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Isolation Of Entomopathogenic Nematodes From Soil By The "Galleria Trap" Method And Their Efficacy Against The Cotton Bollworm (Helicoverpa Armigera)

Karimova Rikhsiniso Miratkhamovna
Doctoral student, Research Institute of Plant Quarantine and Protection, Uzbekistan

ABSTRACT

Entomopathogenic nematodes were isolated by the Galleria trap method from 60 soil samples collected in Urtachirchik and Yukorichirchik districts of Tashkent region, and their efficacy against cotton bollworm (Helicoverpa armigera Hbn.) larvae was assessed. 20.0% of samples were positive (9 Steinernema, 3 Heterorhabditis isolates). Steinernema sp. OCh-2 at 100 IJs/larva caused 92.5% mortality after 72 h (LC_{50} = 20.4 IJs/larva).

KEYWORDS: Entomopathogenic nematodes, Steinernema, Heterorhabditis, Galleria mellonella, Helicoverpa armigera, biological control.

INTRODUCTION

The cotton bollworm (Helicoverpa armigera Hbn.) is a polyphagous pest causing serious damage to cotton, tomato and maize in Uzbekistan. Long-term use of insecticides has led to the development of resistance in this pest and to environmental pollution. Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae, together with their symbiotic bacteria, kill the insect host within 24–48 hours and are safe for warm-blooded animals [1; 5; 9]. However, commercial strains often show low efficacy because they are not adapted to local conditions [6; 8].

The aim of the study was to isolate EPNs from local soils and to determine their efficacy against the cotton bollworm.

MATERIALS AND METHODS

In May–June 2025, 60 composite soil samples (30 from each district) were collected at a depth of 0–20 cm from cotton and alfalfa fields and orchards in Urtachirchik and Yukorichirchik districts of Tashkent region. EPNs were isolated by the "Galleria trap" method [2]: five larvae of Galleria mellonella L. were placed in 200 g of soil and kept at 25±1 °C for 7 days; infective juveniles (IJs) were collected from the dead larvae in White traps [3] and identified to genus level based on cadaver colour and IJ morphometry [7]; to fulfil Koch's postulates, the IJs were re-inoculated into healthy G. mellonella larvae.

In the bioassay, third-instar cotton bollworm larvae were inoculated in 24-well plates at rates of 25, 50 and 100 IJs/larva (4 replicates of 20 larvae each). Mortality was recorded at 24, 48 and 72 h and corrected using Abbott's formula [4]; the data were processed by ANOVA and Tukey's test ($P<0.05$), and LC_{50} and LT_{50} values were determined by probit analysis.

RESULTS

EPNs were detected in 12 of the 60 samples (20.0%); their occurrence was higher in Urtachirchik than in Yukorichirchik (Table 1).

Table 1. Isolation of EPNs from soils

District	Samples	Positive	Occurrence, %	*Steinernema*	*Heterorhabditis*
Urtachirchik	30	7	23.3	5	2
Yukorichirchik	30	5	16.7	4	1
Total	60	12	20.0	9	3

The efficacy of the most virulent isolates, Steinernema sp. OCh-2 and Heterorhabditis sp. YCh-1, increased with increasing rate (Table 2). At 100 IJs/larva, OCh-2 and YCh-1 caused 36.3% and 28.8% mortality, respectively, within 24 h ($P<0.05$).

Table 2. Efficacy of the isolates against the cotton bollworm (72 h, %, M±m)

Isolate	25 IJ	50 IJ	100 IJ	LC_{50}, IJs/larva	LT_{50}, h
Steinernema sp. OCh-2	58.8±3.1	78.8±2.8	92.5±2.0	20.4	33.6
Heterorhabditis sp. YCh-1	52.5±3.2	71.3±2.9	86.3±2.4	25.7	38.9

Note: natural mortality in the control did not exceed 2.5%.

Discussion

The occurrence rate of 20.0% falls within the range of 2–45% reported in the literature [8]. The predominance of Steinernema can be explained by the loamy soils, and the rapid action of OCh-2 by its adaptation to local conditions [1; 6]. The 1.26-fold lower LC_{50} and 5.3 h shorter LT_{50} of OCh-2 compared with YCh-1 indicate the high pathogenicity of its symbiotic Xenorhabdus bacteria [9]. Given that the cotton bollworm pupates in the soil, it is advisable to apply the isolates to the soil surface together with irrigation [5; 9].

Conclusions

1. The “Galleria trap” method proved simple and effective for isolating EPNs from the soils of Tashkent region: 20.0% of the samples were positive, and of the 12 isolates, 9 belonged to the genus Steinernema and 3 to the genus Heterorhabditis.
2. Steinernema sp. OCh-2 (92.5% mortality at 72 h; LC_{50} = 20.4 IJs/larva) is a promising isolate for developing a biological preparation; its molecular identification and field testing are planned at the next stage.

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