Field Application of Entomopathogenic Nematodes Against White Grubs, *Pentodon algerinum* on Strawberry Plants in Egypt

M.S. Hammad[1*], M.H. Mowafi[2], A.A. Abdallah[2] and M.I. Hassan[1]
(1) Plant Protection Research Institute, A.R.C., Dokki, Giza, Egypt;
(2) Agricultural Zoology and Nematology Department, Faculty of Agriculture, Al-Azhar University, Egypt.
*Email address of the corresponding author: mismail1968@yahoo.com

Abstract

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This study aimed to assess the ability of four entomopathogenic nematode strains (two Steinernematid and two Heterorhabditid) to control white grubs, *Pentodon algerinum* (Coleoptera: Scarabaeidae) on strawberry plants in the field, by releasing them through the irrigation water system during 2021 in El-Qualiobia and El-Behera Governorates. Results obtained revealed that nematode application had a significant effect against the white grubs by reducing the number of infested plants. All strains used significantly reduced the number of infested plants after the 2nd week of application, and the effect was increased further three weeks after the treatment, and the EGAZ10 strain was significantly the most effective. Results obtained should encourage workers to further investigate biocontrol agents as potential safe components in pest management.

Keywords: Strawberry, white grubs, entomopathogenic nematodes, field applications.

Introduction

Strawberry, *Fragaria ananassa* Duchesne (Rosales: Rosaceae), is considered one of the most commonly consumed fruit crops worldwide, produced mainly in China, USA, Mexico, Egypt, Türkiye and Spain (Talavera *et al*., 2019). The world production of strawberry is about 9.11 million tons. Egypt occupied fourth place in strawberries production worldwide. Strawberry area in Egypt is about 237,73.8 acres producing 464958 tons, with 164,740 tons exported in 2016, making Egypt the fifth largest strawberry producer after China, the USA, Turkey, and Mexico (FAOSTAT, 2023).

Mild weather is suitable for strawberry plants and, unfortunately also for several arthropod pests. Recently, white grub *Pentodon algerinum* is a universal agricultural polyphagous insect pest, which recently reported to destroy strawberry crop in Egypt (Samah *et al.,* 2021). White grubs larvae feed on plant roots, facilitating their infection with other soil pathogens and subsequent decay. The damage caused to the roots affect the entire plant, eventually leading to the plant's death. White grubs live concealed in the soil that makes it difficult to control, in addition to their ability to develop insecticide resistance (Gao *et al*., 2020). *P. algerinum*, larvae are identified as the waste organic manure larvae (White grubs) with a brown head and a thick large arched yellowish body, the larvae live around roots of plants in a curved shape, and feed on organic matter and plant roots, rapidly causing the plant to wilt and die (Laznik *et al.*, 2012). Their life cycle has 3 instar larvae and pupates in the soil, with two generations per year. Hard-back scarab beetles' mate in spring and remain dormant in the soil during winter. Adults are nocturnal creatures, more active during the night. For many years, white grubs have been mainly controlled by using chemical insecticides, such as phoxim and chlorpyrifos (Liu *et al*., 2017), but their application increased environmental contamination (Swelam, 2021). Therefore, in light of growing concerns about environmental and human safety, there is an urgent need for alternative biological strategies to control white grubs. Entomopathogenic nematodes (EPNs) are recognized as potential biological control agents and have been used to manage a variety of soil-dwelling insects due to their exceptional ability to actively seek out hosts (Georgis *et al*., 2006; Grewal *et al*., 2005; Torrini *et al.,* 2020). Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) against white grubs have been successfully applied (Samah *et al.*, 2021). The natural habitat of entomopathogenic nematode strains is the soil where they infect white grubs and kill them within 48 hours (Gümüşsoy *et al.,* 2022).

The present study aimed to evaluate efficacy of the four entomopathogenic nematode strains against *P. algerinum* (Coleoptera: Scarabaeidae) under field conditions in two Governorates, Egypt.

Materials and Methods

Experimental field design and treatments

Field experiments were conducted on strawberry plants naturally infested with *Pentodon algerinum* in Toukh, El-Qualiobia Governorate and Bader Center, El-Behera Governorate during 2021-2022 season. Soil type was loamy in the first location and sandy soil in the second location.

The experimental design followed was the random complete block design and the plots were planted with strawberry (Varantoura variety) in September 2021. The plants were naturally infested with white grubs, and the infestation rate range was 35-60% on 11/3/2021 before the

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treatment application. An area of one feddan (4000 m^2) was selected for treatments in each Governorate and each location was planted with 40,000 strawberry plants/feddan. The experimental area was divided into four blocks, each block was subdivided into five plots (as replicates) treated with entomopathogenic nematodes (10000 IJs/plant), with one left untreated as a control treatment.

Drip irrigation system was utilized. Twenty plants were randomly examined before treatment to determine the percentage of infested plants and also 7, 15, and 21 days after treatment. The reduction in infestation rate was calculated according to Henderson and Tilton's formula (Henderson & Tilton, 1955).

Source of entomopathogenic nematode strains

Nematodes, *Steinernema carpocapsae* (EGAZ9 and EGAZ10) and *Heterorhabditis* spp. (EGAZ1 and EGAZ3) were obtained from the Pest Physiology Department, Plant Protection Research Institute, Dokki, Giza, Egypt. Nematodes were cultured on larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) at 25±1°C. Infective juveniles (IJs) emerging from the wax moth larvae were collected twice a week, using white traps (Sikandar *et al.*, 2021). Nematode viability was assessed under a stereomicroscope and considered alive when actively moving or showing response after probing with a needle. Entomopathogenic nematode suspensions were used for experiments when most of IJs were viable and active (10000 IJs/plant). All treatments were performed before sunset to prevent excessive evaporation and direct sunlight.

Calculation of pest reduction rate

The pest reduction rate (%) was calculated according to the equation of Henderson & Tilton (1955):

$$\text{Reduction rate (\%)} = 1 - \frac{\text{number of pest in the treated group after treatment} \times \text{number of pests in the treated group before treatment.}}{\text{number of pests in the control group after treatment} \times \text{number of pests in the control group before treatment}} \times 100$$

Statistical analysis

The data from the field study was analyzed using statistical methods. The mean and standard deviation were calculated for the quantitative variables. To compare the differences among the groups being studied, a three-way and two-way analysis of variance (ANOVA) was used with the help of SPSS program. Significance between mean values was determined by the least significant difference at P=0.05.

Results and Discussion

Data presented in Table 1 and Figure 1 show that the mean numbers of infested strawberry plants with white grubs, *P. algerinum* before and after nematodes application throughout 3 successive weeks. The infested strawberry plants of initial records were approximately the same. The mean numbers of nematode-treated infested strawberry plants decreased with time compared to the untreated ones. There was a significant difference among the pest population decrease in infested plants using the four nematode strains compared to the control treatment under field conditions in both locations.

The EGAZ10 entomopathogenic strain produced the highest significant decrease in white grubs *P. algerinum* compared to all other treatments. This strain was the most effective in reducing white grubs on day 15 and day 21, whereas the other three strains EGAZ9, EGAZ1 and EGAZ3 had a close effect to each other, in both locations.

Effect of different treatments at both locations

Bader region- The results obtained showed that the number of plants infested with white grubs began to decrease on the seventh day due to nematode strains, with no significant differences among treatments. Reduction rate range was 19.63-26.14% (Tables 1 & 2 and Figures 1 & 2). The efficiency of nematode strains increased with time, with no significant differences among them, except for the strain EGAZ10, which gave the most significant decrease in the number of infested strawberry plants. The pest reduction rate by the four entomopathogenic nematode strains was in the range 38.25-52.77% during the experimental period.

Similarly, the same trend was recorded and the reduction rate during the twenty-one-day period ranged from 55.64-74.20% (Figure 2 and Table 2) in the four entomopathogenic nematode strains. The reduction in the number of pests gradually increased over time.

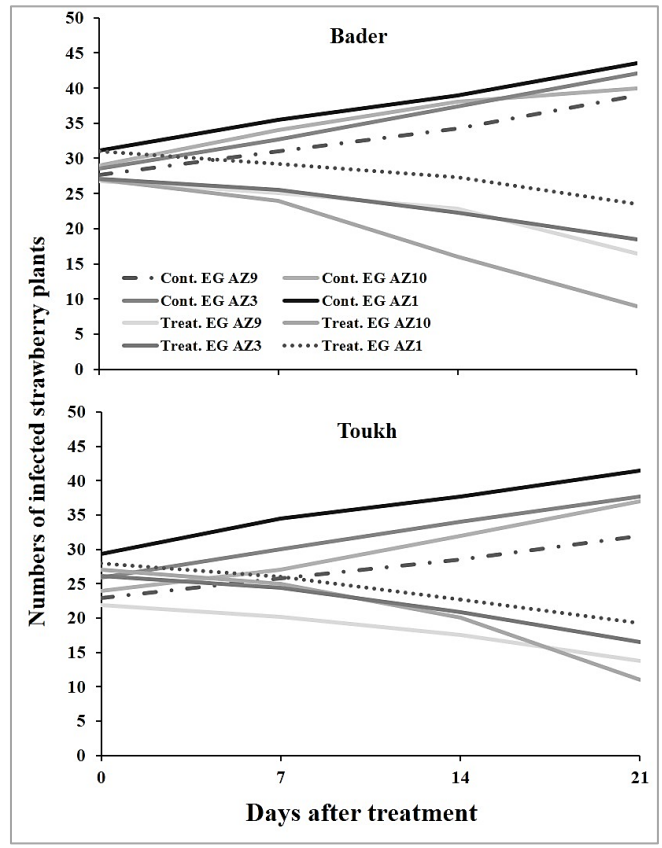


Figure 1. Effect of the four entomopathogenic nematode strains on the reduction of white grubs *Pentodon algerinum* in infested strawberry plants compared to the control treatment under field conditions.

Table 1. Effect of four entomopathogenic nematode strains (EPNs) on mean number of infested strawberry plants with *Pentodon algerinum* compared to untreated plants at Bader and Touch locations.

	Time after application (days)							
	0		7		14		21	
EPN strains	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Location Bader								
EG AZ9	27.60	26.80	31.05	25.05	34.30	22.85	38.95	16.50
EG AZ10	29.00	27.00	34.00	24.00	38.00	16.00	40.00	9.00
EG AZ3	28.55	27.13	32.68	25.55	37.40	22.25	42.05	18.50
EG AZ1	31.10	30.95	35.45	29.20	38.95	27.30	43.55	23.55
Location Toukh								
EG AZ9	22.95	21.90	25.80	20.15	28.55	17.55	31.95	13.75
EG AZ10	24.00	27.00	27.00	25.00	32.00	20.00	37.00	11.00
EG AZ3	25.85	26.10	30.05	24.45	34.05	20.85	37.70	16.55
EG AZ1	29.35	27.95	34.45	26.05	37.70	22.70	41.50	19.30

Table 2. The effect of the four entomopathogenic nematode strains on the reduction rate of white grubs infested plants at 7, 14 and 21 days after treatment.

	Reduction rate (%) after application (days)					
	7		14		21	
Nematode strains	Bader	Toukh	Bader	Toukh	Bader	Toukh
EGAZ9	19.63	21.00	38.36	39.48	61.34	56.17
EGAZ10	26.14	24.90	52.77	49.09	74.20	70.37
EGAZ3	20.37	21.48	41.00	44.10	56.94	59.48
EGAZ1	21.65	24.33	38.25	43.26	55.64	58.39

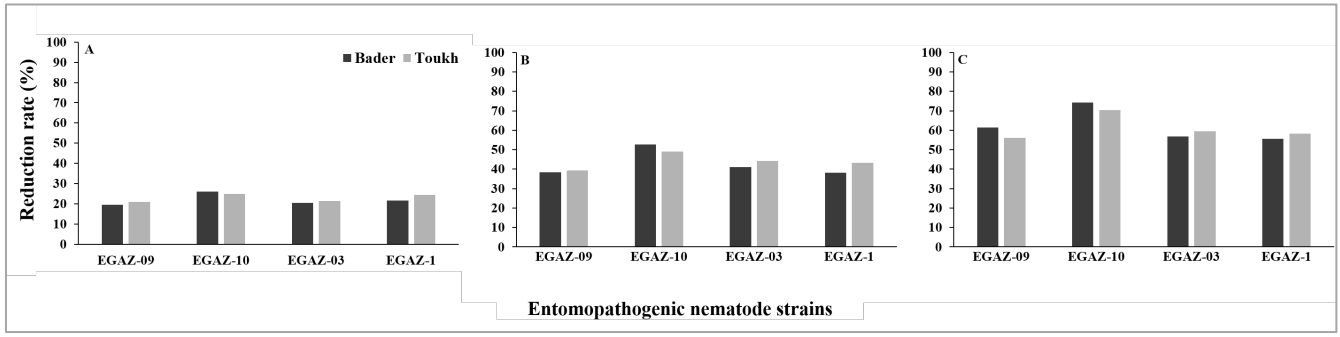


Figure 2. Decrease in the numbers of white grubs infested plants after releasing the four entomopathogenic nematode strains, 7 (A), 14 (B) and 21 (C) days after treatment.

Toukh region- Similarly, in the Toukh region, the results showed that the reduction of white grubs ranged from 21.00-24.90% during the first seven days, but the reduction was not statistically significant among the four entomopathogenic nematode strains EGAZ10, EGAZ9, EGAZ1 and EGAZ3. The reduction of white grub treated with the nematode strains continued to decrease on day 14, with no significant difference among them except for strain EGAZ10, which had the most significant decrease in the number of infested strawberry plants. The reduction rates ranged by the four entomopathogenic nematode strains from 39.48-49.09% during this period. The same trend of pest reduction rate continued during the 21-day period in the range 56.17-70.37%, by the four nematode strains (Figure 2 and Table 2).

In general, the treatment with the nematode strain EGAZ10 showed the highest pest reduction rate in the number of infested plants in both regions (Badr and Toukh), when samples were collected on the 7th, 14th and 21st day after treatment (Figure 2 and Table 2).

Effect of the control (untreated) treatment

In the Bader region, there was an increase in infested plants, observed on 7, 14 and 21 days after treatment, with an increase in infested plants by 13-17% during the first seven days. Infested plants continued to gradually increase in the same direction until it reached 24-31% on day 14 after treatment, and 38-47% on day 21 after treatment. Similar trends were observed in the Toukh region (Figure 1 and Table 1). In general, there were no significant differences

between the increases in the number of infested plants in the two regions (Badr and Toukh) in the untreated plots.

The nematode strains used reduced the numbers of infected plants with white grubs from 50 to 70%. Especially EGAZ10 strain was more effective against these white grubs compared to the other three strains (df =3, F=13.4 and *P*=0.0001) after releasing the strains on days 7, 14 and 21. However, there were no significant differences between both locations (df =1, F=0.7 and *P*=0.4176) in (Table, 3).
Generally, the treatment with the four entomopathogenic nematode strains showed a decrease in the number of plants infested with the white grub populations 7, 14 and 21 days after treatment and were highly significant compared to the control treatment (df=6, F=108.2 and *P*=0.0001) in both locations (Table 3).

Based on the results obtained in this study, it is proposed to control white grubs in strawberry fields by using entomopathogenic nematodes in irrigation water after sunset. Entomopathogenic nematodes can play an essential role in the control of different species of soil-borne pests such as *Pentodon algerinum* (Sikandar *et al.*, 2021). In the current investigation, four entomopathogenic nematode strains belonging to Steinernematidae and Heterorhabditidae were found as highly pathogenic agent against larvae of *Pentodon algerinum*. Similarly, the tested nematode species were reported earlier to have significant pathogenicity against other insect species (Devi *et al.,* 2020).

Table 3. Three away ANOVA analysis.

Main effects	DF	F	P	$LSD_{0.05}$
Location	1	0.7	0.4176	2.5
Strain	3	13.4	<0.0001	3.9
Time after treatment	2	303.9	<0.0001	3.2

It could be concluded that the biological control of the *Pentodon algerinum* with four strains of entomopathogenic nematodes was promising. Further studies to compare the effectiveness of chemical control agents with biological control approaches, keeping in mind cost and effect on the environment. In particular, the application of EPNs to control soil-borne pests is of significance for ecological planning, not only in support of biological control approaches, but also to enhance plant defenses (Helms *et al*., 2019).

الملخص

حماد، سند محمد، مصطفى حلمي موافي، عوض علي عبد الله ومحمد اسماعيل حسن. 2026. التطبيق الحقلي للنيماتودا الممرضة للحشرات ضدّ يرقات الجعال ذو الظهر الجامد (*Pentodon algerinum*) على نباتات الفريز/الفراولة في مصر. مجلة وقاية النبات العربية، 44(3):377−381.
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هدفت هذه الدراسة إلى تقييم حقلي لقدرة أربع سلالات من النيماتودا الممرضة للحشرات (اثنتان من نوع Steinernematid واثنتان من نوع Heterorhabditid) على مكافحة يرقات الجعال ذو الظهر الجامد (*Pentodon algerinum*) (Coleoptera: Scarabaeidae) على نباتات الفريز/الفراولة، وذلك بإضافتها للتربة مع مياه الري خلال موسم 2022/2021 في محافظات القليوبية والبحيرة، مصر. أظهرت النتائج أن للنيماتودا تأثير معنوي ضدّ يرقات الجعال البيضاء من خلال تقليل أعداد النباتات المصابة وذلك خلال 3 أسابيع متوالية بعد المعاملة وكان هذا التأثير واضحاً خلال الأسبوع الثالث. كانت السلالة EGAZ10 هي الأكثر تأثيراً وكان للمدة الزمنية بعد المعاملة تأثير كبير. كانت النتائج مشجعة للغاية لاستخدام هذه الأعداء الحيوية (سلالات النيماتودا الممرضة للحشرات) في مكافحة الحشرة، والتي تعدّ من الطرائق الصديقة للبيئة. كما أشارت النتائج بأن السلالة EGAZ10 كانت الأكثر كفاءة، مما يشجع على استخدامها في حقول المزارعين.

كلمات مفتاحية: الفريز/الفراولة، يرقات الجعال ذو الظهر الجامد، النيماتودا الممرضة للحشرات، تطبيقات حقلية.

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