EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
TOXICOLOGY & PEST CONTROL

F

ISSN
2090-0791

WWW.EAJBS.EG.NET

Vol. 16 No. 2 (2024)

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci.* (F. Toxicology& Pest control) *Vol.16(2) pp23-43 (2024)*
DOI: 10.21608/EAJBSF.2024.371517

Egypt. Acad. J. Biology. Sci., 16(2):23-43 (2024)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/

Biology and Control of the Bean Slug *Sarasinula plebeia* (*Gastropoda, Veronicellidae*): A Newly Recorded Species in Egypt

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REVIEWINFO
Review History
Received:29/6/2024
Accepted:3/8/2024
Available:7/8/2024

***Keywords*:** *Sarasinula plebeia*, life cycle, microbial control, predation, *Eobania vermiculata*.

ABSTRACT

In this study the veronicellid slug *Sarasinula plebeia* was discovered for the first time in Egypt, infesting the nursery plant *Platyruscus hypophyllum* at Ismailia Governorate. The external morphology and anatomic features of the collected specimens coincide with the descriptions of this species. The life cycle, growth rate and biological control of *S. plebeia* by pathogenic microbes and the predatory snail *Eobania vermiculata* were investigated under laboratory conditions. The results demonstrated that slugs began egg laying in December, and deposited eggs in clutches each containing from 12 to 67 oval eggs in the soil. Eggs take from 18 to 21 days to hatch and the hatching period varies between 8 to 11 days. The average life span was 369.95 days and the generation time varied between 287 and 293 days and this indicates that *S. plebeia* has one generation per year. With respect to the biological control of *S. plebeia*, *Trichoderma asperellum* and *Bacillus subtilis* exhibited the highest pathogenicity against both juvenile and adult stages of the slugs at the highest concentration of 6×10^5 CFU/ml. While *Paecilomyces variotii* displayed the lowest influence against the two developmental stages of the slug. In the predation experiment, *E. vermiculata* preferred the young age of *S. plebeia* slug (eggs and juveniles) and concurrently showed no preference towards adults. Generally, these results highlight the possible role of microbial isolates and the predator snail *E. vermiculata* as effective biological control agents against terrestrial slugs, especially the new invasive slug *S. plebeia*.

INTRODUCTION

Veronicellids represent tropical and subtropical terrestrial slugs belonging to Order: Systellommatophora. They often attack ornamental plants and are also considered a serious garden pest causing significant damage and losses of numerous crops (Robinson and Hollingsworth, 2005; Gomes *et al*., 2013). This family is widely distributed in America, southern Asia and the Indian Ocean Islands (Herbert and Kilburn, 2004). The bean slug, *S. plebeia* considered one of the most distinctive species of Veronicellidae, causes great damage to multiple crops, plant nurseries and gardens and it prefers the soft stems and young leaf tissues (Garcia *et al*., 2007; Constantino *et al*., 2010). Moreover, it is a possible vector for pathogens to humans indirectly through their consumption of contaminated fruits and vegetables with mucus and faeces. Studying the biology and life cycle of veronicellid slugs is considered a major aspect of determining the appropriate control program and detecting its suitable application time. The biology of this family's species is still insufficient (USDA-

APHIS, 2010). Few studies have been conducted on the distribution and life history of *S. plebeia*. Breeding of this slug occurs in the rainy seasons which leads to high population densities in the disturbed regions (Brodie and Barker, 2012). It can copulate several times in the same night and release a sexual pheromone after sexual maturation (Rueda *et al*., 2002)**.** The management of slugs has become indispensable to avoid crop damage and has been indicated by many authors **(**Bailey 2002; Henderson and Triebskorn, 2002). Due to the dangerous environmental effects of chemical molluscicides, the need for sustainable management measures focusing on biological eco-friendly substitutes has become urgent (Howlett, 2012). Natural enemies of molluscs have been known as an important slug biological control measure (Schneider, 2016). The predatory snails such as *Euglandina rosea* and *Gonaxis quadrilateralis* attacked and consumed small slugs (Barker, 2004). In addition, pathogenic bacteria and fungi are also of interest for slug biological management (Le Gall and Tooker, 2017; Galvis and Moreno, 2018).

This study aimed to indicate the first presence of the bean slug, *Sarasinula plebeia* in Egypt and explore the life history, growth rate and survival of this slug species. The biological control of *S. plebeia* by pathogenic microbes and predatory snail were also investigated.

MATERIALS AND METHODS

Slug Collection And Rearing:

In October 2022, adult individuals of *Sarasinula plebeia* slug were hand gathered from an infested *Platyruscus hypophyllum* (L.) plant situated in El-Quassasin Horticultural Research Station, Ismailia Governorate, Egypt, and this represents the first occurrence of this slug species in the Egyptian Agro-system. The collected slugs kept in plastic boxes (22 cm × 13 cm) contain a mixture of moistened clay and sandy soil. The boxes were provided daily with fresh cabbage leaves and covered with gauze cloths secured with an elastic band for protection and prevention of slugs from escaping. Slugs were acclimated to laboratory conditions for two weeks prior to experiments (Clemente *et al*., 2008).

Reproduction Experiment:

This investigation was conducted on November 2022, 15 plastic boxes (17 cm diameter) containing moist clay soil were set up. Two individuals of *S. plebeia* slugs were placed into each box, supplied daily with fresh cabbage leaves and remoistened as needed (Ali, 2011). The time of oviposition, the number of egg clutches and the number of eggs per clutch were determined monthly throughout the generation period (Godan, 1983). The eggs of each clutch were transferred to new boxes which prepared as the parent boxes and placed at the same depth of laying. The egg clutches were observed daily until hatching to calculate the incubation period (the period from egg laying to the first egg hatching), hatching period (the period from first egg hatching to the last hatching egg in each clutch) and hatchability (% of hatched eggs). Juveniles were provided daily with fresh leaves of cabbage and soil remoistened as required until the juveniles reached maturity and completed their life span. Ten individuals of the newly hatched juveniles were selected randomly to measure their length monthly until maturity. The oviposition period (the period from beginning egg laying until the last egg deposited by adult slug), fecundity (number of eggs per slug), life span (from the date of hatching slug till its mortality) and the generation period (from egg to egg) were also calculated.

Biological Control of *Sarasinula plebeia* Slugs:

From rearing boxes, the deposited eggs were collected and subdivided into two groups, the first group was used in the subsequent egg experiments and the second group was examined continuously until hatching for the juvenile experiments.

I- Microbial Management:

-Culture and Preparation of Microbial Inocula:

Three identified microbial isolates; *Bacillus subtilis* (bacterium), *Paecilomyces variotii* and *Trichoderma asperellum* (fungi) were obtained from the Insect Pathogen Unit (IPU), Plant Protection Research Institute, Agricultural Research Center, Egypt. These isolates were recommended for pest control and safe for non-target organisms, plants and humans (Butt and Copping, 2000; Samojlov *et al*., 2010). A *B. subtilis* strain was grown on Nutrient broth medium (5 g/l beef extract, 5 g/l bactopeptone, 5 g/l NaCl) and incubated for 48 h. at 37ºC. The cell number was measured by hemocytometer and the final concentrations were adjusted to 2.5×10^6, 5×10^6 and 7×10^6 CFU/ml by adding the required amount of sterile distilled water to the bacterial cells (Mona *et al*., 2017). The fungal strains, *P. variotii* and *T. asperellum* were cultured on potato dextrose agar (PDA) medium for 14 days at 25ºC (Jinhua *et al*., 2013). The fungal spores were supplemented with 10 ml of sterile distilled water mixed with 0.1% Tween-80 and then harvested by scraping the inoculum (El-Husseini, 2019). The number of spores was adjusted to 3×10^5, 4.5×10^5 and 6×10^5 CFU/ml by adding the appropriate amount of sterilized distilled water.

-Bioassay Test:

The juveniles (2 months old) and adult individuals of *S. plebeia* slug were treated with the spores suspension of *B. subtilis* at the concentrations 2.5×10^6, 5×10^6 and 7×10^6 CFU/ml, *P. variotii* and *T. asperellum* at the concentrations 3×10^5, 4.5×10^5 and 6×10^5 CFU/ml under laboratory conditions. The isolates were applied by the spray method for bioassay on juvenile and adult slugs. Plastic boxes (22 cm × 13 cm) were used in this experiment; each box includes 500 gm of moistened sterilized clay soil. Ten juveniles or adults and ten cabbage discs were introduced on the soil surface in each box. The tested concentrations of each bio-agent were sprayed separately at a rate of 20 ml on the cabbage discs and soil (Foster *et al*., 1991). Three replicates were allocated for each bio-agent concentration for both slug stages. The other three control replicates were prepared as the other boxes but without any treatment (20 ml of distilled water was sprayed). To prevent slugs from escaping, all boxes were tightly covered with gauze cloths fixed with elastic bands. The mortality rates of both developmental stages of the slug were recorded daily for 21 days and corrected by Abbott's formula (Abbott, 1925).

II- **Predation Experiment:**

-Predatory Snail:

Adults of the terrestrial snail, *Eobania vermiculata* were collected directly from navel orange trees at Banadf village, Meniet El-Kamh district, Sharkia Governorate, Egypt. Snails were transferred to the laboratory and retained in a glass terrarium (60 × 30 × 30 cm) containing humid mixture of clay and sandy soil covered with gauze cloth fixed with elastic band. The snails were provided daily with fresh leaves of cabbage for 14 days to acclimate prior to any experiment (Genena, 2010).

-The Predatory Potential of *Eobania vermiculata* on *Sarasinula plebeia* slugs in the Laboratory:

The predatory activity of *E. vermiculata* snail on the eggs, juveniles (2 months old) and adults of *S. plebeia* slug was evaluated separately under laboratory conditions. Adult individuals of *E. vermiculata* were starved for three days before the start of the experiment. Plastic boxes (22 cm × 13 cm) were used in this investigation, each box containing ½ kg of moistened mixture of clay and sandy soil. An individual predation experiment was carried out in February 2023 by placing one adult *E. vermiculata* snail with ten newly deposited slug eggs in the same box. Individual predation of *E. vermiculata* adult snails against juveniles (2 months old) and adult slugs was investigated separately. Three replicates were designed

for each developmental stage of the slug. The other three replicates including ten juveniles or ten adults of slug only without any predator were also prepared as a control.

Differences in preference between prey developmental stages were assessed for 15 days by daily counting the number of consumed eggs, juveniles and adults of the slug. The differences in mortality between control and experimental treatments for each developmental stage and survival of the predatory snail were observed (Meyer and Cowie, 2010).

Data Analysis:

All data were statistically analyzed and the difference between means was tested using one-way ANOVA at $P \leq 0.05$ levels (Costat, 2005) and means of data and standard error were computed as appropriate by using the Microsoft Excel Program, expressed as means ± standard error.

RESULTS

In the present study, the bean slug *Sarasinula plebeia* (Fischer, 1868) was recorded for the first time in Egypt on the *Platyruscus hypophyllum* plant in two nurseries of ornamental plants located at El-Quassasin Horticultural Research Station, Ismailia governorate, in late October 2022.

*** Classification**

Kingdom: Animalia
Subkingdom: Metazoa
Phylum: Mollusca
Class: Gastropoda
Order: Systellommatophora
Superfamily: Veronicelloidea
Family: Veronicellidae
Genus: *Sarasinula*
Species: *Sarasinula plebeia* (Fischer, 1868) Thomé, 1975; Thomé, 1989; Thomé, 1993; Gomes and Thomé, 2001; Agudo-Padron, 2008.

*** Description of *Sarasinula plebeian:***

Sarasinula plebeia is a relatively large shell-less slug, its body form is flattened. The upper surface (notum) is thick and its colour is light to dark brown with scattered small punctuations, with the absence of a pale median stripe down the back of the animal (Fig. 1A) as indicated by Alvarez-Cerrillo (2022). The body colour changes to grey after preservations, it becomes longer when the slug is crawling and the body length of a fully grown individual can attain 63.44 ± 1.4 mm (min = 60.3 mm, max = 70 mm, n = 10) (Fig. 1B) as described by Jackson and Mua (2019). The head has two pairs of tentacles; at the ends of the upper pair, the eyes are located. The tentacles are contractile and hidden underneath the notum when the slug is dormant (Fig. 1C), and this is in accordance with Brodie and Barker (2012). The slug's underside has a narrow foot that appears as an inner band down the body centre while the fleshy areas along the side represent the mantle (Fig. 1D). Additionally, the penis in the reproductive system is short and smooth without annular protrusion, with enlarged glands. The digitiform gland with an elongated form with seven tubules varying in length (Fig. 1E), and this finding was confirmed by Cowie *et al*. (2008) who found that the number of digitiform tubules in *S. plebeia* reproductive system is exactly seven. This represents a main distinct between *S. plebeia* and the other veronicellid species like *Laevicaulis stuhlmanni* which was recorded recently in Egypt by Ali (2017); Metwaly and Ali (2024) indicated that the number of digitiform tubules in this slug is around fourteen tubules.

*** Host Plants And Infestation:**

Sarasinula plebeia was recorded for the first time in Egypt at the end of October 2022 in two nurseries of ornamental plants infested the nursery plant, *Platyruscus hypophyllum*. It was found in *P. hypophyllum* pots (Fig. 2A), consuming a large amount of the plant leaves (Fig. 2B) and causing noticeable injuries (Fig. 2C).

In other previous studies, *S. plebeia* was found under stones, decaying wood, grass and ground crevices. In Fiji, it can exist in lowlands, plantations and gardens. Moreover, in Central America, *S. plebeia* damages beans by cutting seedlings and consuming leaves and young pods (Brodie and Barker, 2011). Although this species has been widely spread in tropical and subtropical regions, it is able to endure subfreezing temperatures which indicates that this species is cold-tolerant (Naranjo-Garcia *et al*., 2007). Smith and Dartnall (1976) reported that *S. plebeia* is found in different suburban areas including gardens in the Northern Territory in Australia. On the other hand, in Hawaii, this species causes serious damage to flowers and foliage (Hata *et al*., 1995).

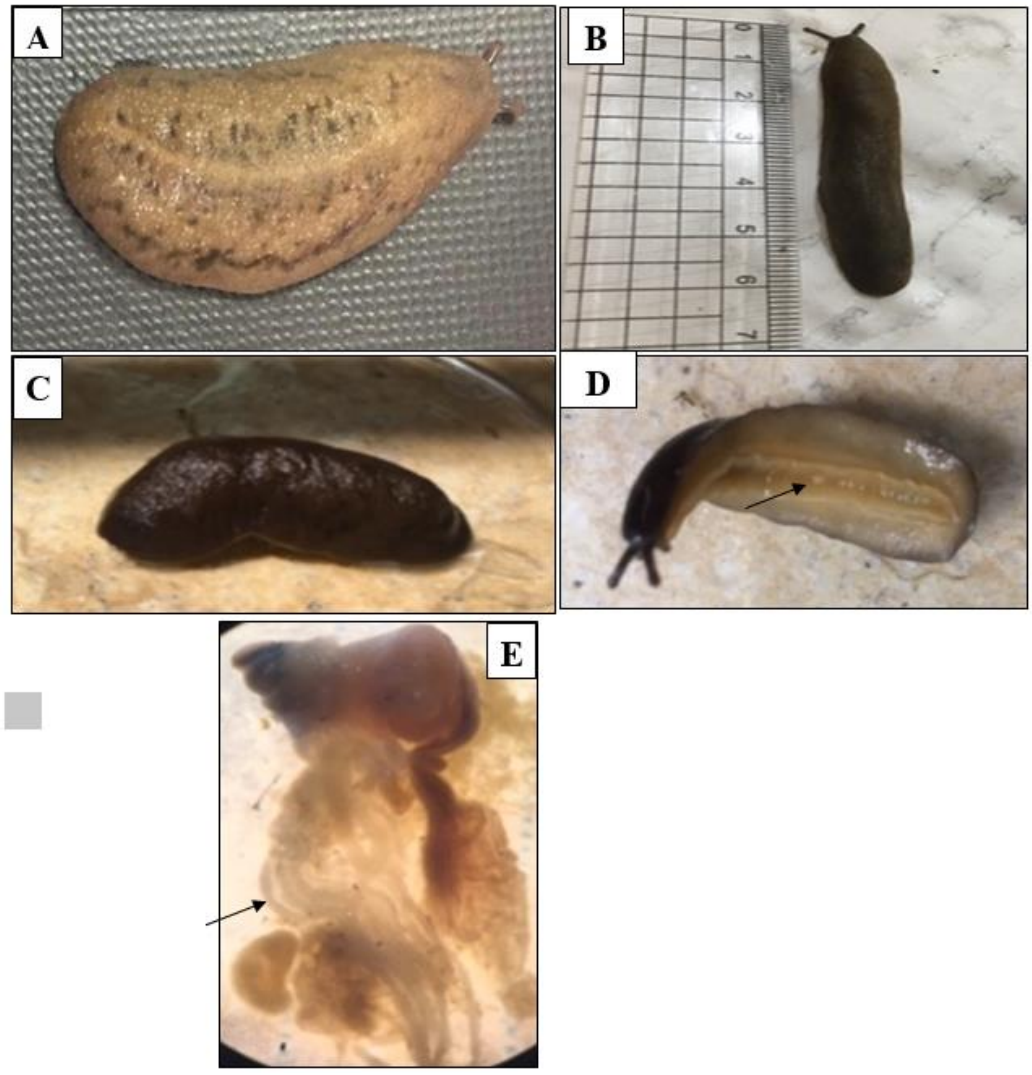

Fig. 1: Description of *Sarasinula plebeia*. (A) Dorsal view of a live specimen showing the flattened body shape and punctuations; (B) Body length of a fully grown individual; (C) Contraction and hidden of the tentacles when the slug is inactive; (D) Ventral view showing the inner band that represents the slug's foot; (E) Part of the reproductive system showing the short penis and elongated digitiform gland with seven tubules (arrow).

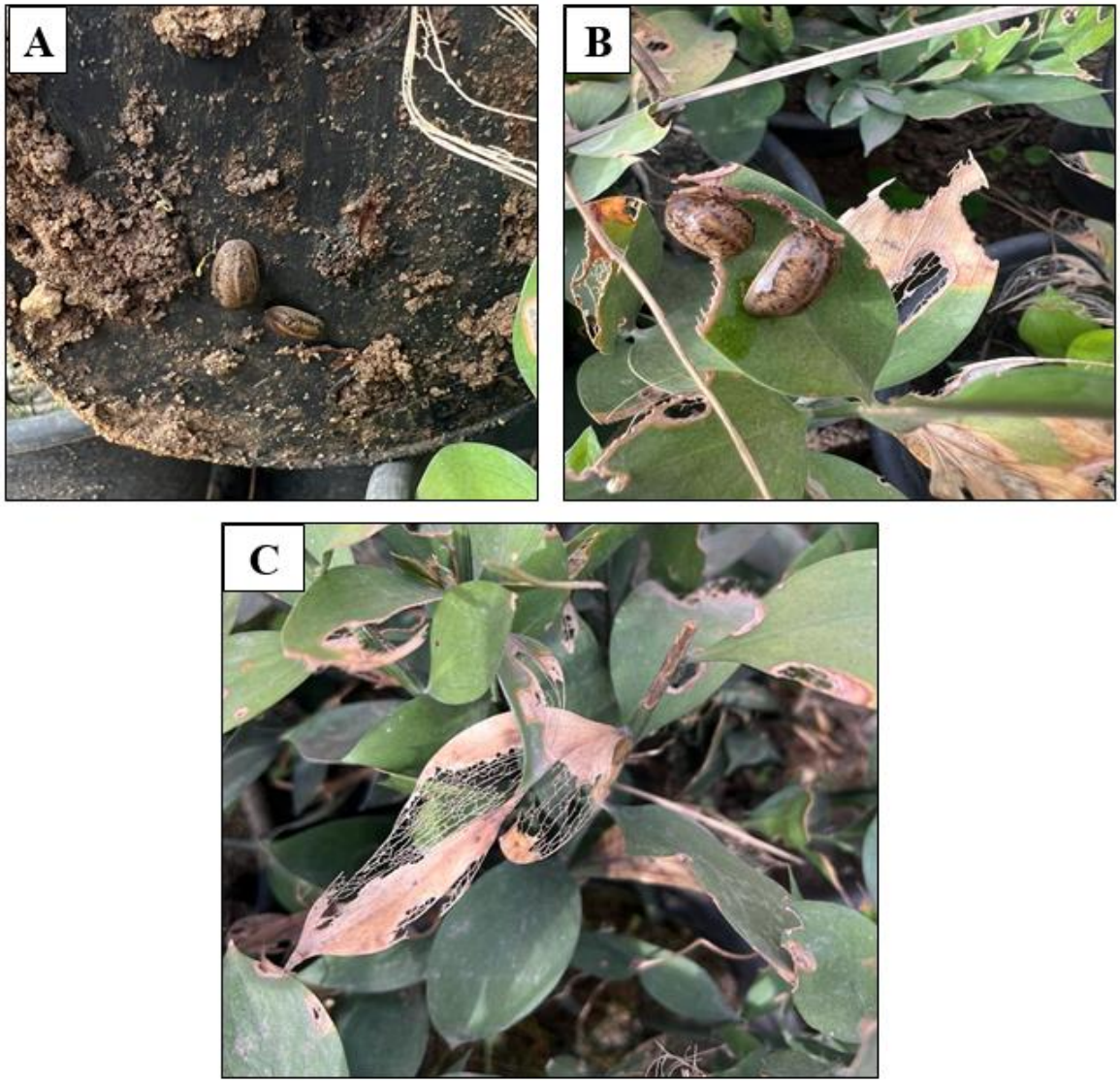

Fig. 2: Infestation of *Platyruscus hypophyllum* with *Sarasinula plebeia*. (A) Presence of the slug in the plant pot; (B & C) Foliar damages and injuries of the plant due to *S. plebeia* infestation.

The life cycle of *Sarasinula plebeia*

I- Eggs:

The adult individual of *S. plebeia* makes a hole in the soil (Fig. 3A) to deposit eggs (Fig. 3B). This slug species laid translucent oval eggs in a spiral, and the eggs usually laid in clusters (Fig. 3C). Moreover, this slug is characterized by depositing faecal-like material with their eggs (Fig. 3D). The egg diameter was 1 mm and its length was about 7 mm (Fig. 3E). After six days of laying, the colour of eggs changed to a light brown (Fig. 3F).

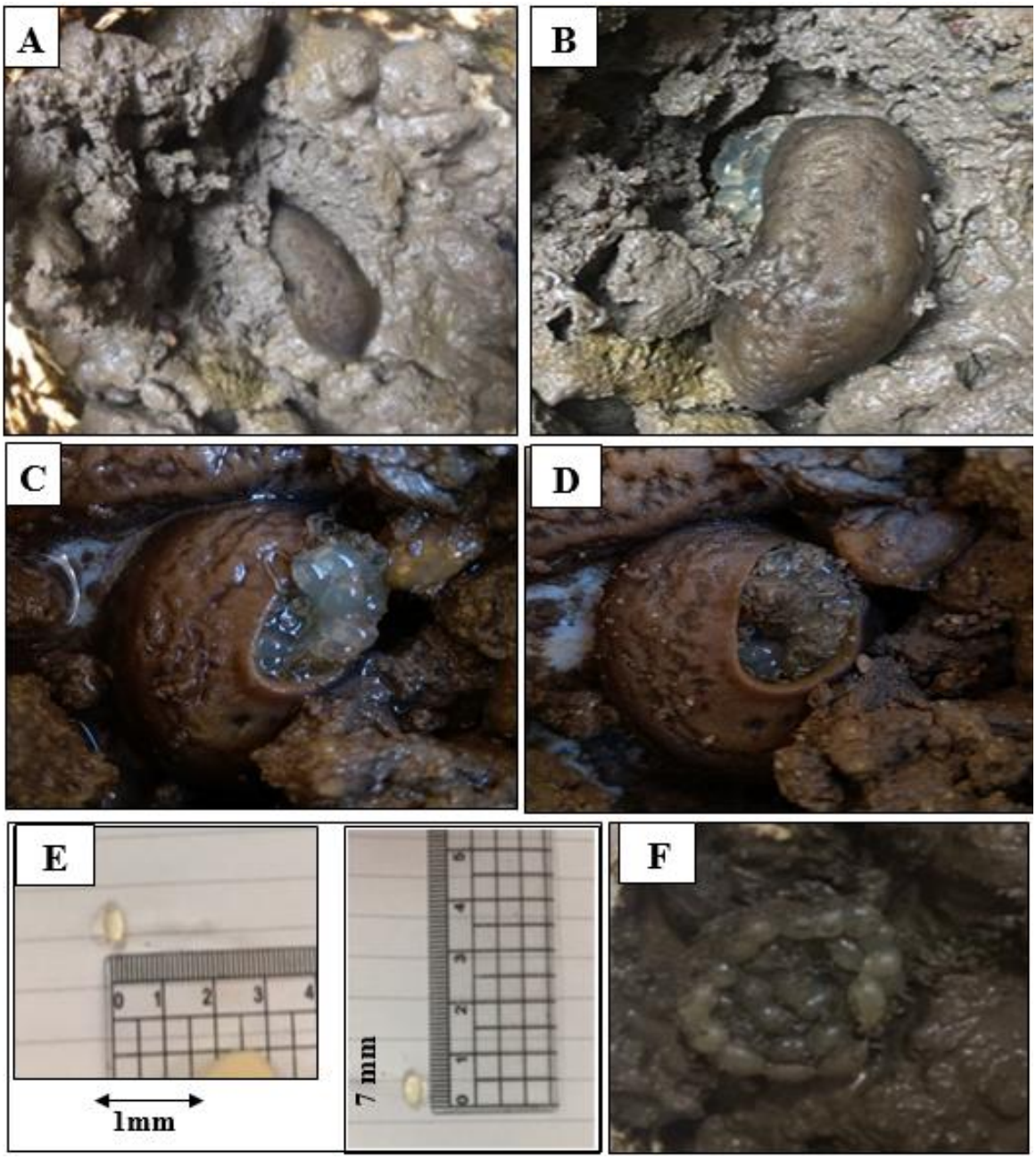

Fig. 3: Characteristics of slug eggs. (A) *S. plebeia* makes a hole in the soil to deposit eggs; (B) The beginning of egg laying; (C) New deposited translucent eggs; (D) Deposition of the eggs with faecal material; (E) Egg diameter and length; (F) Change of the colour of the egg to light brown after six days of laying.

These findings were in accordance with Capinera (2021) who showed that slug eggs are translucent or white and usually have a spherical or oval shape and are laid in clusters. In addition, some slug species deposit fecal material with their eggs and the reason for this action is unknown even now. On the same pattern, Hend (2015) reported that *Deroceras reticulatum* slug laid white oval eggs, the egg's average diameter was 3 mm and measured 2 mm in length. Similarly, Kim *et al*. (2009) indicated that the egg size of *D. reticulatum* was 1.9 × 2.2 mm. On the other hand, the egg size of *Lehmannia marginata*, *Limax maximus* and *Arion intermedius* slugs was 5 × 4 mm, 6 × 4.5 mm and 3.1 × 2.5 mm respectively (Fromming, 1954).

The breeding season of *S. plebeia* slug in the laboratory was determined by the presence of egg clutches. The mean number of egg clutches and eggs was estimated monthly from November 2022 to October 2023 under laboratory conditions. As cleared in Table 1. the slug laid its eggs irregularly throughout the year. At the beginning of egg laying the number of egg clutches and eggs was low. Then the deposited egg clutches and eggs gradually increased to reach the maximum rate in March and April with mean of 1.49 & 30 and 1 & 26.5 eggs, respectively. After that, the rate of deposited eggs decreased in the period from May to August and increased again in September and laid an average egg clutches of 0.83 containing around 24.16 eggs. These findings revealed clearly that slugs did not deposit any eggs during November, February and August months at all. These results were strongly corroborated by Faberi *et al*. (2006) who showed that the reproduction of *S. plebeia* occurs in autumn or spring throughout their lifetime. Moreover, Garcia *et al*. (2007) indicated that the reproduction of *S. plebeia* was high during the rainy season and cooler conditions and laid one to four clutches per year each containing about 30 eggs. Another similar study stated that the breeding of *S. plebeia* occurs in the wet season and produces clutches averaging 37 eggs (Rueda *et al*., 2002). According to the report of Kozlowski (2000) *Arion vulgaris* slug deposited around 400 eggs in several clutches from the end of summer to autumn. On the other hand, the grey field slug *Deroceras reticulatum* lays from 60 to 75 eggs throughout the year (Branson, 1980). In contrast, South (1982) stated that *D. reticulatum* produced up to 500 eggs in a year. The breeding and reproductive activity of some Veronicellidae were observed from June to September in India. While, the breeding of South African populations occurs in the warm, rainy summer months. *Laevicaulis stuhlmanni* slug deposited its eggs in clutches of up to 190 eggs (Herbert and Kilburn, 2004). Under laboratory conditions, the egg-laying period of *L. stuhlmanni* started from March to November. The number of produced egg clutches increases from the end of May until the beginning of September. Moreover, from July to September the number of eggs in clutches increases. During the May and June months, *L. stuhlmanni* laid an average of 50.85 egg clutches and the number of eggs per clutch ranged from 5 to 140 eggs (Ali, 2017).

Table 1. Mean number (± SE) of egg clutches and eggs/pair of *Sarasinula plebeia* from November 2022 to October 2023 under laboratory conditions

Months	Egg clutches	Eggs
Nov.	0 ± 0	0 ± 0
Dec.	0.6 ± 0	13 ± 1.52
Jan.	0.3 ± 0.15	6.7 ± 1.12
Feb.	0 ± 0	0 ± 0
Mar.	1.49 ± 0.14	30 ± 8.54
Apr.	1.0 ± 0.18	26.5 ± 6.95
May	0.49 ± 0.1	12.16 ± 3.92
Jun.	0.33 ± 0	5.5 ± 0.76
Jul.	0.30 ± 0.12	7 ± 1.15
Aug.	0 ± 0	0 ± 0
Sep.	0.83 ± 0.32	24.16 ± 3.87
Oct.	0.33 ± 0.1	5 ± 0.57

II- Biological Parameters And Growth Development Of Juveniles:

As indicated in Table 2. the incubation period of *S. plebeia* eggs ranged from 18 to 21 days with an average of 18.33 days. While 9.66 days was the mean of the hatching period and the hatchability ranged from 78 to 92.6% with an average of 84.57%. The number of eggs per clutch ranged between 12 and 67 eggs with an average of 17.4 eggs and the mean

of fecundity (eggs/slug) was 14.36 eggs throughout the season. On the other hand, the generation period recorded 291 days and this means that *S. plebeia* has one generation per year. 49.28 days was the mean of the oviposition period while the life span (longevity) varied between 368 to 371 days with an average of 369.95 days. Related to this aspect, Jackson and Mua (2019) reported that the eggs of *S. plebeia* slug hatch in 20 - 24 days and the juveniles become adults in approximately 2.5 months. Contrarily, Rueda *et al*. (2002) stated that *S. plebeia* reached to the mature stage after about 6 months.

Table 2. Mean (± SE) of the life history parameters of *Sarasinula plebeia*

Items	**Incubation period (days)**	**Hatching period (days)**	**Hatchability (%)**	**Number of eggs per clutch**	**Fecundity (eggs/slug)**	**Generation period (days)**	**Oviposition period (days)**	**Life span (days)**
Average	18.33 ± 0.33	9.66 ± 0.87	84.57 ± 2.28	17.4 ± 0.83	14.36 ± 2.38	291.00 ± 1.15	49.28 ± 2.55	369.95 ± 0.97
Range	18 - 21	8 - 11	78 – 92.6	12 - 67	6 - 24	287 - 293	49 - 52	368 - 371

The incubation period of *Laevicaulis stuhlmanni* eggs ranged from 10 to 19 days with an average of 13.18 days. Variation in the incubation period may be attributed to the temperature and other environmental conditions. On the other hand, the hatchability of *L. stuhlmanni* eggs was 85.15% and the mean oviposition period was 46.83 days, the post-oviposition period was 13.92 days. The life span ranged between 127 to 188 days and the mean of *L. stuhlmanni* generation period was 102.61 days (Ali, 2017). In a similar study, Herbert and Kilburn (2004) demonstrated that the life span of *L. stuhlmanni* varied between five and six months but, it was recorded around seven months for *Laevicaulis alte* slug. Both *L. stuhlmanni* and *L. alte* reached sexual maturity after about five months. Brodie and Barker (2012) confirmed that *L. stuhlmanni* reached sexual maturity after seven months. The eggs of *D. reticulatum* take from 23 to 27 days to hatch and the mean hatching period was 8.4 days. The hatchability of *D. reticulatum* eggs varied between 79 and 97.3% and the average fecundity was 67.3 eggs throughout the season. Additionally, the life span of *D. reticulatum* ranged from 54 to 57 weeks (Hend, 2015). Juveniles of *Arion vulgaris* hatch after 30 – 50 days and most slugs die after oviposition (South, 1992; Kozlowski, 2000).

The body length of ten juveniles was measured monthly until reaching the adult stage. As shown in Table 3. and Fig. 4., the body length of juveniles increased with increasing age. The maximum rate of monthly length increase was 7 mm, but a significant increase was observed especially from the first, second, third, fourth and ninth-month-old juveniles until they became adults (12 months old) with a rate reaching 11.3 mm.

Table 3. The monthly growth rate of *Sarasinula plebeia* juveniles until maturity

Individuals No.	Body length (mm) / age											
	1 m	2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m	11 m	12 m
1	9.00	20.00	20.30	30.20	30.80	40.10	40.20	40.30	40.70	50.40	60.00	60.50
2	8.00	18.00	20.60	30.30	30.60	40.00	40.20	40.20	40.50	50.90	60.40	70.00
3	9.00	20.20	20.40	30.00	30.60	30.90	40.10	40.30	40.50	50.70	50.80	60.50
4	7.00	20.00	20.80	30.50	30.40	40.20	40.10	40.20	40.80	50.30	60.00	60.30
5	9.00	20.10	20.20	30.20	30.50	40.00	40.30	40.40	40.60	50.00	60.30	60.60
6	8.00	20.30	20.40	30.40	30.70	30.80	30.80	40.00	40.80	40.80	50.70	70.00
7	8.00	20.00	20.60	30.10	30.80	40.20	40.20	40.20	40.90	50.50	60.20	60.90
8	9.00	20.00	20.40	30.00	30.40	30.80	40.00	40.00	40.80	50.00	60.00	60.80
9	7.00	20.10	20.20	30.50	30.60	40.10	40.30	40.30	40.60	40.80	50.80	70.00
10	9.00	18.00	20.30	30.20	30.80	40.00	40.00	40.20	40.30	50.90	60.40	60.80
Average ± SE	8.30 ± 0.20	19.67 ± 0.30	20.42 ± 0.06	30.24 ± 0.05	30.62 ± 0.04	37.31 ± 1.41	39.22 ± 0.93	40.21 ± 0.04	40.65 ± 0.06	48.53 ± 1.2	51.74 ± 1.4	57.36 ± 1.4
Range	7 - 9	18 - 20.3	20.2 -2 0.8	30 – 30.5	30.4 – 30.8	30.8 - 40.2	30.7 – 40.2	40 – 40.4	40.3 – 40.9	40.8 - 50.9	50.7 – 60.4	60.3 - 70

m = months old

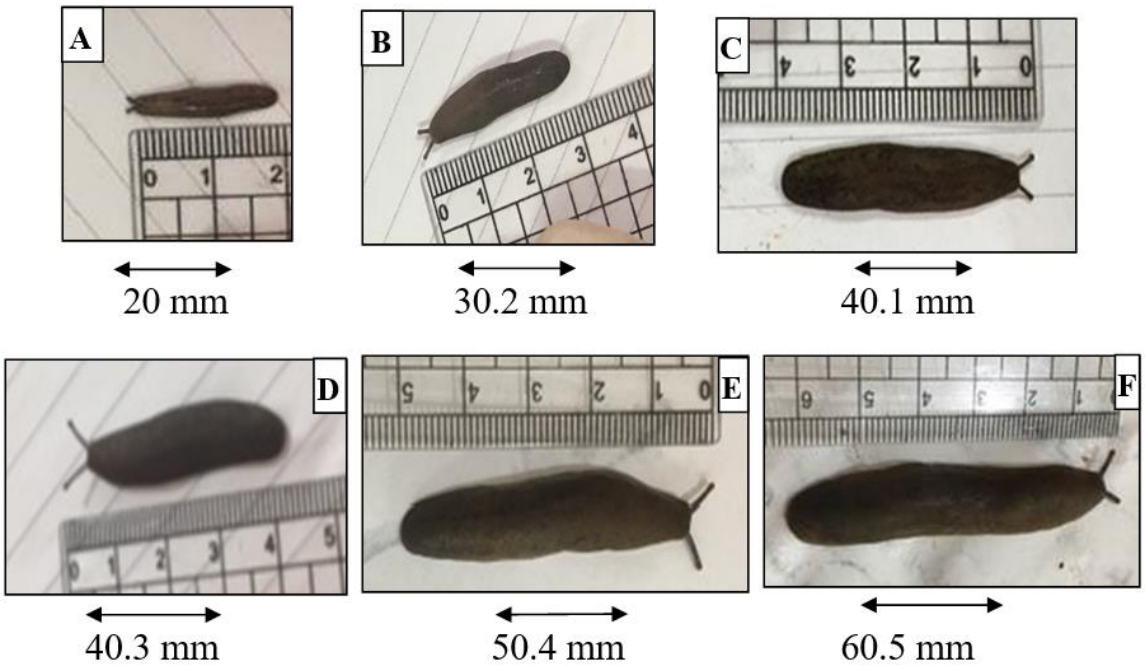

Fig. 4: Growth rate of *Sarasinula plebeia* juveniles. (A) Slug length at 2 months; (B) Slug length at 4 months; (C) Length of Slug at 6 months; (D) Slug length at 8 months; (E) Slug length at 10 months; (F) Length of the fully grown individual (12 months old).

The mean length of the juveniles was 19.67, 30.24, 37.31, 40.21, 48.53 and 57.36 mm at the age of 2, 4, 6, 8, 10 and 12 months, respectively. It is important to mention that the maximum length of a fully grown individual (12 months old) of *S. plebeia* was 70 mm long.

These findings were exactly consistent with Jackson and Mua (2019) who confirmed that the length of the *S. plebeia* adult slug measured 70 mm. On the other hand, Hend (2015) indicated that the mean body length of *D. reticulatum* newly hatched individuals was 5 mm. The length of juveniles increased with increasing age reaching 7 mm, 13 mm, 22 mm and 43 mm at the age of 2, 4, 6 and 9 months, consecutively. The adult length of *D. reticulatum*

measured about 52 mm. In a similar study, Clemente *et al*. (2008) showed that the mean length of the new hatching slugs of *D. reticulatum* was 3.8 mm. While, the lengths of newly hatched individuals of *L. stuhlmanni* slug ranged from 1 to 2 mm (Ali, 2017). Similarly, Herbert and Kilburn (2004) stated that the length of *L. stuhlmanni* hatching ranged from 7 to 8 mm. The average body length of *L. stuhlmanni* adult was 46.2 mm and ranged from 35 mm to 56 mm and the body becomes more elongated when the slug is moving (Metwaly and Ali, 2024).

It is important to indicate that the morphological characteristics, biological parameters and anatomy of the reproductive system represent the main aspects for distinguishing between different species of slugs especially members of the Veronicellidae family.

Biological Control:

I- Microbial Management of *Sarasinula plebeian*:

The molluscicidal potencies of *Bacillus subtilis*, *Paecilomyces variotii* and *Trichoderma asperellum* were investigated against *S. plebeia* juveniles under laboratory conditions. As cleared in Table 4., all tested isolates were pathogenic against juveniles with different virulence. Moreover, the mortality rate of juveniles generally increased with the concentration increase of the tested isolates, although all isolates achieved the lowest influence on juveniles at their lowest concentration. The highest mortality rates 83.33% and 80% were obtained at 6×10^5 CFU/ml concentration in a mean period of 17.3 days post-treatment for *T. asperellum* and 7×10^6 CFU/ml, on the 19th day post-treatment for *B. subtilis*. On the other hand, *P. variotii* caused the lowest mortality rate (76.66%) at 6×10^5 CFU/ml after 18 days of treatment. Concurrently, there was no mortality among the juvenile slugs in the control. It could be concluded that all isolates had the highest mortality rates in an average time of 17 – 20 days after infection.

Table 4. Mean mortality (% ± SE) of *Sarasinula plebeia* juveniles treated with microbial isolates under laboratory conditions

Tested Microorganisms	Tested conc. (CFU/ml)	Mortality (%)	Mortality time (days)
Bacterium strain			
Bacillus subtilis	7×10^6	80 ± 10^{a}	19 ± 0.5^{ab}
	5×10^6	53.33 ± 6.6^{cd}	19.6 ± 0.8^{a}
	2.5×10^6	36.66 ± 8.8^{d}	18.3 ± 0.3^{abc}
Fungal strains	6×10^5	76.66 ± 3.3^{ab}	18 ± 0.5^{bc}
Paecilomyces variotii	4.5×10^5	60 ± 5.7^{bc}	17.6 ± 0.3^{bc}
	3×10^5	36.66 ± 6.6^{d}	17 ± 0^{c}
Trichoderma asperellum	6×10^5	83.33 ± 3.3^{a}	17.3 ± 0.6^{c}
	4.5×10^5	66.66 ± 6.7^{abc}	18 ± 0^{bc}
	3×10^5	53.33 ± 3.3^{cd}	17.6 ± 0.3^{bc}
Control		0 ± 0^{e}	0 ± 0^{d}

In addition, there was a significant difference in mortality rates and mortality times of juveniles caused by tested isolates in comparison with the control.

Few studies were found on *S. plebeia* control by using pathogenic bacteria and fungi. But in this regard, Matthews (2005) indicated that disruption in the life history traits of slugs as reproduction, growth and fitness may lead to slug death. So, any substances affected on these traits can be considered as potential alternatives for slug control and bacteria may affect these traits. Moreover, the life stages of any pest play a major role in the fungal isolates infection process (Sutanto *et al*., 2021). The green fungus, *Metarhizium*

anisopliae is virulent to juvenile snails because it can penetrate the juvenile body, digest its tissues and convert them into diffusible nutrient materials and this is also in addition to fungus releasing toxins (Roberts and Humber, 1981). Similarly, Abd El-Magied (2009) reported that *Aspergillus flavus* caused 80% mortality of *Monacha cartusiana* juveniles within 21 days of infection. It was followed by *Aspergillus terreus* and *Aspergillus kiliense* which attained 60% and 46.67% mortality of juveniles after the same experimental period, respectively. In another study, juveniles of *Cochlicella acuta* and *Theba pisana* at the age of six months were highly susceptible to *Trichoderma harzianum* which caused 83% and 71% mortality of both snails, consecutively. While, *A. terreus* and *Aspergillus phoenicis* attained 71% & 40% and 50% & 37% mortality of *C. acuta* and *T. pisana* juveniles, respectively (Hend, 2007).

Hend (2023) inferred that *Eobania vermiculata* juveniles at the age of two months were significantly affected by *T. harzianum* which recorded 80%, 70% and 66.66% mortality at 6×10^5, 4×10^5 and 2×10^5 spores/ml concentrations consecutively on day 21 post-treatment. In the same trend, Maketon *et al*. (2009) showed that *Paecilomyces lilacinus* had high pathogenicity against *Pomacea canaliculata* juveniles and this can be attributed to its secretion to hydrolysis enzymes which facilitates the penetration of fungal mycelium into the juvenile's cell walls.

The results are in Table 5. showed the mortality rates of *S. plebeia* adult slugs treated with different concentrations of *B. subtilis*, *P. variotii* and *T. asperellum* isolates. According to the findings, increasing the tested concentration of isolates gives a better chance of successful infection. Moreover, there is no mortality of slugs was achieved by all isolates before 10 days of treatment. The highest mortality 63.33% was caused by *T. asperellum* at 6×10^5 CFU/ml concentration (Fig. 5) in a mean period of 19.3 days post-exposure.

Table 5. Mean mortality (% ± SE) of *Sarasinula plebeia* adult slugs treated with microbial isolates under laboratory conditions

Tested Microorganisms	Tested conc. (CFU/ml)	Mortality (%)	Mortality time (days)
Bacterium strain			
Bacillus subtilis	7×10^6	60 ± 10^{a}	18 ± 0^{ab}
	5×10^6	36.66 ± 3.3^{cd}	15.3 ± 0.3^{cd}
	2.5×10^6	23.33 ± 6.7^{d}	14.6 ± 0.8^{d}
Fungal strains			
Paecilomyces variotii	6×10^5	53.3 ± 6.7^{ab}	19 ± 1^{a}
	4.5×10^5	40 ± 0^{bc}	19 ± 0.5^{a}
	3×10^5	26.66 ± 3.3^{cd}	18.3 ± 0.7^{ab}
Trichoderma asperellum	6×10^5	63.33 ± 3.3^{a}	19.3 ± 0.3^{a}
	4.5×10^5	56.66 ± 8.8^{a}	18.6 ± 0.6^{ab}
	3×10^5	40 ± 5.7^{bc}	17 ± 0^{bc}
Control		0 ± 0^{e}	0 ± 0^{e}

Fig. 5: Lysis in the body of adult *Sarasinula plebeia* after 14 days of treatment with *Trichoderma asperellum* at 6×10^5 CFU/ml.

It was followed by *B. subtilis* and *P. variotii* which attained 60% and 53.33% mortality at 7×10^6 and 6×10^5 CFU/ml within 18 and 19 days of infection with both isolates, respectively. Interestingly, *B. subtilis* at the concentration 5×10^6 CFU/ml caused charring and dryness in the slug body and turned its colour to black after 12 days of the treatment (Fig. 6). In contrast, no mortalities were recorded in the control at all until the end of the investigation. Moreover, there was a significant difference in the slug's mortality and mortality times compared to untreated individuals. These results were in accordance with Mona *et al*. (2017) stating that the mortality of *E. vermiculata* adult snails caused by *Bacillus megaterium* and *Trichoderma album* was raised with an increase in the exposure period to these bioagents. Moreover, the molluscicidal potency of *B. megaterium* was more than *T. album* against the snail individuals. In this trend, Shakeri and Foster (2007) explained that increasing the mortality rates of adult snails by increasing the experimental time may be due to the period required for the fungus to establish itself on the soft body of the snail and start infection. The maximum production of extracellular protease which facilitates the fungus invasion to the host surface was reached to ten days for most fungi. According to the report of Genena *et al*. (2008), *Bacillus thuringiensis* was effective against *E. vermiculata* and *M. cartusiana* adult snails at 7×10^6 CFU/ml concentration. It achieved 86.6% and 53% mortality of the two snails consecutively after 28 days of the treatment. On the same pattern, Hend (2023) revealed that *B. subtilis* caused 56.66% and 40% mortality of *E. vermiculata* and *M. cartusiana* adult snails at the concentration of 4×10^5 spores/ml within seven days of the infection, respectively. The two snails also were susceptible to *T. album* and the pathogenicity of this fungus against both snails was increased with the increase in the concentrations and the time elapsed. It was attained 40%, 30% and 26.66% & 33.33%, 26.66% and 23.33% mortality of *E. vermiculata* and *M. cartusiana* adult snails for 1.5×10^6, 1×10^6 and 0.5×10^6 spores/ml concentrations, consecutively. Similarly, Abd El-Atti *et al*. (2020) confirmed that *T. album* had significant potent activity in the control of *M. cartusiana* adults. The pathogenic activity of Trichoderma species likewise *Trichoderma viride*, *Trichoderma harzianum* and the other fungus *P. variotii* can be attributed to their ability to

penetrate the host cuticle by producing the hydrolysis enzymes on the surface of the host body (Abd El-Azem, 2008).

Fig. 6: Dryness and charring in the body of *Sarasinula plebeia* adult after 12 days of treatment with *Bacillus subtilis* at 5×10^6 CFU/ml.

II- **Predation:**

Predation Potency of *Eobania vermiculata* on the Developmental Stages of *Sarasinula plebeian:*

Individual predation, as well as prey preference of *E. vermiculata* adult snail on eggs, juveniles and adults (10 from each stage/replicate) of *S. plebeia* slug, was illustrated in Table 6. After two days of the experiment, two eggs were eaten by *E. vermiculata* snail and then one from both eggs excreted and has been observed in the snail feces. The mean eaten eggs reached 1.3, 1.6 and 2.6 after 4, 7 and 11 days respectively after which no further predation on eggs occurred until the end of the investigation. With respect to juveniles, after only three hours of the experiment, two juveniles from the ten individuals attacked the snail shell near the shell aperture (Fig. 7A) and then the snail animal caught one of them for predation (Fig. 7B). There were 2.6 mean eaten juveniles and other four dead individuals were recorded within the first day of the experiment. On the second day, other four juvenile slugs were eaten by the snail, after which no other juveniles were eaten or died until the end of the experiment. On the other hand, the single snail did not predate any adult slug at all during the experimental period. Moreover, all predator snails remained alive until the end of the investigation and also no death of the juvenile and adult slugs was recorded in the control throughout the experiment. These findings can be concluded that *E. vermiculata* snail preferred the young age of slug; hence, adult slugs were the least preferred stage to the snail. Related to this aspect, Meyer and Cowie (2010) reported that the small slug *Deroceras laeve* was preferred to *Euglandina rosea* snail over *Veronicella cubensis* slug and the other snail *Oxychilus alliarius* completely failed to predate the adult individuals of both slug species. A large number of studies indicated that prey size plays a main role in predator preference (Nishida and Napompeth, 1975; Griffiths *et al*., 1993; Gerlach, 1999).

Table 6. The mean predatory activity of *Eobania vermiculata* on *Sarasinula plebeia* at different developmental stages

Eggs		juveniles			Adults		Control
Duration (days)	**No. Eaten**	**Duration (days)**	**No. Eaten**	**No. Died**	**No. Eaten**	**No. Died**	**No. Died**
2	2	1	2.6	4	0	0	0
4	1.3	2	4	0	0	0	0
7	1.6	7	0	0	0	0	0
11	2.6	11	0	0	0	0	0
Total count	7.5	Total count	6.6	4	0	0	0

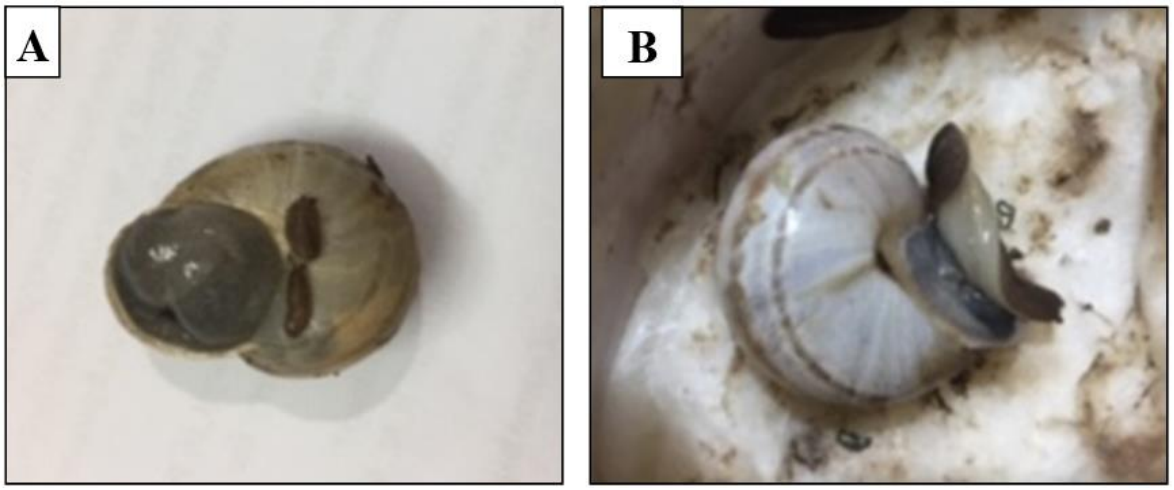

Fig. 7: Predation of *Sarasinula plebeia* juvenile by *Eobania vermiculata* adult snail. (A) Two juvenile slugs attached to the snail shell near to the shell aperture; (B) Catching the snail animal to juvenile slug for predation.

In the same trend, Cook (1989) confirmed that prey size is an important aspect of the predation process; smaller species were preferred to the predator. So, the terrestrial snail *E. rosea* showed significant predation potency on the small individuals of *Achatina fulica* snail, causing a high reduction in its populations (Cowie, 2001). Moreover, the early stages of succineid life history make them preferred to *O. alliarius* snail (Brown *et al*., 2003; Rundell and Cowie, 2003). Knowledge of the feeding behaviour of predators is essential to design the best strategies to control native Mollusca species. In addition, both predatory snails and the introduced prey species have been widely distributed by human activities (Barker and Efford, 2004; Cadiz and Gallardo, 2007). More studies are required to investigate the predatory activity of more snail species against slugs at different ages as an efficient bio-control method.

CONCLUSION

The results of the current study indicated that *S. plebeia* deposited translucent oval eggs in clutches deep in the soil and this slug had one generation through the year. The present findings also proved that microbial isolates *T. asperellum* and *B. subtilis* have strong pathogenic activity against juvenile and adult individuals of *S. plebeia*. In addition, *E. vermiculata* snail showed distinctive predatory potency against eggs and juvenile slugs. More future studies are needed on using the most virulent microbial isolates and predatory snails in the management of *S. plebeia* slug under field conditions.

Declarations:
Ethical Approval: Not applicable
Competing interests: The authors declare that they have no duality of interest associated with this manuscript.
Funding: No specific funding was received for this work.
Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.
Acknowledgements: Not applicable.

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ARABIC SUMMARY

بيولوجية و مكافحة بزاقة الفول ساراسينيولا بلبيا، نوع جديد تم تسجيله لأول مره في مصر

هند شكري غريب و محمد حسن عصام الدين متولي لقمه
معهد بحوث وقايه النباتات – مركز البحوث الزراعيه - الدقي – جيزه – مصر

تم إجراء هذه الدراسة المعمليه بهدف التعرف على دورة حياة و تطور نمو البزاقه ساراسينيولا بلبيا و هي نوع جديد من البزاقات الأرضيه تم تسجيل وجودها لأول مره من خلال هذه الدراسه في مصر على نبات الزينه السفندر في مشتل بمدينة الإسماعيليه. إمتدت الدراسه أيضا إلى إجراء المكافحه البيولوجيه لهذه البزاقه للأفراد الصغيره عند عمر شهرين و أيضا للأفراد البالغه بإستخدام العزلات الفطريه الموصى بها في مجال مكافحه الأفات الزراعية تريكوديرما أسبيريلم و باسيلوميسس فاريوتي عند ثلاثة تركيزات مختبره 6×10^5 و$4،5 \times 10^5$ و 3×10^5 جرثومه / ملل و بكتيريا باسيلس ساتلس عند التركيزات 7×10^6 و 5×10^6 و $2،5 \times 10^6$ جرثومه / ملل. تم إختبار أيضا السلوك الإفتراسي للطور البالغ من القوقع الأرضي إيوبانيا فيرميكيولاتا على البيض و الأفراد الصغيره و البالغه لهذه البزاقه تحت الظروف المعمليه. أظهرت النتائج أن أفراد البزاقات تضع بيض بيضاوي الشكل شفاف على هيئة مجموعات في أعماق التربه و كان بدايه وضع البيض في شهر ديسمبر كما تراوح عدد البيض في الكتله من 12 إلى 67 بيضه. أوضحت النتائج أيضا أن فتره حضانة البيض تتراوح ما بين 18 إلى 21 يوم كما سجلت فتره الفقس من 8 إلى 11 يوم بينما تراوحت نسبه الفقس ما بين 78% إلى 92،6% و وصلت فترة دورة الحياة إلى 293 يوم و هذا يعني أن هذه البزاقة لها جيل واحد خلال العام. بدراسة التأثير القاتل للعزلات الميكروبيه ضد الأفراد الصغيره عمر شهرين و الأفراد البالغه للبزاقة أشارت النتائج إلى أن فطر تريكوديرما أسبيريلم و بكتيريا باسيلس ساتلس قد حققا أعلى تأثير ضد كلا العمرين عند أعلى تركيز مختبر لكلاهما بينما سجل فطر باسيلوميسس فاريوتي أقل تأثير إبادي ضد كلا العمرين من البزاقه. أما فيما يخص السلوك الإفتراسي لقوقع إيوبانيا فيرميكيولاتا ضد الأعمار المختلفه للبزاقه فقد حقق القوقع أعلى معدل إفتراس ضد البيض يليه الأفراد الصغيره عمر شهرين بينما لم يستطيع إفتراس الأفراد البالغه من البزاقه نهائيا. هكذا تشير نتائج هذه الدراسه بقوه إلى وجود وسائل مكافحة بيولوجيه جديده فعاله و أمنه لمكافحة البزاقات الأرضيه خاصه بزاقة ساراسينيولا بلبيا كما ساهم دراسه السلوك البيولوجي للبزاقه في تحديد الوقت الأمثل لإجراء المكافحه في شهري مارس و أبريل و هما الشهرين الذي وصل فيهما النشاط البيولوجي للبزاقه أقصاه.