopathogenic nematode, *S. feltiae* was found effective against different life stages of Mole Cricket. The lower concentration of *S. feltiae* show the lower mortality 20% at 24 hours while the higher concentration of EPNs *S. feltiae* show highest mortality 100% at 72 hours. It was also observed that the mortality of Mole Crickets increased with increasing the concentration of IJs with the increase in time period. The current study agree with Andaló *et al.* (2018) described that the entomopathogenic nematodes are effectively utilized against soil inhibiting insects [3].

4 CONCLUSION

The data demonstrated the potential of EPNs as the viable approach to control the Mole Crickets. However, the concentration and choice of nematode is critical against the different nymphal stages of the Mole Cricket. These findings emphasize the need to find out the pest management strategies that take into account the biology of the target insect and the characteristics of the employed EPNs, further demanding research and development in this area.

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