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BIOLOGY, ECOLOGY AND MOLECULAR IDENTIFICATION OF *Eobania vermiculata* (MÜLLER) PHENOTYPES INFESTING CERTAIN FRUIT ORCHARDS IN BEHAIRA GOVERNORATE, EGYPT

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ABSTRACT: Land snails are considered one of the most destructive agricultural pests. A survey was carried out on three orchards of fruit trees, namely navel orange, mango, and guava, in Behaira Governorate from January 2020 to December 2021. It was found that individuals of the brown garden (chocolate-banded) snail, *Eubania vemiculata* (Muller), were present in the three orchards throughout the entire year with varying values according to season and host plant with two phenotypes: banded brown color and unbanded pale-yellow color. The identified phenotypes varied in percentage occurrence from one orchard to another. The relative percentages of adults with banded brown color and unbanded pale-yellow color on navel orange, mango, and guava during 2020 were 51.22% & 48.18-%, 51.13% & 48.17%, and 56.21% & 43.19%, respectively. While the corresponding values during 2021 were 54.11% & 46.10%, 54.15% & 45.15%, and 52.13% & 47.17%, respectively. Oranges harbored the highest numbers followed by mango and guava. with mean values of 20.51 (21.47), 14.93 (15.57), and 15.13 (15.56) snails per sample, respectively. The values of *E.vermiculata* population density gradually increased after winter months to reach the maximum values during the summer or autumn months. It was observed that adults were more abundant during the summer months, i.e., June, July, August, and September. In contrast, juveniles were more prevalent during February, March, and April. Snails of *E. vermiculata* laid their eggs for about 3.5 months, starting from mid-October 2020 to the end-January 2021. The eggs were spherical with a mean diameter of 3.3 ± 0.35 mm. The number of clutches and eggs laid by *E. vermiculata* significantly ($P \leq 0.05$) changed during the breeding season. Shell measurements of *E. vermiculata* showed a mean shell height of 20.20 mm ranging from 16.60 to 23.80 mm, while the mean diameter was 26.65 mm, with a range of 22.70 to 30.60 mm. Shell thickness ranged between 0.73 to 0.89 mm with a mean of 0.81mm. The mean aperture diameter was 9.04 mm, which ranged between 8.18 to 9.89 mm. On the other hand, the number of whorls ranged between 4.5 to 6.0. and the animal color is banded brown or unbanded pale yellowish. The dendrogram of genetic distances among populations based on band polymorphisms generated by RAPD-PCR using the two primers showed that the two populations (p1& p3) can be identified as the banded brown phenotype, while the two populations (p2 & p4) can be classified as an unbanded pale-yellow phenotype of the land snail, *E. vermiculata.*

Key words: Land snails, *Eubania vermiculata*, Biology, Ecology, Molecular identification, Fruit orchards, Behaira Governorate.

INTRODUCTION

In the last few years, it was noticed that infestation with land snail species has increased markedly and become serious pests causing damage to the agriculture sector all over the world **(Barker, 2002)**. Land gastropods, including snails and slugs, inhabit a wide range of

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environments, from arid to Mediterranean regions **(Schweizer *et al*., 2019)**. Gastropoda is the largest class of Phylum Mollusca containing around 70.000 species including snails and slugs, which are present on every continent **(Pyron and Brown, 2015; Jane *et al*., 2018; Haszprunar, 2020)**.

Recently, in Egypt, land snails have increased dramatically in economic crops causing significant damage to vegetable and field crops, fruit trees, and ornamental plants in several governorates **(Kassab and Daoud, 1964; El-Deeb *et al*., 1996; Hegab *et al*., 1999; Mahrous *et al*., 2002; Ismail *et al*., 2003; Ibrahim *et al*., 2017; Heiba *et al*., 2018; Ali and Robinson, 2020; Abo Zaid *et al*., 2021and Morsy *et al*., 2021)**. Therefore, it is important to shed light on the development of infestation with these pests in the last decade. To accomplish this goal, data concerning the occurrence, population density, oviposition period, and taxonomic characterization of *E. vermiculata* were studied to design effective pest control strategies and reduce the losses of economic fruit trees.

MATERIALS AND METHODS

Field experiments were conducted to gain principal information concerned with the brown garden (chocolate-banded) snail, *E. vermiculata* (Muller), infesting major fruit trees, namely navel orange, *Citrus sinensis* (L.) Osbeck; mango, *Mangifera indica* L., and guava, *Pisidium guajava* L.at Badr Center, Tahrir District, Behaira Governorate, during two successive growing seasons from January 2021 to December 2022. The ecobiological studies in this regard included occurrence and identification, population density, infestation development with temperature and relative humidity, and observation on the oviposition period. Additionally, taxonomic characterization was conducted using DNA isolation and RAPD-PCR techniques.

Ecobiological Consideration on the Brown Garden Snail, *E. vermiculata*

Occurrence and identification of *E. vermiculata*

An extensive survey was carried out from January 2021 to December 2022 concerning the occurrence of land snail species infesting fruit trees, *i.e.*, navel orange, mango, and guava, at Behaira Governorate. Samples from each crop were collected from both plant vegetation and soil surface surrounding these trees in the early morning, according to the method described by **Staikou *et al*. (1988)**. The specimens were then placed in muslin bags with labels containing ecological information and immediately sent to the laboratory for identification. To study the occurrence of *E. vermiculata*, hundred individuals from this species were randomly collected monthly. The shell diameter was measured using a vernier caliper as it is generally accepted as the most reliable morphometric parameter (**Lazaridou-Dimitriadou and Kattoulas, 1985; Staikou *et al*., 1988**).

Population density and development of *E. vermiculata* infestation

Enumeration of *E. vermiculata* infesting fruit trees, *i.e.*, navel orange, mango, and guava at Behaira Governorate. was conducted in three farms. Snails were counted once every month during the period extended from January 2020 to December 2021. Samples were taken from the three fruit orchards. Ten trees were randomly chosen from each orchard, and snails were counted in the early morning in a mean number of four areas 25x25 cm^2 under each tested tree and on the trunk. All snails counted on branches, and soil surface in the samples were left in their initial places. Population density (PD) for *E.vermiculata* was calculated according to (**Norton, 1978**) as follows:

$$PD = \frac{\text{Total number of individuals of a species}}{\text{Number of samples containing this species}}$$

Data concerning temperature and relative humidity during the study period were obtained from the metrological station at Behaira Governorate. The obtained data were subjected to statistical analysis using the (F) test, and the least significant differences test was used at $P < 0.05$. Aditionally, correlation coefficients were also studied between temperature or relative humidity and snail density.

Observations on oviposition of *E. vermiculata*

Field experiments were undertaken in three orchards of fruit trees, i.*e.*, navel orange, mango,

and guava, at Behaira Governorate. Ten replicates of 0.25 m each were examined biweekly from mid-October 2020 to the end of January 2021. The number of clutches in each quadrant and the number of eggs in each clutch were counted. The depth of clutches was also determined. Moreover, two clutches were randomly collected ,mixed together and 20 eggs were measured under a microscope using a stage micrometer. The general mean ± S.E. was calculated for the number of clutches, eggs, clutch size, and the depth of clutches during the oviposition period. Data were statistically analyzed using the F test, and the least significant differences were calculated at 5 %.

Morphological and Morphometric Characterization of the Brown Garden Snail *Eubania vermiculata* Collected from Behiara Governorate

The objective of the trial is to describe the brwn garden *snail E.vermiculata* infested fruit orchards at Behaira Governorate, based on specific morphological and morphometric parameters. Samples were hand collected from the infested fruit trees in early morning. The specimens were then placed in muslin bags and immediately sent to the laboratory. The shells were cleaned and separated from the animal's visceral mass according to **Frandsen (1983)**. The shells were washed and soaked in a 2% oxalic acid solution for 24 hours before being photographed dorsally and ventrally. The main morphological and morphometric characters were shell height, shell diameter, shell thickness, aperture, number of whorls, and color. The snail species was characterized using terminology given by **El-Okda (1979), Godan (1983), Mazon *et al*. (1990), Sharaf (1993) and Awad (2000)**.

Molecular characterization using RAPD-PCR to differentiate *Eobania vermiculata* populations

E. vermiculata populations in orchards of fruit trees at Behaira Governorate exhibit morphological variations, such as banded brown and unbanded pale-yellow coloration. To distinguish between these populations, random amplified polymorphism DNA isolation (RAPD-PCR) was employed. The populations tested were *Eobania* p1 (banded brown), *Eobania* p2 (unbanded pale-yellow), *Eobania* p3 (banded brown), and *Eobania* p4 (unbanded pale-yellow).

DNA isolation and RAPD technique

DNA was isolated from 50 mg of snail using a Qiagen kit for DNA extraction. The extracted DNA was dissolved in 100 µl of elution buffer. The concentration and purity of the obtained DNA were determined using the Gen quanta system from Pharmacia Biotech. The purity of the DNA for all samples was between 90-97 %, and the ratio was between 1.7 – 1.8. Concentration adjusted to 6 µg/for all samples using TE buffer pH 8.0.

Random amplified polymorphism DNA technique (RAPD)

Thirty ng of the extracted DNA were used for the amplification reaction. The polymerase chain reaction (PCR) mixture contained a PCR beads tablet (manufactured by Masham Pharmacia Biotech), containing all the necessary reagents except the primer and the DNA added to the tablet. The kits from Masham Pharmacia Biotech also included the flowing primers. Five microliters of the primer (10 Mer) were added. The sequences of the primers used are as follows; RAPD analysis primer 1:6-d (GGTG CGGGAA)-3 and RAPD analysis primer 6:6-d (CCCGTCAGCA)-3. The total volume was adjusted to 25 µl using sterile distilled water. The amplification protocol was carried out as follows using PCR unit II biometric.

A-Denaturation at 95 ˚C for 5 min.

B-45 cycles each consist of the following steps:

1-Denaturiation at 95 ˚C for 1min.

2-Annealing at 36 ˚C for 1min.

3-Extension at 72 ˚C for 2 min.

C-Final extension at 72 ˚C for 5 min.

D-Hold at 4 ˚C. 7 µl of 6 X tracking buffer (manufactured by Qiagen) was added to 25 µl of the amplification product.

Amplification product analysis

The amplified DNA from all samples was electrophoresed (15µl) using an electrophoresis unit (WIDE mini-sub-cell GT Bio-Rad) on 1%

agarose containing ethidium bromide (0.5 µg/ml) at constant voltage of 75 volts and visualized with a UV transilluminator.

Gel analysis

Agarose were scanned for band R_f using a gel documentation system (AAB Advanced American Biotechnology 1166 E. Valencia Dr. Unit 6 C, Fullerton CA 92631). The different molecular weight (M.W.) bands were determined against PCR marker Promega G317A using unweighted pair-group method based on the arithmetic mean (UPGMA).

RESULTS AND DISCUSSION

Ecobiological Consideration on the Brown Garden Snail, *E. vermiculata*

Ecobiological information is essential to design successful control programs and protect crops from damage caused by land snail species such as follows:

Occurrence and identification of *E. vermiculata*

The survey was carried out on three orchards of fruit trees, namely navel orange, mango, and guava, in Behaira Governorate from January 2020 to December 2021. It was found that one species of terrestrial snails belonging to the Order Pulmonata was present in the three farms. This species was identified as the brown garden snail, *E. vermiculata*, with two phenotypes: banded brown color and unbanded pale-yellow color, as shown in Fig. 1. The identified phenotypes varied in percentage occurrence from one orchard to another. The percentages of banded brown color and unbanded pale-yellow color of *E. vermiculata* females were calculated monthly during each year of the study period (Table 1). Moreover-, adults were also counted based on the reflexed lip formation at the mouth of the shell. It was observed that adults were more abundant during the summer months, i.e., June, July, August, and September. In contrast, juveniles were more prevalent during February, March, and April. The relative percentages of adults with banded brown color and unbanded pale-yellow color on navel orange, mango, and guava during 2020 were 51.22% & 48.18-%, 51.13% & 48.17%, and 56.21% & 43.19%, respectively. While the parallel values during 2021 were 54.11% & 46.10-%, 54.15% & 45.15%, and 52.13% & 47.17%, respectively.

Seasonal population dynamics of *E.vermiculata* on navel orange, mango and guava at Behaira Governorate

Data in Table 2 showed that three orchards of fruit trees, *i.e.*, navel orange, mango, and guava, at Behaira Governorate were highly infested with *E. vermiculata*. The inspection was carried out from January 2020 to December 2021. It was found that snails of *E. vermiculata* infested all tested trees with varying degrees.

Population density data clearly showed that individuals of *E. vermiculata* were present on navel orange, mango, and guava throughout the entire year with varying values according to season and host plant. The lowest population density values were observed on guava during February, mango during January, and navel orange during December with 4.69 (3.67), 7.52 (4.25), and 8.07(11.34) snails counted per sample in 2020 and 2021, respectively. In contrast, the highest population density values were found on navel oranges during September, mango during August, and guava during October, with 34.66 (33.35), 24.74 (25.72), and 23.00 (24.63) snails counted per sample in 2020 and 2021, respectively.Also,range of *E. vermiculata* population density varied between host plants ranging from 11.34 to 34.66 on navel orange, 4.25 to 25.72 on mango, and 3.67 to 24.63 on guava per sample.

Generally, oranges harbored the highest numbers followed by mangoes and guava. The total number of counted snails on these crops during 2020 and 2021 were 246.14 (257.69), 179.23 (186.94), and 181.61 (186.69), with general mean values of 20.51 (21.47), 14.93 (15.57), and 15.13 (15.56) snails per sample, respectively. On the other hand, the data revealed that population density values of *E.vermiculata* gradually increased after the winter months to reach the maximum values during the summer or autumn months. Peaks of *E. vermiculata* differed from one host plant to another and from month to another.

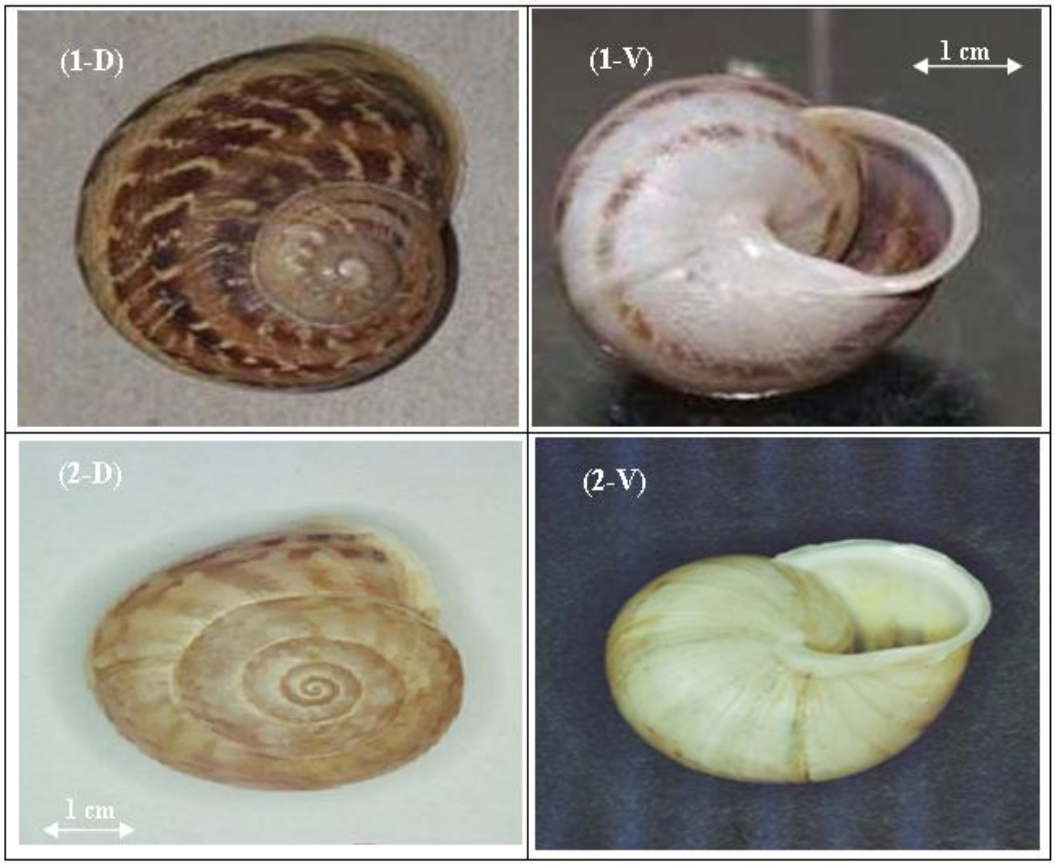

Fig. 1. Morphology of *E. vermiculata* shell types infesting certain fruit orchards at Behaira Governorate

1- *E. vermiculata* (banded, brown color). 2- *E. vermiculata* (unbanded, pale-yellow color)

D- Dorsal view. V- Ventral view.

Table 1. Percentage occurrence of the identified *E. vermiculata* phenotypes infesting certain fruit orchards at Behaira Governorate from January 2020 to December 2021

Snail phenotypes		Number of counted adult snails /year					
		Orange		Mango		Guava	
		2020	2021	2020	2021	2020	2021
***E. vermiculata* (banded)**		233.71	270.18	167.10	189.19	201.12	175.18
***E. vermiculata* unbanded)**		222.42	230.14	158.18	158.18	157.16	160.13
Total adults/ year		456.13	501.21	325.18	348.17	358.19	336.10
General mean/ sample		22.81	25.21	16.13	17.14	17.19	16.18
(%) Occurrence	**(Banded)**	51.22	54.11	51.13	54.15	56.21	52.13
	(Unbanded)	48.18	46.10	48.17	45.15	43.19	47.17

Table 2. Population density of *E. vermiculata* infesting certain fruit orchards at Behaira Governorate from January 2020 to December 2021 in relation to temperature and relative humidity

Date	The mean number of snails/samples						Temp. (˚C.)		R.H. (%)	
	Orange		Mango		Guava					
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Jan.	11.77	12.64	7.52	4.25	6.32	7.74	14.23	15.54	62.04	64.14
Feb.	14.5	15.37	5.89	7.74	4.69	3.67	16.35	17.35	61.04	60.84
Mar.	15.48	16.46	10.14	8.94	10.25	11.34	18.06	19.68	58.04	60.54
Apr.	18.42	18.97	12.43	12.86	12.64	13.73	24.62	25.73	53.25	55.54
May.	21.15	19.84	13.95	14.06	13.41	15.04	25.63	26.94	51.75	52.45
Jun.	22.13	24.53	17	18.75	17.99	16.57	29.06	28.66	55.64	57.04
Jul.	24.42	26.27	18.31	19.73	20.06	18.86	29.46	28.76	59.84	61.74
Aug.	28.67	27.69	24.74	25.72	21.91	20.93	28.35	30.37	60.24	62.34
Sep.	34.66	33.35	21.36	22.67	24.31	22.24	27.14	28.66	57.24	59.24
Oct.	27.14	28.45	21.91	22.24	23	24.63	24.72	25.12	60.14	59.84
Nov.	19.73	22.78	15.91	18.86	17.66	20.82	20.48	21.49	64.04	60.74
Dec.	8.07	11.34	10.07	11.12	9.37	11.12	16.75	15.84	64.34	61.14
Total	246.14	257.69	179.23	186.94	181.61	186.69				
Mean	20.51	21.47	14.93	15.57	15.13	15.56				
L.S.D. 0.05	1.97	1.50	2.72	3.01	1.89	2.12				

- F. test for the general mean of population density in the two years 2020 and 2021 on each crop was not significant at (0.05%).

Data in Table 3 showed a correlation between temperature or relative humidity and the population density of *E. vermiculata* during 2020 and 2021. This revealed that temperature and relative humidity had a variable effect on the population density of the land snail *E. vermiculata*. The correlation coefficients of temperature with *E. vermiculata* on navel orange, mango, and guava trees were highly significant or significant with values 0.992, 0.883 and 0.543 in 2020, respectively, while the corresponding values in 2021 were found to be highly significant on navel orange (0.832), significant on mango (0.722), and insignificant on guava (0.441). Regarding the correlation between relative humidity and the population density of *E. vermiculata* on the same fruit trees, relative humidity showed negative or positive insignificant effects on the three fruit species in 2020 and 2021, respectively. The correlation coefficients on navel orange, mango, and guava trees were -0.263 (0.330), -0.160 (0.250), and -0.293 (0.349) in 2020 and 2021, respectively.

Observations on oviposition of *E. vermiculata*

This experiment was designed to detect the breeding season of *E. vermiculata,* which was recognized by the presence of egg clutches in the field. Therefore, the number of clutches and eggs, clutch size, and depth of clutches were determined in 10 replicates of 0.25 m^2 in the infested orchards of fruit trees, *i.e.,* navel orange, mango, and guava at Behaira Governorate.

Data in Table 4 revealed that snails of *E. vermiculata* laid their eggs for about 3.5 months, starting from mid-October 2020 to the end-January 2021. Clutches were laid in humid, shady places in small holes dug by the parent snail in the soil. The eggs were spherical with a mean diameter of 3.3 ± 0.35 mm. The number of

Table 3. Effect of temperature and relative humidity on the population density of *E. vermiculata* infesting certain fruit orchards at Behaira Governorate from January 2020 to December 2021

Host plants	Temperature (˚C.)		Relative humidity (%)	
	2020	2021	2020	2021
Orange	0.992^{**}	0.832^{**}	$-0.263^{n.s}$	$0.330^{n.s}$
Mango	0.883^{**}	0.722^{*}	$-0.160^{n.s}$	$0.250^{n.s}$
Guava	0.543^{*}	$0.441^{n.s}$	$-0.293^{n.s}$	$0.349^{n.s}$

- Each value represents the correlation coefficient. *= Significant at 0.05 level.
n.s = not significant. **= Highly significant at 0.01 level.

Table 4. Number of clutches and eggs per (50 x 50) cm^2; clutch size and depth of egg laying (cm) of *E. vermiculata* infesting certain fruit orchards at Behaira Governorate from mid-October 2020 to end-January 2021

Variables	Sampling time								General mean ± S. E	L.S.D (0.05)
	Oct. 16	Oct. 30	Nov. 14	Nov. 29	Dec. 16	Dec. 30	Jan. 15	Jan. 30		
Clutches	2.54	2.76	3.29	4.45	5.09	3.71	2.97	1.80	3.33 ± 0.18	1.31
Eggs	69.13	88.69	112.54	190.68	219.25	136.50	63.85	37.59	114.78 ± 7.6	12.42
Clutch size	27.17	32.18	34.25	42.83	43.09	36.79	21.51	20.86	32.34 ± 2.1	1.25
Clutch depth	1.54	1.64	1.74	1.83	1.89	1.31	1.06	0.94	1.49 ± 0.07	0.45

clutches and eggs laid by *E. vermiculata* significantly (P≤0.05) changed during the breeding season. At the beginning of the egg-laying period, the number of clutches and eggs per 50 ×50 cm2 was relatively low. Then, the number of clutches and eggs gradually increased to reach its peak values of 5.09 clutches and 219.25 eggs per 0.25 m^2 in mid-December. After that, the number of clutches and eggs decreased recording values of 1.80 clutches and 37.59eggs per sample at the end of the breeding season. The same trend was noticed with clutch size, since at the beginning of the egg-laying period clutch size was 27.17 eggs/clutch in mid-October. This value was increased to reach the maximum in mid-December, recording 43.09 eggs/clutch. Then, clutch size was decreased to 20.86 eggs/clutch at the end of the breeding season. On the other hand, in the first half of the breeding season depth of clutches ranged between 1.54 to 1.89 cm, while in the second half, it ranged between 0.94 to1.31 cm.

Morphological Remarks and Shell Measurements of *E. vermiculata*

Data in Table 5 illustrate specific measurements of *E. vermiculata* collected from certain infested orchards of fruit trees at Behaira Governorate. The measurements taken were as follows . The mean of shell height is 20.20 mm ranging from 16.60 to 23.80 mm, while the mean shell diameter is 26.65 mm, with a range of 22.70 to 30.60 mm. The shell thickness ranged between 0.73 to 0.89 mm with a mean of 0.81 mm. The mean aperture diameter is 9.04 mm ranging between 8.18 to 9.89 mm. The number of whorls ranged between 4.5 to 6.0, and the animal color is banded brown or unbanded pale yellowish.

The question of specifying its taxonomic status was raised due to variations in measurements and descriptions of *E. vermiculata* species. Therefore, morphological and morphometric characteristics accomplished in this study indicated that two phenotypes of *E. vermiculata*

Table 5. Shell measurements of *E. vermiculata* adult snails infesting certain fruit orchards at Behaira Governorate

Measurements	*E. vermiculata*		
	Range		Mean ± S. E
	from	to	
Shell height (mm) SH	16.60	23.80	20.20 ± 0.32
Shell diameter (mm) SD	22.70	30.60	26.65 ± 1.41
SH / SD	0.73	0.78	0.76 ± 0.03
Shell thickness (mm)	0.73	0.89	0.81 ± 0.12
Aperture diameter (mm)	8.18	9.89	9.04 ± 0.22
Number of whorls	4.50	6.00	5.50 ± 0.50

were distributed. These types were banded brown or unbanded pale yellowish. This result was supported through the application of RAPD-PCR in identifying this species.

Molecular Characterization by RAPD-PCR to Differentiate *Eobania* Species Phenotypes

The rapid progress in biotechnology has provided taxonomists with a wide range of new tools. In this study, the RAPD-PCR technique was used to differentiate phenotypes of *E. vermiculata* collected from infested orchards of fruit trees at Behaira Governorate. Four populations were selected for this study including. The banded brown phenotype and unbanded pale-yellow phenotype. These populations were designed as follows: *Eobania* p1 (banded brown phenotype), *Eobania* p2 (unbanded pale-yellow phenotype), *Eobania* p3 (banded brown phenotype), *Eobania* p4 (unbanded pale-yellow phenotype).Many authors have applied different tools of molecular biology to differentiate species of land snails and slugs (**Backeljau *et al.*, 1994; Voss *et al.*, 1999; Parmakelis *et al.*, 2003; Desouky and Awwad, 2004**).

Genetic diversity as indicated by RAPD markers presented in Fig.(2)was analyzed using the primer 1:6-d (GGTGCGGGAA)-3. The banding patterns of RAPD fragments produced with primer 1:6-d showed fragment size of PCR products ranging from 280 to 1000 bp in p1 and p3, 180 to 570 bp in p2, and 280 to 1000 in p4. The 1:6-d primer produced three common bands in all populations with sizes of 280, 480, and 570 bp. A band of size 320 bp was observed in p2 but was absent from all populations. On the other hand, primer 6:6-d (CCCGTCAGCA)-3 showed banding patterns of RAPD fragments with fragment sizes of PCR products ranging from 500 to 1000 bp in p1 and p3, 200 to 1000 bp in p2, and 100 to 1000 in p4. The 6-d primer produced two common bands in all species with sizes of 500 and 1000 bp. A band of size 750 bp was observed in p2 but was absent from all populations. Bands of size 200, 380, and 430 bp were observed only in p2 and p4.

The dendrogram of genetic distances among populations based on band polymorphisms generated by RAPD-PCR using the two primers is shown in Fig. 3. It is not surprising that p1 and p3 are segregated in one cluster, while p2 and p4 are grouped in the second cluster. The RAPD marker data indicate a close relationship between p1 and p3 and between p2 and p4. Generally, based on molecular characterization by RAPD-PCR, the two populations (p1& p3) can be identified as the banded brown phenotype while the two populations (p2 & p4) can be classified as an unbanded pale-yellow phenotype of the land snail, *E. vermiculata*.

Before discussing the previous results with land snail species, it is important to mention that many authors reported problems caused by snail pests on crops in most countries around the world, *i.e.*, Australia **(Baker, 1989)**, Switzerland

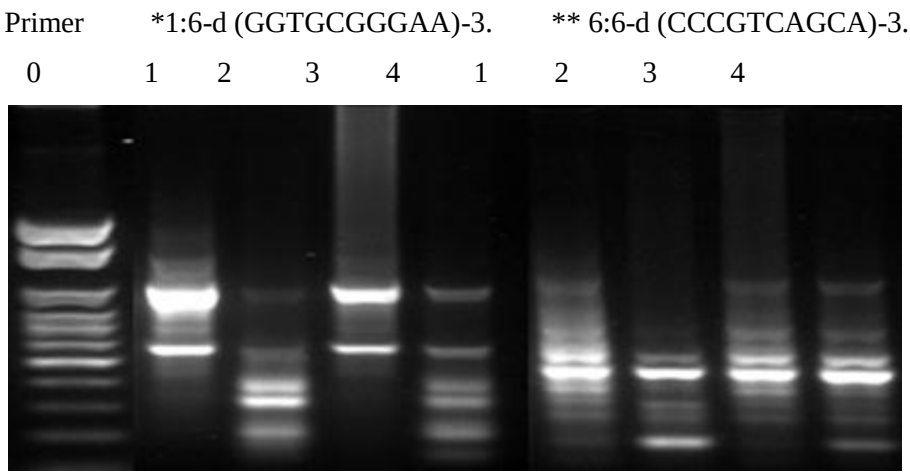

Fig. 2. The sequences of used primers; RAPD analysis primers; 1:6-d (GGTGCGGGAA)-3 and 6:6-d (CCCGTCAGCA)-3 of *E. vermiculata* types at Behaira Governorate

Where; 0 - Primer.
*The sequences of primer: 1:6-d (GGTGCGGGAA)-3.
**The sequences of primer: 6:6-d (CCCGTCAGCA)-3.
1- *Eobania* p1 (banded, brown color). 2- *Eobania* p2 (unbanded, pale-yellow color).
3- *Eobania* p3 (banded, brown color). 4- *Eobania* p4 (unbanded, pale-yellow color).

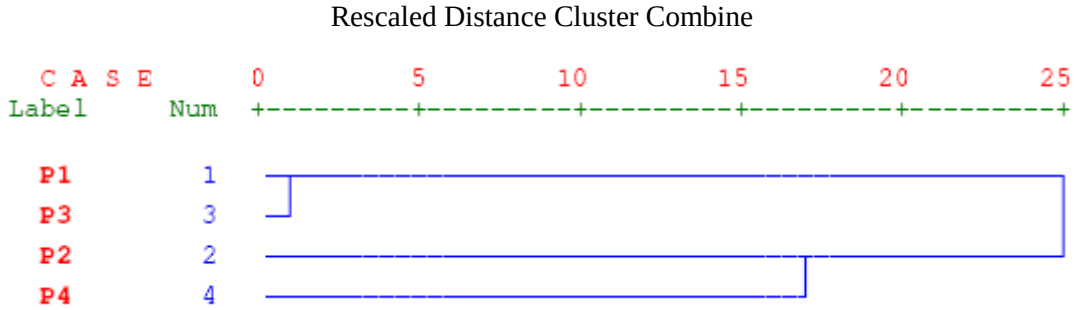

Fig. 3. Linkage dendrogram using two primers for *E. vermiculata* phenotypes; (banded, brown color) and (unbanded, pale-yellow color) based on combined analysis of amplified RAPD fragments

Where; 1- *Eobania* p1 (banded, brown color). 2- *Eobania* p2 (unbanded, pale-yellow color).
3- *Eobania* p3 (banded, brown color). 4- *Eobania* p4 (unbanded, pale-yellow color).

(**Baur and Baur, 1993**), China (**Chen, 1994**), England (**Newman *et al.*, 1994**), Spain (**Castielleio *et at.*, 1996**), and in Egypt, where land snails have observed at different Governorates attacking many economic crops. For instance, *T. pisana* was found to be most damaging in northern Egypt (**Kassab and Daoud, 1964**). Moreover, the incidence severity of land snails has been mentioned by many authors (**Baker, 1988; Hegab *et al.*, 1999; Asran *et al.*, 2011; Abd El-Aal and Arafa, 2019**).

Our results showed that the brown garden snail, *E. vermiculata* infested fruit trees, i.e., navel orange, mango, and guava, at Behaira Governorate. These results are consistent with previous findings that indicated the presence of snails invarious Governorates; including Alexandria, Behera, Ismailia, and Sharkia Governorates (**Mahrous *et al.*, 2002; Ismail *et al.*, 2011**). Additionally, **Rady *et al.* (2014) and Kadry *et al.* (2018)** identified four species of gastropods in Sharkia Governorate. **Abd El-Haleim *et al.***

(2022) reported that the incidence and level of infestation of *E. vermiculata* varied according to the locality, and it was the prevalent land snail species in Beheira Governorate.

The population density of *E. vermiculata* increased during March compared to the other months, which is in line with **(Hegab *et al*., 1999)**, while it was low to moderate during the winter and autumn months **(Mahrous *et al*., 2002; Ismail *et al*., 2011)**. The correlation between climatic factors and the population density of land snails is in harmony with those reported by **Abd El-Haleim *et al*. (2022)**. Comparing numbers of *E. vermiculata* on four host plants showed insignificantly different results in the two successive growing seasons, as many authors reported in different land snail species **(Nakhla *et al*., 1993; Nakhla and Tadros, 1995; Philippe *et al*., 2011; Baker, 2012)**.

The literature reports concerning the biology of *E. vermiculata* under field conditions are very limited. However, some attempts have been made under laboratory conditions. **Mohamed, (2006)** mentioned that the immature period, life cycle, and life span of *E. vermiculata* differed according to temperature and food type, with the life cycle and life span lasted 379.4 ± 5.2 and 921.4 ± 8.9 days at the same temperature and relative humidity. The oviposition period of *E. vermiculata* averaged 15.2 and 24.9 days during seasons 2003/2004 and 2004/2005, respectively **(Wanninger and Wollesen, 2019)**.

Many authors measured and described several land snail species in different countries around the world **(Cowie, 1990; Flint, 2011; Heryanto, 2011; Scheil *et al*., 2013; Aydin Örstan, 2014; Psonis *et al*., 2014; Sean *et al*., 2015)**. In Egypt, **Awad (2000)** redescribed some land snail and slug species based on morphological features in the Nile Delta. Furthermore, **Mohamed (2006)** showed that all measurements of *M. cartusiana* differed according to host plant and locality. For example, the average shell diameter increased to 13.5 mm/snail on Sudan grass in Oseam, while it decreased to 11.0 mm on cabbage and Cogon grass,. Finally, many authors applied different tools of molecular biology to differentiate species of land snails and slugs **(Backeljau *et al*., 1994; Voss *et al*., 1999; Parmakelis *et al*., 2003; Desouky and Awwad, 2004; Uit De Weerd and Gittenberger, 2005; Schultes, 2011; Johnson, 2012; Scheil, *et al*.,2012)**.

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بيولوجي، ايكولوجي وتعريف جزيئي للأنماط الظاهرية لقوقع *Eubania vermiculata* (Müller) الذي يصيب بعض بساتين الفاكهة في محافظة البحيرة، مصر

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تعتبر القواقع الأرضية من أكثر الآفات الزراعية تدميراً للمحاصيل المختلفة، فقد تم إجراء حصر على ثلاثة بساتين لأشجار الفاكهة، وهي البرتقال بسرة والمانجو والجوافة، في محافظة البحيرة من يناير 2020 إلى ديسمبر 2021. حيث وجد أن أفراد القوقع البني (الشيكولاتي) (Muller) *Eubania vemiculata* كانت موجودة في البساتين الثلاثة طوال العام بقيم متفاوتة وفقًا للموسم الزراعي والنبات العائل بنمطين ظاهريين: الأول اللون البني المخطط والثاني اللون الأصفر الباهت غير المخطط. حيث تباينت الأنماط الظاهرية في نسب ظهورها من بستان إلى آخر. وبلغت النسب المئوية للأطوار البالغة ذات اللون البني المخطط واللون الأصفر الباهت غير المخطط على البرتقال والمانجو والجوافة خلال عام 2020 ما يلي 51.22% و 48.18% و 51.13% و 48.17% و 56.21% و43.19% على التوالي. بينما كانت القيم المقابلة خلال عام 2021 ما يلي 54.11% و 46.10% و 54.15% و 45.15% و 52.13% و 47.17% على التوالي. وسجل البرتقال بسرة أعلى الأعداد تواجد يليه المانجو والجوافة بمتوسط قيم مقدارها 20.51 (21.47) و 14.93 (15.57) و15.13 (15.56) قوقع لكل عينة على التوالي. ثم ارتفعت قيم كثافة أعداد قوقع *E.vermiculata* تدريجيًا بعد أشهر الشتاء لتصل إلى أقصى قيمها خلال أشهر الصيف أو الخريف. ولوحظ أن أعداد الأفراد البالغة كانت أكثر وفرة خلال أشهر الصيف، أي يونيو ويوليو وأغسطس وسبتمبر. وعلى النقيض من ذلك، كانت أعداد الصغار أكثر انتشارًا خلال أشهر فبراير ومارس وأبريل، حيث وضعت افراد قوقع *E.vermiculata* بيضها لمدة 3.5 شهر تقريبًا، بدءًا من منتصف أكتوبر 2020 حتى نهاية يناير 2021. وكان البيض كروي الشكل بمتوسط قطر مقدارة 3.3 ± 0.35 مم. حيث تغير عدد كتل البيض وعدد البيض لكل كتلة الذي وضعته افراد *E.vermiculata* بشكل ملحوظ ($P \leq 0.05$) خلال موسم التكاثر. وأظهرت قياسات صدفة *E. vermiculata* ارتفاعًا متوسطًا للصدفة مقدارة 20.20 ملم يتراوح من 16.60 إلى 23.80 ملم ، بينما كان متوسط القطر 26.65 ملم ، بمدى يتراوح من 22.70 إلى 30.60 ملم. وتراوحت سماكة الصدفة بين 0.73 إلى 0.89 ملم بمتوسط 0.81 ملم. وكان متوسط قطر فتحة الصدفة 9.04 ملم، والذي تراوح بين 8.18 إلى 9.89 ملم. ومن ناحية أخرى تراوح عدد لفات الصدفة بين 4.5 إلى 6.0 ، لفة ولون الحيوان من بني مخطط الي مصفر باهت غير مخطط. وقد أظهر مخطط شجري لدرجات القرابة الوراثية بين التعداد على أسس تعدد أشكال النطاقات الناتج عن تفاعل البوليميرز باستخدام البادئين أنه يمكن تحديد التعداد p1 و p3 على أنهما النمط الظاهري البني المخطط، في حين يمكن تصنيف التعداد p2 و p4 على أنهما النمط الظاهري الأصفر الباهت غير المخطط للقوقع الأرضي *E. vermiculata* .

الكلمات الإسترشادية: القواقع الأرضية، *Eubania vermiculata*، بيولوجي، ايكولوجي، التعريف الجزيئي، بساتين الفاكهة، محافظة البحيرة .

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