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Body mass and cuticular transpiration of the pulmonate slug *Milax rusticus* treated with spinach homogenate filtrate

G. Achuthan Nair[1], Nouara El-Azirag El-Ammari[2] and Saleh Salem Bushaiba[2]
[1]Deprtment of Environmental Sciences, University of Kerala, Kariavattom, Thiruvananthapuram-695 581, India
[2]Department of Zoology, Faculty of Science, University of Garyounis, Post Box: 9480, Benghazi, Libya

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Abstract: *The effects of four concentrations (25, 50, 75, 100%) of spinach homogenate filtrate over a period of 96 hours on the body mass and transpiration rates of the pulmonate slug Milax rusticus, a crop pest found in Benghazi, Libya, are reported. A decrease in body mass and transpiration rate in filtrate treated slug over time was evident and the differences in these factors between control and treated slug were significant ($p<0.01$). Tendencies for regaining lost weight and increase in transpiration were observed in the filtrate treated slugs during their seven days of recovery period in normal conditions. Application of spinach homogenate filtrate as a possible contact slugicide of M.rusticus is suggested.*

Key words: *Milax rusticus, Spinach homogenate filtrate, Body mass, Transpiration rate.*

Introduction

Milax rusticus (Millet, 1843) (Mollusca : Gastropoda, Milacidae) is widespread in Benghazi (32º 10'N, 20º 06'E) and its neighborhood in Libya (Nair *et al.*, 1996). These slugs appear on the soil surface during winter from November/ December until March/ April. They were found to feed on the leaves and the tender stems of a variety of crop plants causing extensive damage. Previously we observed that these slugs when exposed to spinach (*Spinacia oleracea*) homogenate filtrate, showed symptoms of irritation, stress, poor feeding and assimilation of food (Nair *et al.*, 1994). They become totally lethargic, movements become slow and the feeding rates are severely curtailed after exposure to spinach homogenate filtrate. Secretion of mucus from the body is also a common phenomenon. Their mortality rates were also high after five days of exposure in the filtrate. The present study investigated the effect of this filtrate on the body mass and transpiration rates of *M. rusticus,* (which are more pronounced in these slugs when compared with other parameters studied) under controlled laboratory conditions for 96hr, and later their recovery rates in normal conditions.

Materials and Methods

The homogenate was prepared by macerating thoroughly 2g of spinach leaf in 1ml distilled water in a blender. The contents were filtered and the filtrate was designated as 100%. The other concentrations (25, 50, 75%) of the filtrate were prepared by suitably diluting the 100% filtrate with distilled water.

Whatman filter papers (11cm diameter) and fresh lettuce (*Lactusa sativa*) leaves, which served as food for these animals, were dipped in 100, 75, 50, 25% of homogenate filtrates for 30 minutes and kept at the bottom of glass vessels (10.5cm length x 8.5cm width x 5.5cm height) and covered with lids. The filtrate was applied on the inner sides of the vessels and cover lids using a brush. *M. rusticus*, weighing 9.0 to 9.9g, were acclimatized in the laboratory for three days and were individually introduced to each vessel. Ten replicates of each homogenate filtrate concentration were prepared. Controls (0% filtrate) (10 replicates) were also set up where filter papers and lettuce leaves were dipped in rain water. The animals were kept in the dark at 15± 2ºC. The body mass and the transpiration rate of each animal were determined at 0 hr, and after 24, 48, 72 and 96hr of exposure. After 96hr, 5 animals each from the control and filtrate concentrations were weighed separately and kept at 55ºC in an oven for 96hr to determine their moisture contents. The remaining 5 slugs from each of the control and filtrate concentrations were transferred individually back to the vessels containing filter papers and lettuce leaves dipped in rain water for seven days in order to determine their recovery rates in body mass and transpiration rates.

The methodology used by Cloudsley-Thompson (1969); Nair *et al.,* (2001) to measure the transpiration rates of different species of woodlice were adopted in the present study. Each slug after measuring its body mass was exposed for one hour over calcium chloride before re-weighing. The result of transpiration was expressed as a $mg.cm^{-2}h^{-1}$, the surface area of the animal being calculated from the formula

$$S = kW^{2/3}$$

Where, S = surface area, W = initial weight of the animal, k = constant. Value of k was 192.4 in the study. To calculate k, the surface areas of a large number of specimens were initially calculated using Aub and DuBois (1917) formula

$$\text{Surface area} = wt^{0.425} \times ht^{0.725} \times 71.84$$

and later the surface area values were submitted in the formula

$$S = kW^{2/3}$$

and the k value had been tabulated.

Results and Discussion

There was a gradual increase in body mass of the control animals during the 96hr experimental period. However the slugs kept at different spinach filtrate concentrations showed a decrease in body mass during this period and this decrease

Table – 1: Body mass and transpiration rate of *Milax rusticus* treated with control and spinach homogenate filtrate.

Total animals: 10 each in control (0%) and in different filtrate concentrations

Time (hr)	Body mass (g)					Transpiration rate (mg. cm^{-2} h^{-1})				
	Concentration of filtrate (%)					Concentration of filtrate (%)				
	0	25	50	75	100	0	25	50	75	100
0	9.7	9.30	9.10	9.79	9.27	1.27	1.29	1.28	1.25	1.26
24	10.05	8.81	8.85	7.37	6.88	1.13	1.10	0.94	0.56	0.49
48	10.23	7.39	6.16	5.98	5.62	0.63	0.45	0.30	0.29	0.34
72	10.79	6.57	5.14	5.37	4.69	0.88	0.37	0.36	0.30	0.29
96	10.87	6.74	4.94	4.46	3.39	0.79	0.45	0.39	0.41	0.24
After 7 days of recovery	10.60	7.23	11.69	11.38	8.96	1.13	0.57	0.63	0.93	0.95

was more pronounced in them after 48hr of exposure (Table 1). A significant effect of different filtrate concentrations ($F=11.12$; $p<0.01$) and the time course of exposure (0-96hr) ($F=7.25$; $p<0.01$) on the body mass of *M. rusticus* was evident. The loss of body weight appeared to be due to extensive release of mucus at higher filtrate concentrations. During the seven days recovery period, the slugs regained weights remarkably well, and in those kept at 50 and 75% filtrate concentrations previously, the weight gains surpassed those of controls (Table 1).

A decrease in transpiration rate during the initial 48 hr exposure period was discernible in control and in 50% and 75% filtrate treated slugs. However, signs of increase in values of transpiration were evident in them after 72hr of exposure. On the other hand, the transpiration rates of 25% filtrate treated slugs began to decrease from 0 to 72hr with an increase thereafter, whereas a decrease in transpiration rate from 0 to 96hr was noted in those kept at 100% spinach filtrate. Time of exposure (0-96hr) ($F=35.25$;$p<0.01$) and different filtrate concentrations ($F= 6.75$; $p<0.01$) had significant impacts on the transpiration rates of *M. rusticus*. There were signs of reversal towards normal values of transpiration rates in slugs kept in different concentrations.

The moisture content of control slugs after 96hr was 91.59%, which decreased to 88.5%, 83.36%, 82.89% and 77.52% in those kept at 25, 50, 75 and 100% filtrate concentrations respectively. The differences in moisture between the control and filtrate treated *M. rusticus* were highly significant ($F= 98.10$; $p<0.01$). Spinach contains appreciable quantities of ascorbic acid, riboflavin and a small quantity of thiamine, it is also rich in iron, calcium and vitamin A, and calcium is thought to be unavailable as it reacts with oxalic acid to form calcium oxalate (Thompson and Kelly, 1957). The application of the filtrate of spinach homogenate as a possible contact pesticide either alone or after mixing with low concentrations of commonly used slugicides against *M. rusticus* could be investigated in detail. This will minimize the effects of harmful pesticide sprayings on the ecosystem since slugs are known to be powerful concentrators of organochlorine residues and organophosphorus compounds in their tissues before showing any toxic effects (Edwards and Thompson, 1973; Brown, 1978).

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Correspondence to:
Dr. G.A. Nair
Easwari Vilas, Sasthamangalam
Thiruvananthapuram – 695 010 (Kerala), India
E-mail: gachuthannair@yahoo.com
gachu@wmcmail.com
Tel.: +91-471-2721332, 2725726