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Control of Pest Insect of June Beetle (*Amphimallon solstitialis*), with Entomopathogenic Nematodes

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Abstract:- The June beetle (*Amphimallon solstitialis*), a significant pest that affects grass, vegetables, and ornamental plants, causes substantial damage through its root-feeding larvae and foliage-feeding adults. This study evaluates the use of entomopathogenic nematodes as biological control agents against the fourth instar larvae of the June beetle. Two nematode species, *Heterorhabditis bacteriophora* and a local strain, *Steinernema tsagveriensis sp.n.*, were tested for efficacy at various concentrations (2000 and 3500 infective juveniles per milliliter) and temperatures (25°C and 35°C). Results showed that *H. bacteriophora* achieved a mortality rate of 78%, while *S. tsagveriensis sp.n.* resulted in a mortality rate of 56%. The optimal conditions for nematode application included a suspension concentration of at least 2000 infective juveniles per milliliter and a temperature of 25°C. These findings underscore the effectiveness of both nematode species as ecologically sustainable solutions for controlling *A. solstitialis*, with *H. bacteriophora* demonstrating higher efficacy.

Keywords:- *Heterorhabditis Bacteriophora, Amphimallon Solstitialis, Biological Control, Temperature. Concentration of Nematode Suspension.*

I. INTRODUCTION

The June beetle (*Amphimallon solstitialis*), commonly referred to as the summer chafer or European June beetle, is a significant pest affecting turfgrass, vegetable crops, and ornamental plants. These beetles emerge annually from late spring to early summer, with adult beetles feeding on foliage and larvae damaging roots. Their life cycle consists of four stages: eggs, larvae (grubs), pupae, and adults. Severe infestations by larvae can cause extensive turf damage, leading to yellowing, loosening of the grass, and eventual death. The presence of larvae is often indicated by increased digging activity from animals such as raccoons and skunks searching for grubs. Biological control, particularly the use of entomopathogenic nematodes, offers an ecologically friendly alternative to chemical pesticides for managing June beetle populations [5,6]. These nematodes, members of the *Steinernematidae* and *Heterorhabditidae* families, are microscopic, unsegmented roundworms that parasitize insects. Two nematode species, *Heterorhabditis bacteriophora* and a locally isolated *Steinernema tsagveriensis sp.n.*, have demonstrated potential for controlling June beetle larvae. The nematodes release symbiotic bacteria (*Photorhabdus* spp. for *Heterorhabditis*, and *Xenorhabdus* spp. for *Steinernema*), which infect and kill larvae within 48,72 hours [1,2,3,4].

This study evaluates the efficacy of these nematodes under controlled laboratory conditions. Testing was conducted at two temperatures (25°C and 35°C) and at two suspension concentrations (2000 and 3500 infective juveniles per milliliter). The objective was to identify optimal application parameters and assess the effectiveness of both nematode species in managing *A. solstitialis* larvae, contributing to sustainable pest management strategies [7].

Fig 1 *Amphimallon solstitiale* Imago on young oak Leaf

Fig 2 *Amphimallon solstitiale* Imago on the Tree Betula Spp.

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Fig 3 *Amphimallon solstitiale* Forth Instar Larvae in Soil

Fig 4 Using EPN H. *bacteriophora* on the A. *solstitialis*

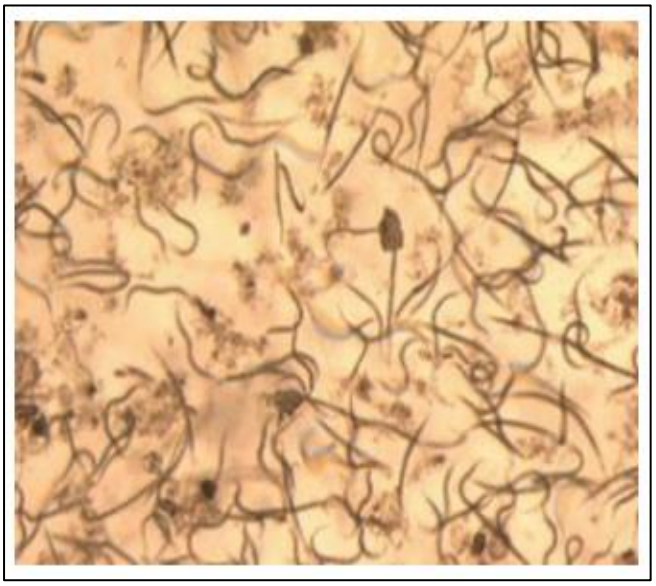

Fig 5 Using EPN isolate S. *tsagveriensis* on the A. *solstitialis*

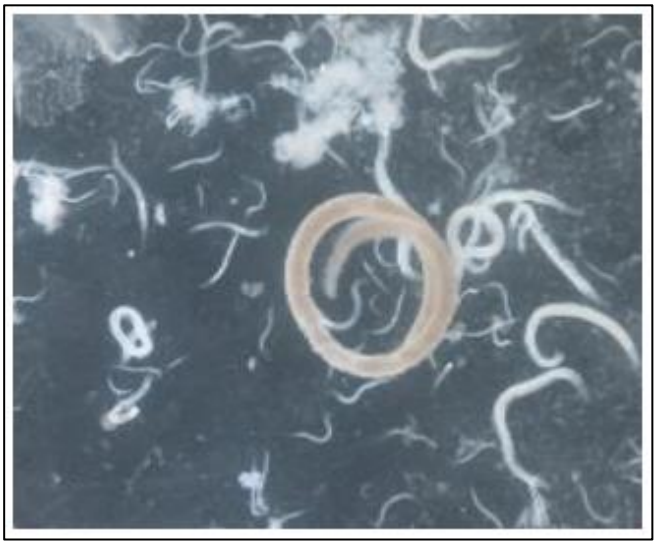

Fig. 6. Using EPN isolate S *tsagveriensis* on the A. *solstitialis*

II. MATERIALS AND METHODS

The target pest for this study was the June beetle (*Amphimallon solstitialis*), specifically its fourth-stage larvae. The nematode species used for biological control included both a commercial strain of *Heterorhabditis bacteriophora* and a local strain of *Steinernema tsagveriensis*. The infective juveniles (IJs) of both nematode species were obtained in suspension form. Two different concentrations of nematode suspension were tested: 2000 infective juveniles (IJs) per milliliter (ml) of water, and 3500 IJs per ml of water. The nematodes were stored and prepared according to the manufacturer's instructions for the commercial strain and were propagated under laboratory conditions for the local strain. The experiment was conducted in a controlled laboratory environment at two different temperatures: 25°C and 35°C. The nematode suspension was applied to containers with soil, where the fourth-stage larvae of *A. solstitialis* were placed. The larvae were collected and placed in the soil at the appropriate stage of development. The nematode suspension was applied to the soil surface or the thatch layer of the containers to ensure uniform distribution. Each treatment consisted of the two nematode species, tested at two different concentrations (2000 and 3500 IJs per ml of water). The nematode suspension was introduced into the experimental setup by evenly spraying it across the surface. After the nematode application, the larvae were monitored for mortality over a 48-hour period. Dead larvae were removed and examined for signs of nematode infection. The effectiveness of the treatments was assessed by calculating the mortality rate of *A. solstitialis* larvae in each treatment, which was based on the number of dead larvae observed [8].

III. OBTAINED RESULTS

The efficacy of both nematode species, *H. bacteriophora* and *S. tsagveriensis*, in controlling *A. solstitialis* larvae was assessed under various experimental conditions. Mortality rates were recorded for each treatment based on the number of dead larvae observed after 48 hours till 72 hr. of exposure to the nematode suspensions. The

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results showed that both nematode species were effective in killing the fourth-stage larvae of *A. solstitialis*, but there were noticeable differences in the effectiveness of the two species. *H. bacteriophora* exhibited higher mortality than *S. tsagveriensis*. At a concentration of 2000 IJs per ml, the mortality rate for *H. bacteriophora* was 78%, while *S. tsagveriensis* caused a mortality rate of 56%. Increasing the concentration of nematodes to 3500 IJs per ml resulted in increased mortality for both species, with *H. bacteriophora* achieving a mortality rate of 82%, and *S. tsagveriensis* achieving 63%. Temperature was also a significant factor in the effectiveness of the nematodes. The optimal temperature for nematode activity and larvae mortality was found to be 25°C. At this temperature, both nematode species showed higher efficacy, with *H. bacteriophora* achieving a mortality rate of 82% at 3500 IJs/ml. At the higher temperature of 35°C, the effectiveness of both species decreased, although *H. bacteriophora* still showed better performance 61% than *S. tsagveriensis* 44 %. Overall, the results indicate that *H. bacteriophora* is a more effective biological control agent against *A. solstitialis* larvae, particularly at higher nematode concentrations and optimal temperatures. However, both species demonstrated potential for pest control, with *S. tsagveriensis* still showing significant efficacy under certain conditions [9,10].

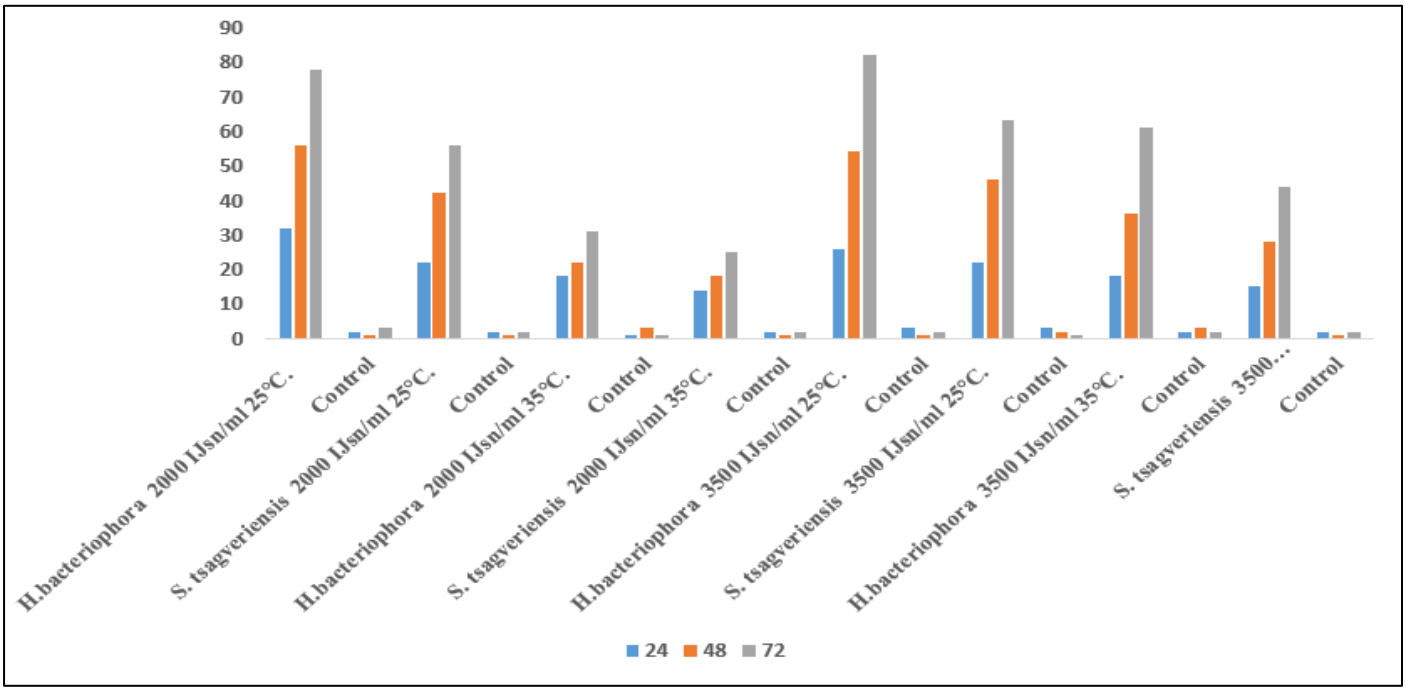

Fig 7 Virulence of *S. tsagveriensis* (Georgia) and commercial strain *H. bacteriophora* against 4th instar larvae June beetle (*A. solstitialis*) on the temperature 25 and 35^{0}C with 2000 and 3500 n/ml concentration

IV. DISCUSSION

The results of this study demonstrate the potential of entomopathogenic nematodes (*H. bacteriophora* and *S. tsagveriensis*) as biological control agents against *A. solstitialis* larvae. Both nematode species were effective in causing mortality in the fourth-stage larvae of the June beetle, with *H. bacteriophora* showing significantly higher efficacy than *S. tsagveriensis*. The higher mortality rate observed for *H. bacteriophora* at both concentrations and temperatures suggests that this species may be more suitable for controlling *A. solstitialis* larvae under typical field conditions. The mortality rate increased with higher nematode concentrations, particularly for *H. bacteriophora*, which reached 82% at 3500 IJs/ml. This finding highlights the importance of using an adequate nematode concentration to achieve effective pest control. At lower concentrations, such as 2000 IJs/ml, both nematode species still demonstrated significant effectiveness, although the mortality rate was lower. Therefore, optimizing the concentration of nematodes is essential for ensuring high levels of mortality in the target pest population.Temperature was a decisive factor influencing the effectiveness of both nematode species. The optimal temperature for nematode activity was found to be 25°C, where *H. bacteriophora* achieved the highest mortality rates. This conclusion aligns with previous studies that report optimal nematode performance within moderate temperature ranges. At higher temperatures (35°C), both species showed reduced efficacy, which could be due to stress induced by elevated temperatures on either the nematodes or the target larvae. This decrease in efficacy at high temperatures emphasizes the necessity of considering environmental conditions when applying nematodes for pest control, as extreme heat may limit their effectiveness. The comparison between *H. bacteriophora* and *S. tsagveriensis* in this study provides valuable insights into the selection of appropriate nematode species for pest control. Although both species were effective, *H. bacteriophora* consistently demonstrated higher mortality rates and a broader range of effective concentrations and temperatures. This makes *H. bacteriophora* a more reliable choice for pest management. However, *S. tsagveriensis* still showed significant effectiveness, especially at lower concentrations, indicating that it may play a role in integrated pest management

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programs, particularly in situations where *H. bacteriophora* is unavailable or unsuitable. The use of entomopathogenic nematodes as a biological control method offers several advantages over chemical pesticides. Nematodes are ecologically clean, non-toxic to humans and animals, and can specifically target insect pests, minimizing harm to beneficial organisms. Furthermore, nematodes can penetrate the soil and target pests at their underground stages, such as larvae, which are difficult to control with traditional pesticide methods. However, the success of nematode application depends on several factors, including the species of nematode, concentration, temperature, and soil conditions. These factors must be optimized to ensure effective control of *A. solstitialis* and other similar pests.

V. CONCLUSION

In conclusion, *H. bacteriophora* is a promising biological control agent against *A. solstitialis* larvae, particularly under optimal concentrations and temperature conditions. However, further research is needed to explore the potential of *S. tsagveriensis* and other nematode species under different environmental conditions. Additionally, field trials should be conducted to assess the effectiveness of these nematodes in real-world settings and to evaluate their potential role in integrated pest management systems for controlling June beetles and other root-feeding pests.

STATISTICAL ANALYSIS

To perform a basic ANOVA test on this data, I can provide a conceptual overview based on its structure:

Overview of the ANOVA Test for This Data.

The data consists of response measurements for two nematode species *(Heterorhabditis bacteriophora* and *Steinernema tsagveriensis*) under varying conditions:

- *Factors:*

- Species: *H. bacteriophora* vs. *S. tsagveriensis.*
- Concentration: 2000 IJs/ml and 3500 IJs/ml.
- Temperature: 25°C and 35°C.
- Time: 24, 48, and 72 hours.
- Responses: Measured values for nematodes under these conditions compared with controls.

- *Expected Results:*

- *Main Effects:*

- Species: Possible differences in responses between *H. bacteriophora* and *S. tsagveriensis.*
- Concentration: Higher concentrations may show higher responses.
- Temperature: Optimal temperatures could enhance effectiveness.
- Time: Responses may increase over time.

- *Interactions:*

Interplay between species, concentration, temperature, and time.

This provides a structured framework for conducting an ANOVA test with this data.

The mortality assessment data were statistically analyzed to compare the effectiveness of the different nematode species, concentrations, and temperature conditions. The results were used to determine the optimal conditions for nematode application in controlling *A. solstitialis* larvae.

This section outlines the methodology used to test the effectiveness of *H. bacteriophora* and *S. tsagveriensis* against the larvae of the June beetle. It describes the experimental setup, nematode preparation, application methods, and data collection procedures in detail.

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