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Morphology of radula and shell in land snail *Caucasotachea lencoranea* (Mousson, 1863) and slug *Drusia ibera* (Eichwald, 1841) in the east of Tehran province

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Abstract
Caucasotachea lencoranea and *Drusia ibera* are important agricultural and garden pests in Iran, so that in some years their high population causes serious damages in gardens and agricultural fields. In order to better control these pests and improve their management strategies, morphological characteristics of these animals is important. The target species were collected from the walnut orchards of Targhian village and Roudehen city during 2021 and 2022. The samples were preserved in 70% alcohol after being killed in cold water. In the laboratory, the radula of the samples was extracted and stained using Mallery II dye, and the dental formula, the teeth, and shell morphology were examined. The length of the central tooth was different in these two species. *Drusia ibera* had one central tooth, forty lateral teeth, and 15 marginal teeth in 14,842 tooth rows. *Caucasotachea lencoranea* had one central tooth, eighteen side teeth and 23 marginal teeth in 1152 tooth rows. The average length and width of shells of adult species *Caucasotachea lencoranea* are equal to 21.5±1.75mm and 30.75±0.45mm respectively. *Drusia ibera*, the length of the shell is 5 ± 0.15 and the width is 10 + 0.45 mm. Also, a significant correlation was observed between the environmental factors of temperature and humidity with changes in the population of the studied species.

Keywords: *Caucasotachea lencoranea*, *Drusia ibera*, radula, shell, population fluctuations.

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INTRODUCTION

The snail *Caucasotachea lencoranea* (Mousson, 1863) belongs to Helicidae family and the slug *Drusia ibera* (Eichwald, 1841) from the Parmacellidae family are among the most important pests of agricultural and garden crops. They cause damage to all kinds of fruit trees, especially citrus fruits, leafy vegetables and ornamental plants. Feeding on leaves, stems, fruits, flowers, seeds of plants (quantitative damage) cause a decrease in marketability (qualitative damage) by causing corrosion.

The Helicidae and Parmacellidae are among the most important agricultural pests and damage-causing families (Tomaz et al., 2018). They are polyphagous and attack various agricultural products (Mahjoub, 2015). The species *C. lencoranea* was first discovered in 1968. The species *D. ibera* was reported in citrus orchards in the north of the country in 1972 (Mirzaee, 1972). These species are found in fields and gardens, as well as in greenhouses that have moderate temperature and high relative humidity,

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in moist, shady areas, under rocks and fallen bushes and tree trunks, or take shelter under the soil. They search for food and cause damage (South, 1992). Many parasitic diseases that can be transmitted to humans through snails and slugs. Dicrosyliasis disease caused by the parasitic worm *Dicrocoelium denderiticum* (Rudolphi, 1819) has two intermediate hosts, the first intermediate host is snails and slugs (Mansourian, 2005), which species are *C. lencoranea* and *D. ibera* can play a role as an intermediate host in the development cycle of the above-mentioned parasitic worm. They cause the spread of disease in livestock and humans, which have been noticed in medicine and veterinary medicine. Also, the parasitic nematode, *Angiostrangylus cantonensis* can be transmitted to humans and animals by the above species (Malek, 1980; Mansourian, 2005; Pilate *et al*., 2012). Considering the intermediate host of molluscs and transmission of parasitic diseases, the important role of malacology studies is clearly visible as a necessity in public health.

The elimination of diseases that can be transmitted through snails and slugs is mainly done by their control (Mirzaei, 1972). Therefore, awareness of the morphology and distribution of their population in each region and the parasitic diseases transmitted by them is one of the basic requirements for dealing with snails and slugs. This can help improve the level of community health (Imani Baran, 2016).

Biological monitoring is the center of environmental assessment and can be an early warning in changes that threaten ecosystems. Biomarkers are able to evaluate and check the trend of ecosystem conditions and provide essential information about the health of the ecosystem. Snails and slugs from Helicidae and Parmacellidae are one of the most important biomarkers, which are suitable for detecting heavy metal-contamination places (Laskowski & Hopkins, 1996; Gomot de Vaufleury & Pihan, 2000; Ibrahim et al., 2006; Moolman et al., 2007).

The aim of this research is to identify the land snail *Caucasoeachea lencoranea* and the *Drusia ibera* slug from the Helicidae and Parmacellidae families based on the morphological structure of radula and shell. Also, determining the pattern of distribution, abundance and population changes of each species. Along with the identification of the ecological factors involved in the changes observed in the spring and summer seasons in the eastern regions of Tehran province. In this way, comprehensive information can be obtained to provide the control of snails and slugs.

MATERIAL AND METHODS

Study area

Tehran province with an area of about 12981 square kilometers is located in the south of the central part of the Alborz Mountain range. The current extent of Tehran extends from 900 to 1800 meters above sea level; This height decreases from north to south province (50° to 53°E and 34° to 36.50° N).

The gardens of Roudehen city (51°54′4″ E; 35°43′34″ N) and Targhian village (51°41′50″ E; 35°45′18″ N), in the east of Tehran province, were selected. First, the desired areas for sampling were determined from the map (Fig. 1). The location of each of the station was determined and recorded by GPS device. Sampling region was done in the spring and summer seasons of 2021-2022.

Collecting and identifying snails and slugs

Sampling of snails and slugs were done by manual method and unaided eye using a net and in some cases by hand. Locating and sampling stations (Fig. 1) were determined based on the natural condition of the area, vegetation, placement of gardens and fields of soil or sub-soil, fodder beds, under stones and tree trunks, weeds, and leafy vegetables. It was carried out in different seasons (spring and summer) in selected stations in the eastern areas of Tehran province. The collected snails and slugs, were killed in cold water, placed in 70% ethanol alcohol and then transferred to the mollusc's laboratory of the Institute of Plant-Medicine Research. The length shell of snails and slugs were measured with the help of calipers. The visceral part was obtained for morphological study of radula and reproduction system. Samples of snails and slugs based on the characteristics of shape, size, patterns on the shell, the number of whorls, umbilicus, aperture, whether the shell is dextral or sinistral, body size, digestive system (radula) and the reproductive system were studied using a stereomicroscope and were identified using the available keys

FIGURE 1. Sampling areas of the gardens of Roudehen city and Targhian village in the east of Tehran province.

(Mahmoud et al. 2011; Kantor et al. 2010; Brown, 2003; Pfleger, 1999; Gittenberger, 1991). In addition, air temperature and humidity (thermo-hydrograph device) were measured. The thermo-hydrograph device was placed in a meteorological box according to meteorological standards. The tapes of the device were changed weekly.

Staining of radula

The buccal mass of snails and slugs of *C. lencoranea* and *D. ibera* species was completely removed (Kristensen, 1984). The tissue around radula was placed in NaOH 7.5% for 24 hours. Then, was stained using Mally II dye (Mansourian, 1986). Neutralized in 15% acetic acid for 5 minutes, placing the slide containing the radula in a diluted solution of Mally II dye for 5 minutes, washed in 2% oxalic acid and dehydrated with 96% alcohol, washing the radula with one to two drops of oil and mounted on glass slide using Canada Balsam.

Population frequency and statistical analysis

The population abundance of the mentioned species was investigated during the spring and summer seasons of 2022, from April to September, in the east of Tehran province. Sampling was monthly between 5-8 in the morning during the activity of snails and slugs. The quadrate method was used to collect eggs, larvae and adults (Miranda et al. 2014). Quadrats were made of wood and samples covering was one square meter. For each sampling time, 30 quadrats were randomly thrown on the ground. Then the snails and slugs in the area marked were collected and counted. The changes in egg, immature and adult population of snails and slugs were statistically analyzed. Morphometric data was analyses using Excel 2016.

RESULTS

A total of 635 specimens of *Caucasotachea lencoranea* and *Drusia ibera* were collected and identified. 353 specimens were related to *C. lencoranea* and 282 specimens were related to *D. ibera.*

Family Helicidae Rafinesque, 1815

***Caucasotachea lencoranea* (Mousson, 1863)**

Shell (Fig. 2A) relatively large with a blunt conic spiral, 21.5x30.75 mm, hard walled, fairly lustrous, dextral, depressed, length less than width but always greater than that of the peristome, usually containing band in whorls, no umbilicus, the edges of the peristome widely thick and reflected and a dark brown color inside the aperture. Reticulate sculpture present only on parts of the shell. The dart spindle-shaped

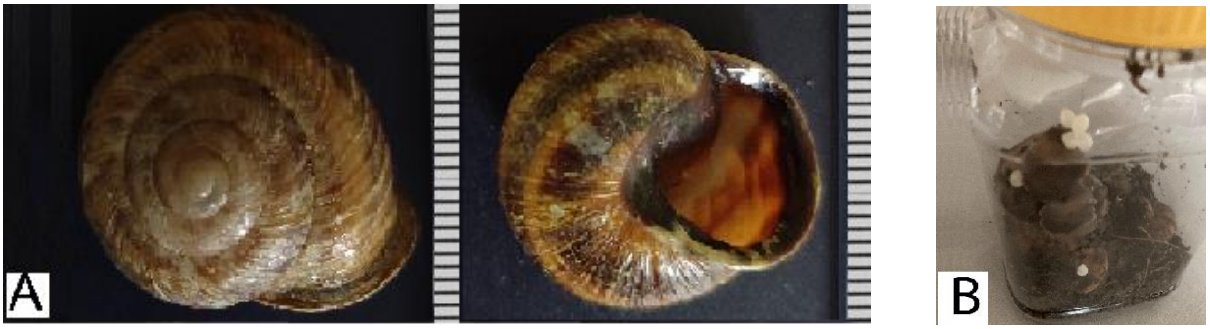

FIGURE 2. A) The dorsal and ventral surface of snail *Caucasotachea lencoranea*; B) The egg mass.

and narrows at the base, giving the appearance of a neck. The edges of dart blades without thick ends; 5.5 to 6 whorls in the length of the shell which evenly increase in size, the embryonal turns (1 ¼) are smooth, light gray and monochromatic; the ultimate turn less than 1 ½ times the width of the penultimate one and descends abruptly at the peristome. The peristome low oval and oblique (the angle of inclination more than 45°). In terms of the shell of this species in the investigated areas, we can mention their size that in the adult *C. lencoranea* species, it has a large shell with an average 21.5 ± 1.75 mm long by 30.75 ± 0.45 mm wide.

Older and mature snails had larger shell sizes than immature snails. The average length and width of the shell and the length and width of the aperture of 80 mature *Caucasotachea lencoranea* were 22.1±1.0mm, 31.5±0.5mm, 1.5±0.4mm, 1.80±0.2mm in Roudehen station and 20.9±2.5mm, 3.0±0.4mm, 1.3±0.2mm and 1.7±0.3mm in Targhian village station, respectively.

Parmacellidae P. Fischer, 1856

***Drusia ibera* (Eichwald, 1841)**

Body up to 69 mm long (Fig. 3A), gray in color with two dark stripes, several spots and a sharp tip on the dorsal side of the body; shell small; Pneumostome in the posterior part of the mantle. In adults, the shell completely under the mantle, but in young specimens, looks like a small cap protruding from the posterior edge of the mantle. Genital atrium lack of a glandular cover, with two large muscular copulatory appendages. A sucker inside at the posterior end of the penis; the prostate gland free and not connected to the uterus. The seminal receptacle nearly spherical. Its damage (Fig. 3B.) can be seen in the form of multiple shredding and scrapping on the leaves of the trees. In terms of the shell of this species in the investigated areas, we can mention their size, in the adult *D. ibera* species, the length of the shell is 5.0± 0.15 mm and its width is 10.0 ± 0.45 mm.

The average length and width of the slug and the length and width of the shell of 80 mature *Drusia ibera* were 69.0±1.5mm, 8.0±0.5mm, 5.0±0.2mm, 1.0±0.7mm in Roudehen station and 70±1.1mm, 9.0±0.7mm, 5.0±0.1mm and 10.0±0.2mm in Targhian village station in the east of Tehran province, respectively.

Radula tooth structure

Morphological differences of radula between the two species of snail *Caucasotachea lencoranea* and slug *Drusia ibera* in Figures 4A and 4B shown. Radula morphology is considered as one of the most common sources of information used for the systematic study of molluscs. The shape and form of the radula teeth of molluscs is usually unique for a genus or species (Pfleger, 1991). The radula is composed of three types of teeth arranged linearly, including central teeth (CT), lateral teeth (LT), and marginal teeth (MT). 80 radula slides were prepared from 80 samples of each species. *Caucasotachea lencoranea* had one central tooth, 18 latteral teeth and 23 marginal teeth, that's mean that the radular formula is (1C+18L+23M) in 152 ± 2 tooth rows. The snails with larger shell size had larger teeth size. Accordingly, the mean length of radular teeth increased with increasing of the body size in all examined specimens.

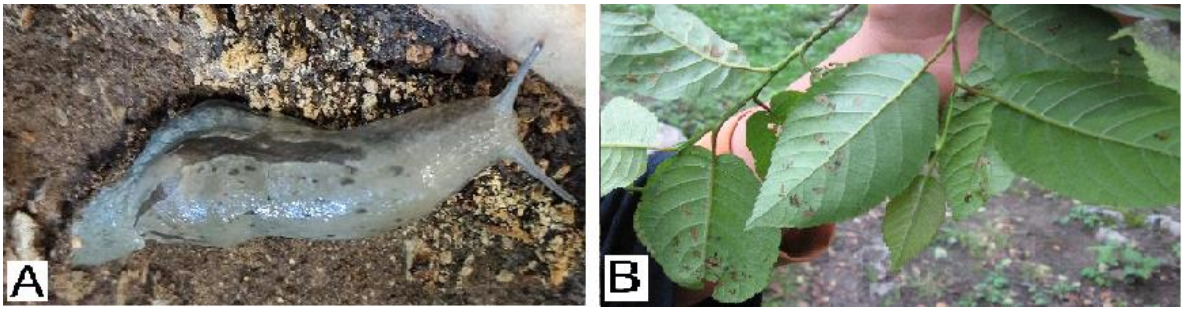

FIGURE 3. A) Adult slug *Drusia ibera*; B) Damage of *Drusia Ibera*, shredding and scrapping on the leaves of walnut trees.

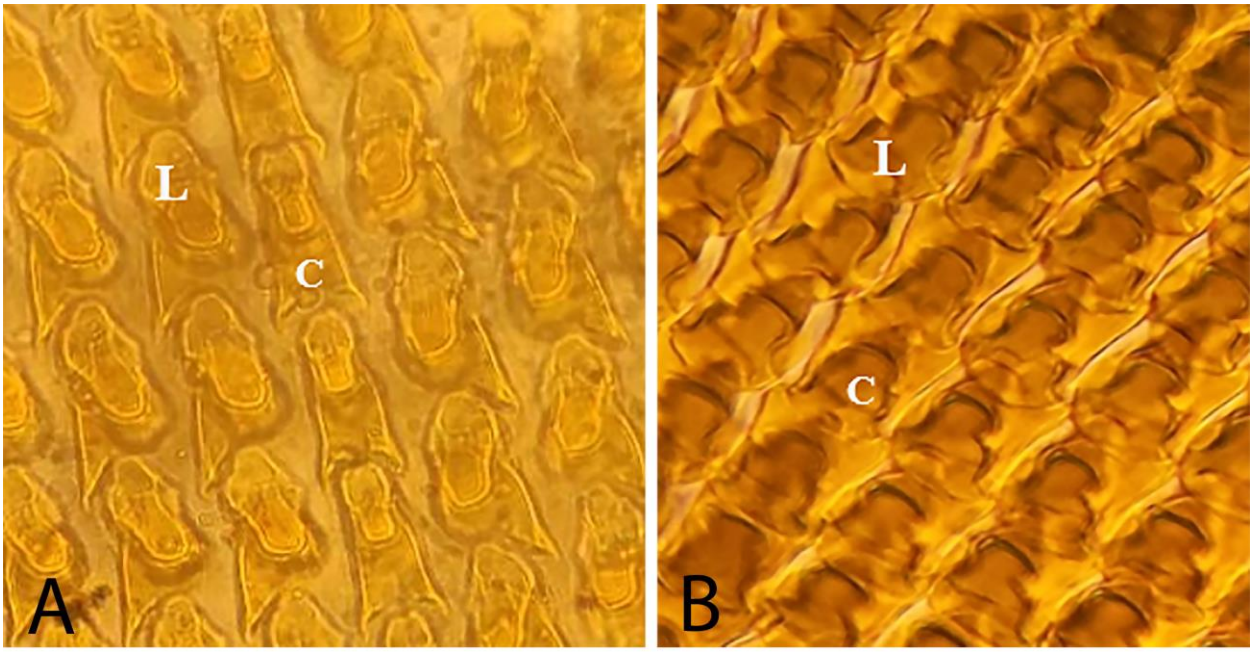

FIGURE 4. Radula teeth. A) *Caucasotachea lencoranea* x 100, B) *Drusia ibera* x 100. C= Central teeth, L= Lateral teeth.

The average length and width of radula teeth in *Caucasotachea lencoranea* from Roudehen station are: central (31.56±0.8µm, 15.01±0.65µm) lateral (31.72±1.1µm, 20.09±0.51µm) and marginal (22.20±1.20µm, 12.31±0.61µm.

The average length and width of radula teeth in *Caucasotachea lencoranea* from Targhian village station in the east of Tehran province are: central (31.20±0.50µm, 14.90±0.49µm) lateral (31.69±0.90µm, 19.98±0.49µm) and marginal (21.90±1.1µm, 12.29±0.70µm) radula teeth.

Drusia ibera had one central tooth, forty lateral teeth and 15 marginal teeth, that's mean that the radular formula is (1C+40L+15M) in 115 ± 2 tooth rows. The number of transverse rows of radula teeth is always greater than the longitudinal rows. The left and right parts of the radula are usually symmetrical. The central tooth (C) had a narrow cusp and two short and wide cusps. The length of the central tooth of the radula (C) in the samples from Roudehen station is 115.7±10.1 µm and its width is 60.2±3.9 µm and Targhian village station, it has a length of 115.5±9.1 µm and a width of 7 was 60.3 ± 3 µm, the lateral tooth (L) was larger and the mesocone was larger and had a cut. The ectocones of the lateral teeth, which were adjacent to the marginal teeth, were shorter.

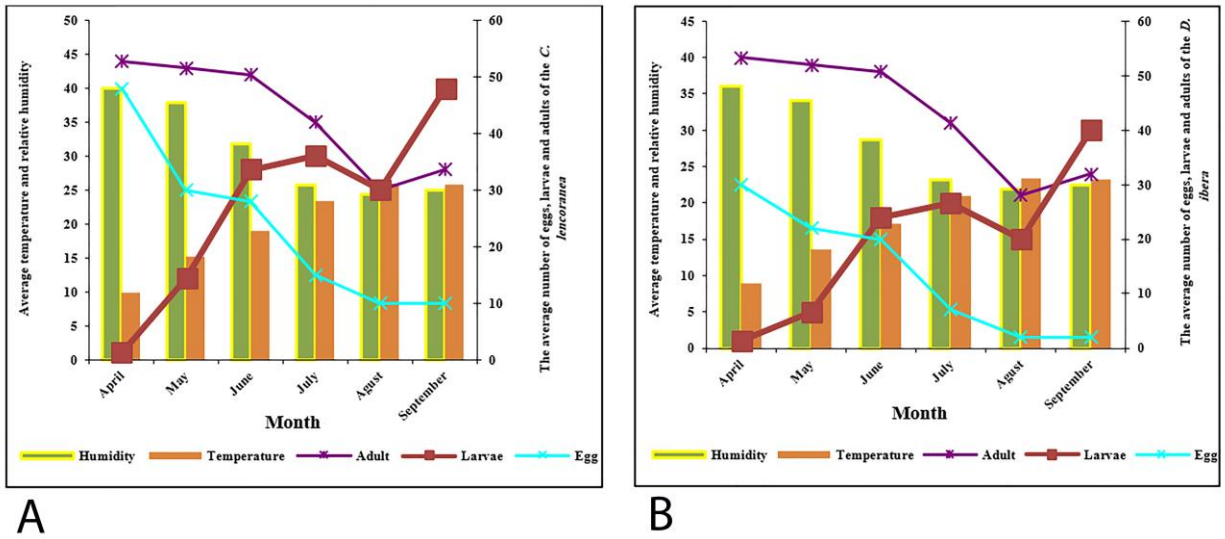

FIGURE 5. The average fluctuations of the population of mature, immature and eggs of *Drusia ibera* (A) and *Caucasotachea lencoranea* (B) with environmental factors, temperature and relative air humidity during the years 2021-2022 in Roudehen station of Tehran province.

Abundance of sampled species

The study of population fluctuations of mature and immature slugs *Drusia ibera* (Fig. 5A) and mature and immature snails *Caucasotachea lencoranea* (Fig. 5B) during the two seasons of spring and summer of 2021-2022 showed that there is a significant correlation between the temperature and relative humidity of the air and the number of samples caught. The monitoring of the population of the examined species showed that snails and adult snails and their eggs were frequent at April than other months. Immature snails and slugs were observed in greater numbers in September. During the hot air and low relative humidity in the summer season, mature snails and slugs are in aestivation. Their behaviour during this dormancy is quite different between species. Mature snails *Caucasotachea lencoranea* close their shell mouth (aperture) with a membrane of dried mucus, after settling at their place of dormancy. Adult slugs *Drusia ibera* produce extra slime, dig hole and bury themselves under leaves in the hole and minimize biological activities.

During the investigated seasons, there was a significant correlation between the changes in the population of life stages of *Caucasotachea lencoranea* snail species with the temperature factor.

The number of mature snails and eggs decreased uniformly with increasing air temperature. The correlation coefficient (R) calculated for these regressions shows that a positive and significant correlation was observed between the environmental factors, temperature and humidity, with changes density of eggs, immature and mature species of *Caucasotachea lencoranea*. The correlation coefficient (R) calculated with the temperature factor is 97, 81 and 80 (Egg: Slope=-1.89, R^2=0.97; Larva: Slope=1.62, R^2=0.81; Adult: Slope= -0.94, R^2=0.80) and with humidity 90, 77 and 80% (Egg: Slope=1.69, R^2=0.90; Larva: Slope= -1.48, R^2=0.77; Adult: Slope=0.88, R^2=0.80).

There was a significant correlation between the average density of eggs, immature and mature population of *Drusia ibera* species with temperature and relative humidity in spring and summer seasons at Roudehen station in the east of Tehran province (Egg: Slope=-1.49, R^2=0.96; Larva: Slope=5.05, R^2=0.80; Adult: Slope=-0.94, R^2=0.80). The correlation coefficient calculated for these regressions showed that the density changes of different biological stages of the pest, i.e. eggs, immature and matures, were correlated with temperature by 96, 80 and 80%, respectively. which means that at lower air temperature, the slug population was added. Also, the correlation coefficient (R) calculated for these regressions showed that the changes in density of eggs, immature and mature *Drusia ibera* with the

relative humidity factor were correlated by 93, 72 and 80%, respectively (Egg: Slope=1.37, R^2=0.93 Larva: Slope=-1.08, R^2=0.72; Adult: Slope=0.88, R^2=0.80).

DISCUSSION

Land snails and slugs are important agricultural pests and act as carriers and intermediate hosts of a large and important group of parasitic worms in medicine and veterinary medicine. They damage various agricultural and garden crops and are able to feed more than 40% of their body weight daily (Barker, 2002). Also, when they move, they create an unpleasant smell that prevents humans and even animals from eating contaminated plants and reduces the marketability of agricultural products (Sallam et al. 2009). In addition to damage to gardens and farms, by swallowing the eggs of parasitic worms and salmonella Gram-negative bacteria and disposing of their excrement, they play an important role in spreading them. On the other hand, by joining the slime secretions of snails and slugs and the movement of these molluscs on garden products (tree fruits) and agricultural products such as leafy vegetables, lettuce and strawberries, it causes their pollution. If there is no proper washing, it endangers human health (Godan, 1987). Millions of people in 90 countries suffer from diseases transmitted by molluscs (Lu *et al*., 2018). Snails and slugs lead to the death of tens of thousands of people every year. The World Health Organization has announced that the two diseases Fascioliasis and Dicrosliiasis, which are transmitted by snails and slugs, are among the most common parasitic diseases common to domestic animals, and both are included in the list of important human food infections by This organization is located (Kiani et al. 2021).

Most of the parasitic diseases that can be transmitted to humans are diseases transmitted through snails and slugs. Therefore, knowledge about the distribution of the population of snails and slugs is one of the basic requirements to deal with snails and raise the level of community health (Linnaeus, 1958).

In systematics of Molluscs, variations and arrangement of the teeth in radula are key characters of information to identify species or genera. Therefore, variations intraspecies in radula characteristics important (Malek, 1962; Pfleger, 1999; Demai tenon, 2004). Radula possess unique morphology and structure species of any mollusk (Yakhchali et al. 2012). In several studies, the importance of radula for the differentiation of different species has been shown (Coma, 2005; Pfenninger et al. 2006). For the first time, the current study used the morphological charateristics to differentiate different radula of *Caucasotachea lencoranea* and *Drusia ibera* in the samples collected from the walnut orchards of Targhian village and Roudehen city in the east of Tehran province during 2021 and 2022.

The radular shape and size are used to differentiate the genus or species of Mollusca (Franklin et al. 2007). The radula in each species of Mollusca has a unique morphology and structure (Yakhchali & Jamshidi Deillami, 2012). The characteristics the radula of most pulmonate species is very similar. All species possess a universal bauplan: base, stylus and cusp, the latter containing denticles. Radula have been categorized by the number, type and arrangement of teeth (Kruglov, 2005).

In the samples collected from *Caucasotachea lencoranea* and *Drusia ibera*, with the increase in relative humidity and moderate temperature, the number of eggs decreased and the number of soil molluscs increased along with the increase in shell length and body weight.

The results show air temperature and relative humidity have a significant effect on the dynamics and fertility of the population of snails and slugs of the species *Caucasotachea lencoranea and Drusia ibera*, Which is similar to results of the research Liew et al. 2021. Also, air temperature is effective in the decrease number of mature, immature and egg-laying species. In the summer season, high temperature has caused the studied species to fall asleep, which is consistent with the research of Kadry *et al*. (2018).

Knowledge of the population growth potential of *Caucasotachea lencoranea* and *Drusia ibera* species and their biology is also one of the basic and important elements in the integrated pest management program, which can be effective in detecting and monitoring pest infestations and growing useful crops. Comparison of the population density of eggs, larvae and adults of snails and slugs of *C. lencoranea* and *D. ibera* species on walnut trees in the east of Tehran province showed that the population snails and slugs are more in spring than in summer. Therefore, for appropriate and effective management of these pests, more attention should be paid to their life cycle. The findings of the present research can

provide useful information for designing a comprehensive integrated management program for the above mollusc species in Tehran province.

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