ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14557610

Evaluation of Virulence of *Steinernema carpocapsae* and a Local strain *Steinernema* sp. against *Melolontha melolontha* Larvae

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Abstract:- This study evaluates virulence of *Steinernema carpocapsae* and a locally isolated *Steinernema* strain against *Melolontha melolontha* larvae, as a significant agricultural pest. Comparative bioassays were conducted to determine mortality rates, lethal concentration (LC50), and lethal time (LT50) under controlled conditions. Results indicate that both nematode strains effectively infect and kill the larvae, with significant differences in efficacy depending on nematode concentration and larval instar stage. The findings support the potential use of local *Steinernema* strains for sustainable pest management. In laboratory conditions, the effectiveness of pathogenic nematodes from the genus *Steinernema* was evaluated for controlling harmful insects. The experiment included the commercial strain *Steinernema carpocapsae* and a local species, *Steinernema spp.*. The study was conducted at temperatures of 25°C and 30°C, using two concentrations of nematode suspensions: 2500 and 3000 infective juveniles (IJs) per 1 ml of water. The findings revealed that the effective concentration against the May beetle (*Melolontha melolontha*) should be at least 2500 nematodes per 1 ml of water, with an optimal temperature of 25°C. Based on insect mortality rates and temperature indices, it was confirmed that both *Steinernema carpocapsae* (commercial strain) and the local *Steinernema spp*. exhibit high pathogenicity. Therefore, their application against the pest *Melolontha melolontha* is well-justified.

Keywords:- *Steinernema carpocapsae, Melolontha melolontha, Biological Control, Temperature. Concentration of Nematode Suspension.*

I. INTRODUCTION

One of the most significant and damaging pests in Georgia is the May beetle (*Melolontha melolontha*), which belongs to the scarab beetle family (*Coleoptera: Scarabaeidae*). Its root-feeding larvae are particularly harmful to turfgrass and ornamental nursery plants. These larvae cause extensive damage to turfgrass, especially under warm and dry conditions in late spring and summer. May beetle larvae mainly feed on plant roots, while adult beetles feed on foliage. In a laboratory experiment, the effectiveness of local entomopathogenic nematodes (*Steinernema* sp.) and a commercial strain of *Steinernema carpocapsae* was studied against the third-stage larvae of the May beetle (*Melolontha melolontha*) [2,9,12]. (Figures 1–6). Entomopathogenic nematodes (*EPNs*), belonging to the family *Steinernematidae*, are considered important natural enemies of insects (Kaya, 1990). These soil-dwelling organisms form a mutualistic relationship with bacteria of the genus *Xenorhabdus* (Burnell and Stock, 2000). When nematodes invade an infected insect, their symbiotic bacteria are introduced into the host's circulatory system. By releasing toxins, they cause the insect's death within 24–72 hours (Forst and Clarke, 2002).

The objective of our research was twofold: first, to evaluate the efficacy of the local strain (*Steinernema* sp.) compared to the commercial strain (*Steinernema carpocapsae*) against the third-stage larvae of the May beetle, and second, to determine the influence of temperature and nematode suspension concentration on the activity of these biological agents [3,4,5].

Fig 1: Melolontha Melolontha Imago on the Oak Leaf

ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14557610


Fig 2: Melolontha melolontha Third Instar Larvae in Soil


Fig 3: Melolontha melolontha on the Root of Strawberry


Fig 4: Female and Male Steinernema sp

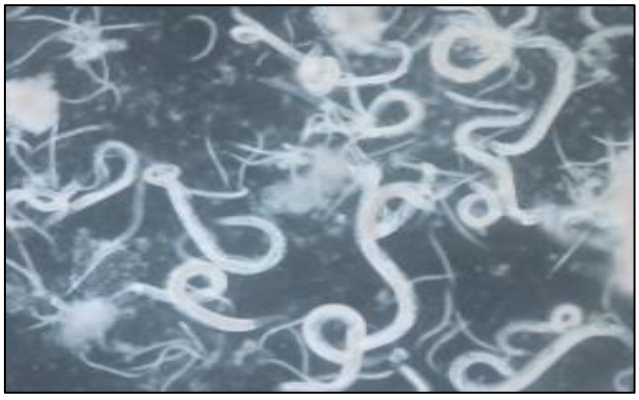
Fig 5: S.carpocapsae against Melolontha melolontha

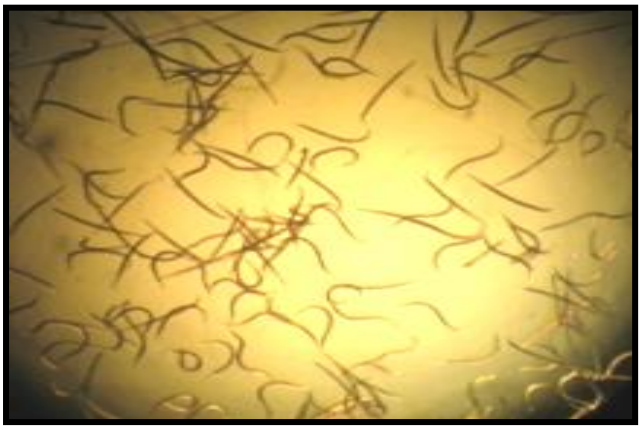
Fig 6: S.carpocapsae against Melolontha melolontha

II. MATERIALS AND METHODS

M. melolontha Larvae were collected from infested agricultural fields. They were sorted by instar stage (L3) and maintained in a controlled environment (20°C,70% RH) with moist soil and plant roots as food sources until experimentation. Nematode Strains *Steinernema carpocapsae* obtained from a commercial source. Local *Steinernema* Strain: isolated from soil samples using the *Galleria* baiting technique and identified morphologically and molecularly *M.melolontha* Larvae were placed in petri dishes (90 mm diameter) or soil-filled containers. Nematodes were applied at concentrations of 2500 and 3000 infective juveniles (IJs) per larva. Control groups were treated with sterile water. Each treatment included 10 larvae per replicate, with three replicates per treatment. Environmental Conditions was temperature: 20°C, relative humidity: 70%. Larvae were monitored daily for 14 days. Dead larvae were dissected to confirm nematode infection. Mortality rates were recorded, and nematode reproduction within the cadavers was assessed. Was established mortality rates: mortality was calculated for each treatment.LC50 and LT50:LC50: The concentration of nematodes required to kill 50% of larvae.LT50: The time required to kill 50% of larvae at a fixed concentration. Data were analyzed using one-way ANOVA test to identify significant differences between treatments.Probit analysis was used to calculate LC50 and LT50 values.

III. OBTAINED RESULTS

Both nematode strains caused significant larval mortality compared to the control. Mortality increased with higher nematode concentrations. The local *Steinernema* strain demonstrated comparable efficacy to *S. carpocapsae*. LT50 values were lower for *S. carpocapsae,* indicating faster action compared to the local strain. Both strains successfully reproduced within the host larvae, with no significant difference in progeny production.

A higher mortality rate of *Melolontha melolontha* on the temperature 250C concentration 2500 IJs for imago (22%), for third instar larvae 48% was obtained by *Steinernema spp,* but for the commercial specie for imago *Steinernema carpocapsae* (49%), for larvae 84%. On the temperature

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250C, and concentration 3000 IJs, higher mortality rate for imago of *Mololontha melolontha* by S. carpocapsae it was 69%, for larvae, it was 95 %. For *Steinernema spp*. higher mortality rate of imago it was 51%, for third instar larvae was 89%. On the temperature 300C, and concentration 3000 IJs *Steinernema carpocapsae* higher mortality rate for imago of *Mololontha melolontha* it was 42%, for larvae, it was 64 %. By nematoda *Steinernema spp* higher mortality of *M. melolontha* imago it was 25%, for larvae 52 %. We determined that in the second option, where high nematode suspension concentrations were used (3000 nematodes in 1 ml of water) on the temperature 250C as for *S. carpocapsae* and also for new species *Steinernema spp*. The nematode concentration was lower (2500 nematodes in 1 ml of water). According to percentage of insect mortality and temperature index, we determined that species *Steinernema carpocapsae*, commercial strain and also new local species *Steinernema sp*. It is distinguished by high efficiency and their use against said pest insect *M. melolontha* is fully justified [11,13].

IV. DISCUSSION

The study demonstrates that both *S. carpocapsae* and the local *Steinernema* strain are effective against *M. melolontha* larvae. While *S. carpocapsae* acted faster, the local strain showed a comparable mortality rate, suggesting its adaptability to the region's ecological conditions. Host factors, such as larval instar stage, influenced susceptibility. Environmental conditions, including soil type and moisture, likely impacted nematode mobility and infection rates. The use of local *Steinernema* strains offers an advantage due to their inherent adaptation to regional conditions, potentially reducing the need for external inputs and enhancing sustainability in pest management strategies. Field trials are necessary to validate laboratory findings. Synergistic effects of combining nematodes with other biological agents should be explored.

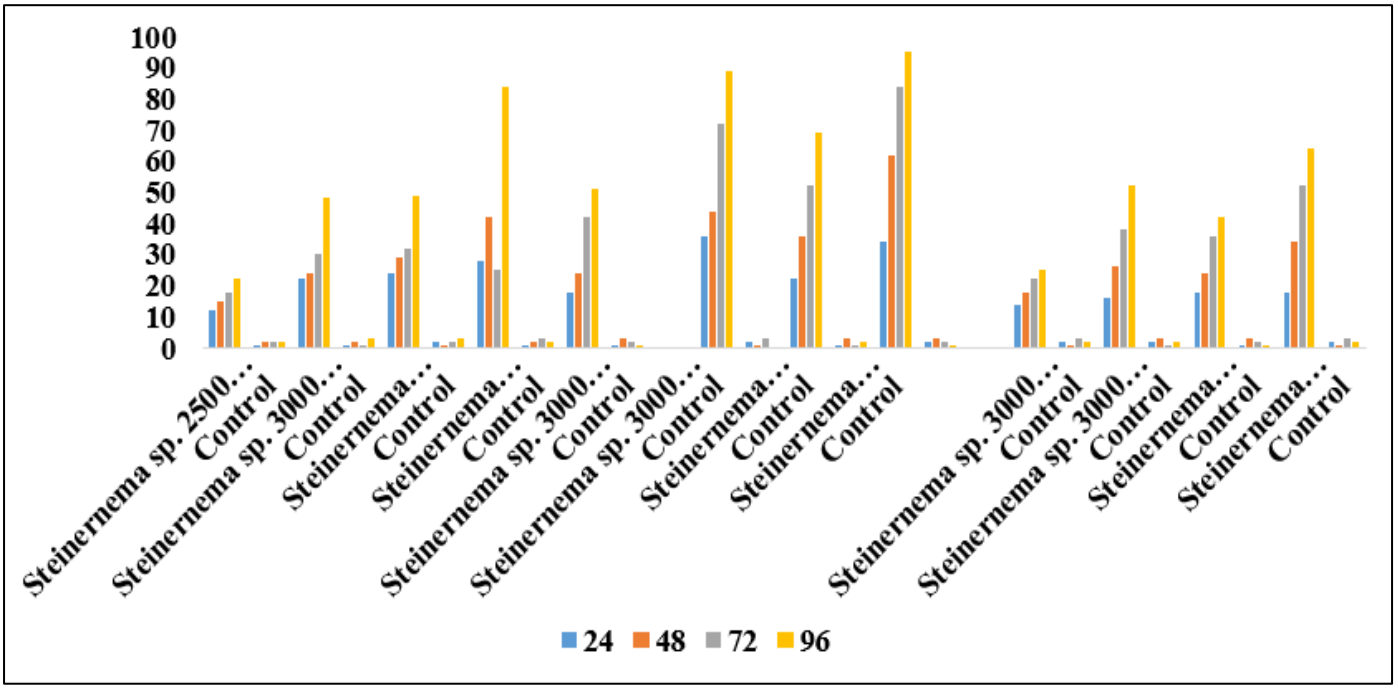

Fig 7: Virulence of *Steinernema sp* (Georgia) and Commercial Strain *Steinernema carpocapsae* against Third Instar Larvae May beetle (*Melolontha melolontha*) on the Temperature 25 and 30^{0}C with 2500, and 3000 n/ml Concentration

V. CONCLUSION

In conclusion, It was determined that, May beetle (*Melolontha melolontha*) can be controlled by *Steinernema sp*. and commercial strain *Steinernema carpocapsae*. Further studies we will continue at field and greenhouse conditions. This study highlights the potential of both *Steinernema carpocapsae* and a local *Steinernema* strain as effective biological control agents against *M. melolontha* larvae. The local strain's efficacy underscores the importance of utilizing region-specific EPNs for integrated pest management.

VI. STATISTICAL ANALYSIS

Example structured dataset (based on extracted raw data) # Columns: Time points (24, 48, 72, 96 hours), Condition, and Values structured_data = {
"Condition": ["Steinernema sp. 2500 n/ml M. melolontha imago", "Control",
"Steinernema sp. 3000 n/ml M. melolontha larvae", "Control",
"Steinernema carpocapsae 2500 n/ml M. melolontha imago", "Control",
"Steinernema carpocapsae 3000 n/ml M. melolontha larvae", "Control",
"Steinernema sp. 3000 n/ml M. melolontha imago", "Control",

ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14557610

"24_hours": [12, 1, 22, 1, 24, 2, 28, 1, 18, 1],
"48_hours": [15, 2, 24, 2, 29, 1, 42, 2, 24, 3],
"72_hours": [18, 2, 30, 1, 32, 2, 25, 3, 42, 2],
"96_hours": [22, 2, 48, 3, 49, 3, 84, 2, 51, 1],
Create a DataFrame from structured data df = pd.DataFrame(structured_data)
Summary statistics summary_stats = df.describe()
Display summary statistics for the raw data summary_stats

	24_hours	48_hours	72_hours	96_hours
count	10.000000	10.000000	10.00000	10.0000 00
mean	11.000000	14.400000	15.70000	26.500000
std	11.105554	14.645439	15.61374	29.534349
min	1.000000	1.000000	1.00000	1.000000
25%	1.000000	2.000000	2.00000	2.250000
50%	7.000000	9.000000	10.50000	12.500000
75%	21.000000	24.000000	28.75000	48.750000
Max	28.000000	42.000000	42.00000	84.000000

➢ *Results of the ANOVA Test:*

- F-statistic: 23.84
- P-value: 4.41×10^{-9}

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