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New Approaches to Improve the Attractiveness and Consumption of *Rattus norvegicus* for Forges Mixed with Grinded Land Snail, *Eobania vermiculata,* in Addition To 4-Ethyl Phenol Substance

[91]

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Abstract

This study carried out to clarify the effect of adding grinded *Eobania vermiculata* to improve the properties of forages used against different ages of *Rattus norvegicus* (immature males and females, mature males and females and pregnant and lactating females). Obtained results confirmed that this addition of ground snail had a clear effect on the attractiveness of the forages for all tested ages as the number of visits and the time spent next to the forages were increased. Different concentrations (10, 15 and 20%) of grinded *E. vermiculata* mixed with the formulated feed of *R. norvegicus*. Our results showed that there was a positive relationship between the increasing in concentration and the amount of forage consumed by all tested ages. The effect of 4-ethyl phenol and ground *E. vermiculata* on the properties of rat forages compared. The higher effect on attractiveness recorded for 4-ethyl phenol, but the mature male and female of *R. norvegicus* preferred feeding more on the snail forages. Formulated feed with 20% ground *E. vermiculata* mixed with 1 and 2.5% of 4-ethyl phenol. This led to positive results as the amount of food consumed increased compared to the control. However, when the concentration of this substance increased to 5% the feed consumption for male and female rats decreased. An analysis was made of some of the chemical components of *E. vermiculata*, and found that it contains large amounts of protein, carbohydrates, sugars and calcium.

Keywords: *Rattus norvegicus, Eobania vermiculata*, 4-ethyl phenol, Feed attractiveness, Food consumption, Chemical analysis.

1 Introduction

Rodents are among the most dangerous pests of mammalian vertebrates of the tribe of Chordates that exists in many environments. They infect various agricultural crops (Sarwar 2015), starting from the stage of seed and seedling cultivation to the whole plant, feeding on all parts of the plant causing great damage which at the end means heavy economic losses (Nils et al 2003, Desoky 2015 and 2018). In addition, they attack grain stores feeding on various types of grains, contaminating the stored grains with urine, feces and falling hair. Besides, these animals are considered important medical and veterinary pests, as they attack many agricultural animals, causing the transmission of many diseases to them, which may lead to death, and the most dangerous is their ability to transmit the most dangerous and deadly diseases to humans such as the plague (Thomas 1993, Grant et al 2003, Hamidi 2018). Many methods used to combat these pests, including cultural, mechanical and chemical methods. However, the most common is the use of good poison bait. For poison baits to be good and effective they should contain the following characters: forage taste should be acceptable to the majority of rat community, rats should eat the amount lead to their death and rats should prefer this specific food more than other foods in their environment. Therefore, some substances are usually added to the

original bait to increase its preference, acceptability, attracteness and finally its effectiveness (Jackson et al 2015, Rady et al 2015).

The mollusk tribe includes nearly 85,000 species around the world, including aquatic, terrestrial and amphibious animals. Terrestrial mollusks cause many injuries to different plants and their different parts (Zala et al 2018). Recently, land snails have become one of the most important invertebrate pests in Egypt, that affect many crops, causing great damage either by feeding or by leaving mucous material on the plant, which increases the severity of infection. These organisms are used in various fields that are very beneficial to humans and other animals such as medicine (Paulo et al 2015, Matusiewicz et al 2018), treat water pollution (Udeozor and Evbuomwan 2014, Ambali et al 2015), industry (Genevive and Isaac 2011, Adeosun et al 2015, Orodu et al 2015, Mohamed 2016) and food source for many other animals (Nahid et al 2013, Rabha et al 2014, Kadir et al 2019, Benno and Rochow 2019, Das 2020) as their bodies are rich in nutrients important for growth (Gomot 1998, Sogbesan et al 2006).

The aim of this research is to exploit the beneficial aspect of land snails to improve the efficiency of rat forages by adding grinded *Eobania vermiculata* to *Rattus norvegicus* formulated feed, which may lead to an increase in the attractiveness and amount of consumed bait.

2 Materials and Methods

This work was carried out in the Plant Protection Department, Faculty of Agriculture, Benha University.

2.1 Preparing *Rattus norvegicus* in the laboratory

Fifteen healthy adults of *Rattus norvegicus* (five males and ten females) were collected from nearby fields and transported to the laboratory where managed and maintained under good hygienic conditions for two weeks prior to the experiment. The animals were housed in metallic cages (five cages, every cage contained one male and two females), and given a reference number, then supplied with enough food which contain of 21% protein, 4.59% fat and 4.20% fiber, this food was purchased from Cairo Company for animal feed, the water was available continuously in clean taps **Fig 1**. Animals observed daily until mating. Pregnant females separated in other cages to take care of their pups from birth until weaning.

2.2 *Eobania vermiculata* Snail body grinded Preparation

Many individuals from land snail *Eobania vermiculata,* from different plants in different places, were collected and transported, in cloth bags to the animal laboratory of the Plant Protection Department at the Faculty of Agriculture, Benha University. Snails then washed in strainers with running water to clean them and put in large plastic basins feeding on lettuce for seven days before grinding. Healthy adult individuals selected and placed in paper bags into an electric drying oven at 70°C for 72 hours, then milled by hand well, and then sterilized under 121°C with 1.5 bars, for 20 minutes. The dry grinded snail bodies kept in the refrigerator until used. Grinded Parts of *E. vermicul*ata sent to the analysis unit in the research laboratories complex of the Faculty of Agriculture, Benha University, to analyze the most important nutrients in it.

Fig 1. *R. norvegicus* breeding cages

2.3 Effect of grinded *E. vermiculata* body on the attractiveness of *R. norvegicus*

E. vermiculata grinded body was tested using Y-maze apparatus **Fig 2**, measuring 150 x 15 x 15cm, with three arms (main arm 80 x 15 cm and the two passage choice arms 1 and 2 each 75 x 15 cm), arms made metal sheets, while the top side made of wire mesh (El-Bath 2015, 2019). The tested individuals of rats were immature males and females, mature males and females, pregnant and lactating females. The grinded snails were put in passage 1 (P1) and rat formulated feed in passage 2 (P2). Each stage of *R. norvegicus* placed separately in the main arm and given the opportunity to enter any of the two choice passages. Numbers of visits and the time spent in each of the two test arms were measured during the 15 minutes test duration. This experiment repeated three times for each stage by a new individual to avoid adaptation.

2.4 Effect of mixing grinded *E. vermiculata* and re-formulated feed on food consumption

Grinded *E. vermiculata* was tested in different concentrations using 45, 42.5 and 40 g of rat formulated feed were separately mixed with 5, 7.5 and 10 g of grinded snail and put in plastic container and efficiently mixed to prepare 10, 15 and 20% of mixed foods, respectively. The tested *R. norvegicus* stages were immature males and females, mature males and females, pregnant and lactating females. 50 g from untreated food put in one corner of the rat cage and another 50 g from treated food were put in the opposite corner for 24 hours (three cages were used as replicates for each stage). The daily food intake estimated per 100 g of each rat body weight.

Fig 2. Y-maze apparatus

2.5 A comparison between the effect of grinded *E. vermiculata* and 4-ethyle phenol on the efficiency of *R. norvegicus* forages

The first part of the experiment done using a Y-maze apparatus, to compare the rat's response to the ground snail compared with 4-ethyl phenol. A glass slide was used and dipped in 4-ethyl phenol substance then placed in one arm (El-Bath 2019); while in the other arm 5 g of pure dry grinded *E. vermiculata* was placed. The number of visits and the time spent for each visit next to each transaction counted during the 15 minutes test period. The experiment repeated 3 times with new individuals of both mature *R. norvegicus* males and females.

After that, forages with a concentration of 20% 4-ethyl phenol and 20% of grinded *E. vermiculata* were separately prepared to compare the effect of each on the amount consumed of food. 10 ml of 4-ethyl phenol to 50 g of formulated food mixed well to prepare forage with 20% of the substance. On the other hand, 10 g of grinded *E. vermiculata* mixed with 40 g of the plain food to prepare forage with 20% of grinded snail. The transactions placed in a test cage next to 50 g of pure regular food as control in front of one adult of *R. norvegicus* males and females for 24 hours to calculate the amount of food consumed. This experiment repeated 3 times with three individuals for each sex.

2.6 Effect of grinded *E. vermiculata* and 4-ethyl phenol together on food consumption

To complete this experiment, six healthy and adult individuals (3 males and 3 females) of *R. norvegicus* selected. 0.5, 1.25 and 2.5 mL of 4-ethyl phenol substance were added to 50 g of pre-prepared grinded *E. vermiculata* forage at a concentration of 20% of it and mixed well to make a final forages containing 20% grinded *E. vermiculata* and (1, 2.5 and 5% 4-ethyl phenol), respectively. Three cages used for each sex separately, and each cage contained only one individual. With each sex, three replicates made for each concentration, along with three replicates of the control (formulated food only). The amount of food consumed calculated as mentioned above.

2.7 Statistical analysis

The Statistical analysis carried out using ANOVA with two factors under significance level of 0.05 for the whole results using SPSS (ver. 22). Data treated as complete randomization design according to (Steel et al 1997). Multiple comparisons carried out applying LSD.

3 Results and Discussion

3.1 Effect of ground *E. vermiculata* on the attractiveness of *R. norvegicus* food

The results in **Table 1** showed the effect of using land snail *E. vermiculata* ground body to improve the attractiveness of rat forages. They indicated significant difference between ground snail forage and control. All stages of *R. norvegicus;* immature male or female, mature male or female, pregnant and lactating female; found attracted significantly to ground *E. vermiculata* snail forage. This was indicated by the increase in the number of visits to forages (6.67, 7.00, 6.33, 6.33, 6.00 and 6.00), and the increase in the time per visit (67.78, 62.08, 77.78, 60.76, 63.30 and 83.51) seconds. As well as theincrease in the total time for all visits per each rat stage (446.67, 433.33, 490.00, 381.67, 375.00 and 493.33) seconds, respectively, compared to the visits to the formulated feed. It is also evident from the results that immature male and female stages were the most attracted to ground *E. vermiculata* compared to the rest rat stages. However, the highest time per visit and total time for all visits registered by lactating females and mature males, followed by immature males.

In general, the previous results cleared that ground *E. vermiculata* snail had a great effect on the attraction of all ages of *R. norvegicus* rat, and this might be due to the smell emanating from sail grinded body.

Many types of food as vanilla, chocolate, peanuts and hazelnuts used in *Mus musculus*, *Rattus norvegicus* and *Rattus rattus* baits to increase bait attractiveness. Our results showed that the flavors of these foods might increase the attractiveness, but they reduced the feeding of bait for *M. musculus*, and for the other two types, they avoided the attraction to these materials (Schmolz and Kalle 2010). Phenol, phenol-2-ethyl-(CAS), phenol-3-ethyle, 4-ethyl phenol, 2-Cyclohexen-1-ol-3-methyle, 5-Octadecenal, Octatecane-5methyl and 4-azidoheptane were also used to test how they might increase the attractiveness of *M. musculus* and *R. norvegicus* forages, from the previous chemical compounds 4-ethyl phenol was the most attractive to males and females of both species (El-Bath 2019).

3.2 Effect of ground *E. vermiculata* on food consumption of *R. norvegicus* forage

Data in **Table 2** clarified the extent of the additive effect of ground *E. vermiculata* to *R. norvegicus* formulated feed. Results indicated that all tested stages of *R. norvegicus* (immature male and female, mature male and female, pregnant and lactating female) had shown to prefer ground *E. vermiculata* forages where they ate most of it when compared to control. In addition, it turns out that, there was a positive relationship between the increase in the percentage of grinded *E. vermiculata* in the forage and the amount consumed of it for all tested stages. As well as the most amounts of forages were eaten by immature male and female under all tested concentrations, especially 20% with [(18.33, 20.60, 28.17) and (21.67, 23.67 and 25.87) g], respectively, compared to other stages of rats.

Finally, the high consumption of *E. vermiculata* forage , by *R. norvegicus,* might be due to their high contents of important organic elements **Fig 3** such as 23.44% proteins, 14.93% carbohydrates, 8.01% sugars, 3.48% fats and some other important elements as 8.51% calcium (Ca), 3.33% Potassium (K), 0.65% phosphorus (P), 0.43% sodium (Na), 0.25% magnesium (Mg), 0.11% iron (Fe), 0.05% manganese (Mn), 0.02% zinc (Zn) in this ground land sail body, which had an effective role in building the body of a living organism, and leads to the attraction of animals to feed to benefit from them.

Other species of snails such as *Helix pomatia*, *Cornu aspesa maxima* and *Cornu aspersum aspersum* snails also used as main protein sources in growing rat's tissues (Radzki et al 2017). The garden snail, *Limicolaria aurora,* used as protein source in meat meal of *Clarias gariepinus* fish feeds (Sogbesan et al 2006). Land snails are preyed by mice and birds in nature, especially large individuals by 36.8% and 26.5% especially *Helix pomatia* and *Cepaea nemoralis* species (Rosin et al 2011). The bodies of land snails very rich in protein and many minerals, essential amino acids other than methionine, cysteine, taurine and possibly tryptophan usually abundant and the amount of unsaturated fatty acids reaches 50% or more of the total fatty acids witch are very important for the growth of living organisms (Fagbuaro et al 2006, Engmann et al 2013, Benno and Rochow 2019).

Table 1. Effect of *E. vermiculata* snail ground on attractiveness of *R. norvegicus* rat under laboratory conditions.

R. norvegicus **stage**	**Av. number of visit times (N.V)**		**Av. time per visit (second) (T/V)**		**Average total time (second) (T.T)**	
	P1 ***E. vermiculata***	**P2 formulated feed**	**P1** ***E. vermiculata***	**P2 formulated feed**	**P1** ***E. vermiculata***	**P2 formulated feed**
Immature male	6.67±0.33^{aA}	6.00±0.58 ^{aB}	67.78±9.22^{bA}	26.79±0.66^{bB}	446.67±43.72^{bA}	160.00±11.55^{abB}
Immature female	7.00±0.58^{aA}	6.33±0.33 ^{aB}	62.08±1.50^{bA}	30.44±1.80^{abB}	433.33±29.63^{bA}	191.67±4.41^{aB}
Mature male	6.33±0.33^{aA}	4.00±0.58^{bB}	77.78±3.92^{aA}	38.33±3.33^{aB}	490.00±2.89^{aA}	150.00±12.58^{bB}
Mature female	6.33±0.33^{aA}	4.67±0.33^{bB}	60.76±5.14^{bA}	39.83±1.36^{aB}	381.67±16.91^{cA}	185.00±7.64^{abB}
Pregnant female	6.00±0.58^{aA}	4.00±0.58^{bB}	63.30±4.85^{bA}	39.42±4.12^{aB}	375.00±18.03^{cA}	155.00±18.93^{abB}
Lactating female	6.00±0.58^{aA}	4.67±0.67^{bB}	83.51±6.74^{aA}	37.50±3.82^{aB}	493.33±8.82^{aA}	171.67±16.41^{abB}
LSD at 0.05 for	Stage (s)		P		S*P	
N.V	1.03		0.60		1.46	
T/V	39.90		23.03		56.42	
T.T	9.35		5.40		13.22	

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

Table 2. Impact of mixing ground *E. vermiculata* snail and formulated feed on food consumption by different *R. norvegicus* stages under laboratory conditions.

Stage / **Snail grinded %**	**Food consumption (g)**					
	immature males	**immature females**	**mature males**	**mature females**	**pregnant females**	**Lactating female**
10 %	18.33±0.88^{b}	21.67±0.88^{b}	12.73±0.15^{b}	11.57±0.07^{b}	10.80±0.15^{c}	14.13±0.19^{b}
15 %	20.60±1.14^{b}	23.67±0.67^{b}	13.23±0.15^{b}	12.57±0.07^{a}	12.03±0.32^{b}	14.30±0.15^{ab}
20 %	28.17±2.24^{a}	25.87±0.13^{a}	14.70±0.15^{a}	12.87±0.13^{a}	13.40±0.56^{a}	15.17±0.44^{a}
Control	10.33±1.20^{c}	9.17±0.60^{c}	9.57±0.30^{c}	9.40±0.21^{c}	9.10±0.21^{d}	10.17±0.60^{c}
LSD at 0.05 for	4.77	2.06	0.64	0.43	1.13	1.28

a,b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

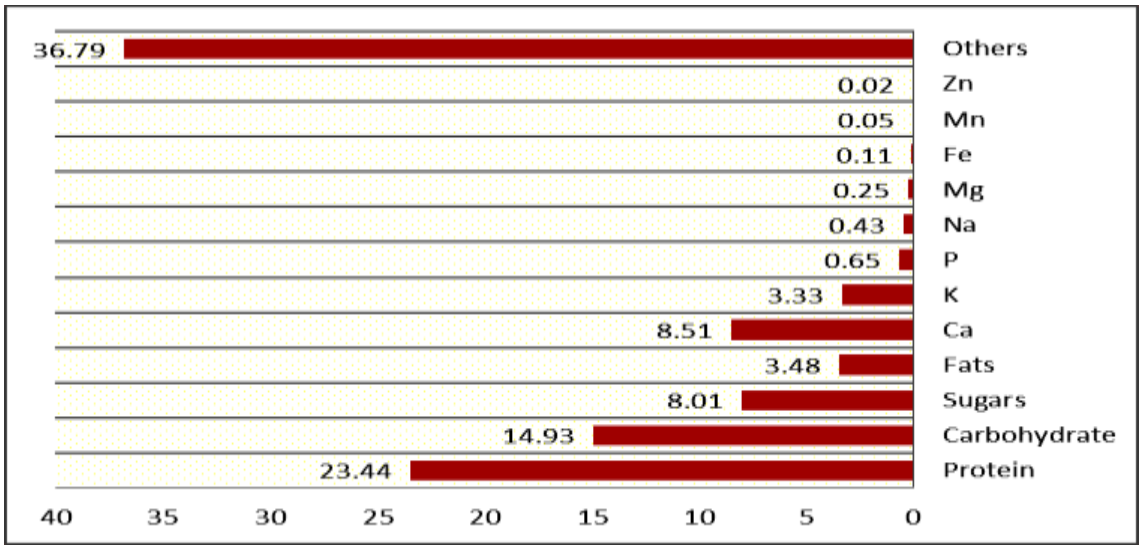

Fig 3. The percent's main elements of grinded *E. vermiculata* body

3.3 Comparison between grounded *E. vermiculata* and 4-ethyl phenol substance in rat forage

The results in **Table 3 and Figs 4, 5 and** 6 explained the comparison between the addition of both ground *E. vermiculata* and 4-ethyl phenol in the formulated feed and the effect of each on forage attractiveness, as well as the amount of food consumption by mature male and female stages of *R. norvegicus.* The results confirmed that 4-ethyl phenol had the largest effect on the attractiveness of both males and females, **Figs 4 and 5**, especially females that had visited the forage for 7.33 times **Fig 4** and spent around 69.38 seconds per visit **Fig 5**. Concerning the amount of food consumption of forage, data in the same **Table and Fig 6** confirmed that both mature males and females preferred to feed on a greater quantity of ground *E. vermiculata* forage compared to 4-ethyl phenol forage when they were added to formulate feed with means (15.47 and 12.60 g) and (9.03 and 7.97g) respectively.

3.4 Effect of mixing *E. vermiculata* grinded body with 4-ethyl phenol substance on food consumption

The results that listed in **Table 4 and Fig 7** prove that there was a clear effect due to combining *E. vermiculata* with 4-ethyl phenol substance on the amount of forage consumed from compared to control by both sexes. For male rats, there were no statistically significant differences between the amount consumed from the snail forage with concentrations of 1 and 2.5% of 4-ethyl phenol, as it was 14.69 and 14.49 g for the two concentrations, respectively. But when 4-ethyl phenol ratio was increased to 5% and mixed with *E. vermiculata* forage, the amount consumed from the food decreased up to 11.28 g. But for *R. norvegicus* females, the largest amount of food consumed (12.09 g) was recorded with a concentration of 2.5%, but also this amount decreased up to 10.53 g with 20% snail ground and 5% 4-ethyl phenol forage.

Generally, it is clear from our results that mixing the ground land snail with 4-ethyl phenol in the rat foods did not prevent the rat from feeding on them, so it is better to mix 4-ethyl phenol in small proportions, because with high proportions of phenol with grinded snail, this leads to a decrease in the amount of food consumption.

Many scientific experiments had done to improve the attractiveness of rat forages by using different sources of pheromones, urine as a famous source of pheromones had a clear effect on stimulating and attracting adult male and female individuals, as well as on the amount of food consumed (El-Bath 2015). Selvaraj and Archunan 2006 proved that the use of some pheromones extracted from some glands of *Rattus rattus* as well as urine had a

positive effect on increasing the acceptance of the toxic baits as well as increasing the amount consumed by males and females, which led to an increase in the mortality rates among individuals. The use of vanilla powder in *Mus musculus, Rattus norvegicus* and *Rattus rattus* baits had a positive and effective effect on the amount of food consumed, while chocolate powder showed a negative effect on attraction and consumption (Schmolz and Kalle 2010).

Table 3. A comparison between grinded *E. vermiculata* and 4-ethyl phenol in formulated feed and its effect on attractiveness and food consumption by *R. norvegicus* under laboratory conditions.

Stage	Food attractiveness				Stage	Food consumption (g)		
	E. vermiculata		4-ethyl phenol			*E. vermiculata*	4-ethyl phenol	Control
	N.V	T/V	N.V	T/V				
Mature male	5.33±0.33^{a}	51.17±3.06^{a}	6.67±0.33^{a}	49.18±1.62^{b}	Mature male	15.47±0.52^{aA}	9.03±0.87^{aB}	5.77±0.62^{aC}
Mature female	4.00±000^{b}	36.55±1.90^{b}	7.33±0.33^{a}	69.38±3.48^{a}	Mature female	12.60±0.97^{bA}	7.97±0.32^{bB}	5.13±0.52^{aC}
LSD at 0.05	0.93	10.00	1.31	10.67	LSD at 0.05 for	Stage (S)	Treatment (T)	S*T
						1.20	1.47	2.08

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

Table 4. Effect of merge grinded *E. vermiculata* and 4-ethyl phenol substance in the formulated food on the food consumption.

R. norvegicus sex	Food consumption of *E. vermiculata* forages			
	4-ethyl phenol concentrations			
	1.0 %	2.5 %	5.0 %	control
Male	14.69±1.21^{aA}	14.49±0.19^{aA}	11.28±0.53^{aB}	6.43±0.56^{a}
Female	11.32±0.50^{bAB}	12.09±0.33^{bA}	10.53±0.40^{aB}	5.18±0.24^{b}
LSD at 0.05 for	Sex (S)	Treatment (T)	S*T	-
	0.87	1.22	1.73	-

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter

Effect of *E. vermiculata* grinded and 4-ethyl phenol on the attractiveness

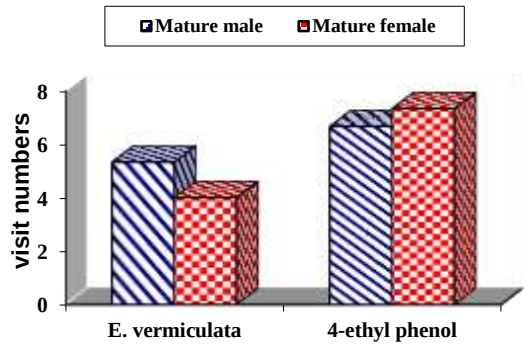

Fig 4. Visits number for forages by *R. norvegicus* (N.V)

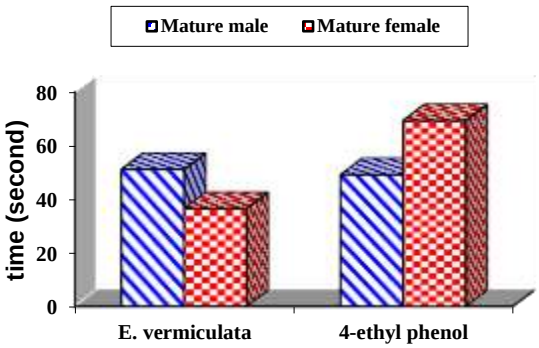

Fig 5. Time spent by *R. norvegicus* next to the forage (T/V)

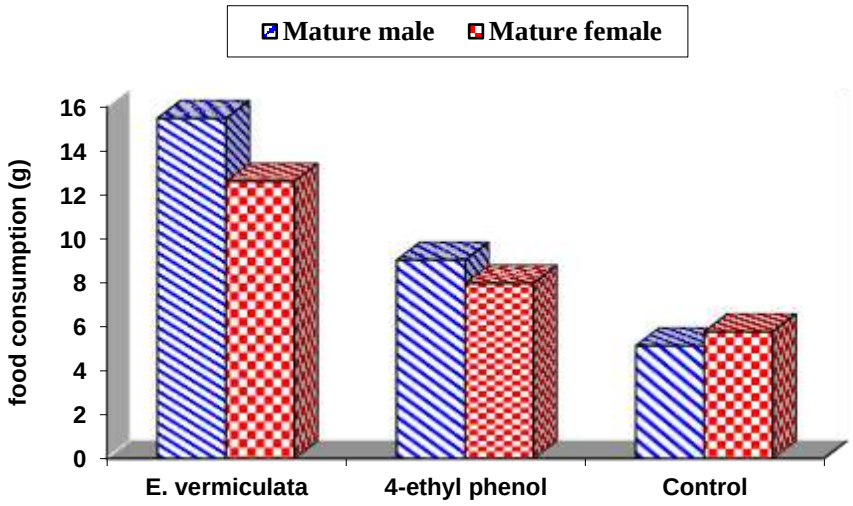

Fig 6. Food consumption of *E. vermiculata* and 4-ethyl phenol forages by *R. norvegicus*.

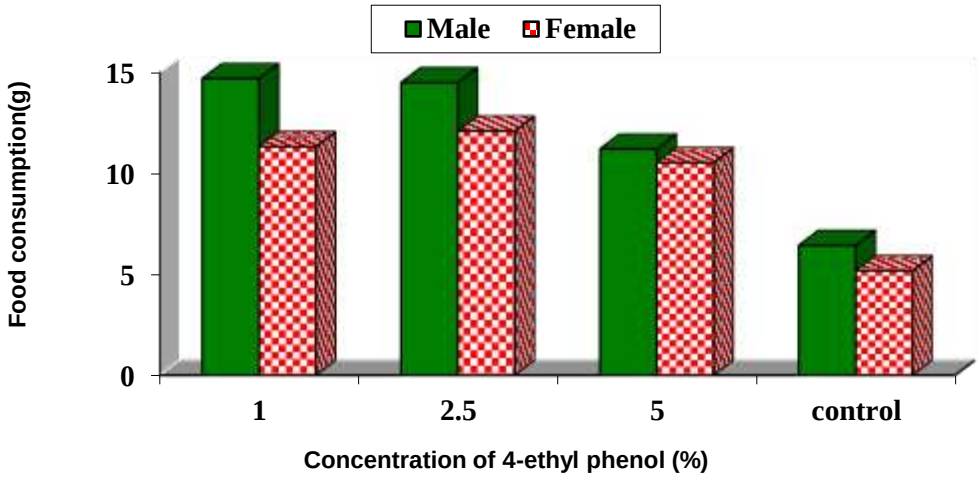

Fig 7. Effect of merge grinded *E. vermiculata* and 4-ethyl phenol on the food consumption.

4 Conclusion

Grinded body of Land snail, *E. vermiculata,* proven to have an effective role in improving the qualities of rat forages, i.e. the attractiveness and the food consumption. Results indicated that 4-ethyl phenol had the highest effect in attracting, while ground snail had the most effect on food consumption when they compared to each other. When grinded snail mixed with 4-ethyle phenol, it found that the amount of food consumed from forage was almost the same as the grinded snail forage alone. Besides that, 4-ethyl phenol is an expensive and difficult substance to obtain. So from the economic point of view, it could be said that using of *E. vermiculata* grinded body alone in forages is the best, as it can be obtained easily, inexpensive, and gives a very good results for the attributes of rat's baits.

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الأتجاهات الحديثة لتحسين جاذبية وأستهلاك *Rattus norvegicus* للأعلاف بأستخدام مطحون القوقع الأرضى *Eobania vermiculata* و مادة 4-ethyl phenol

[91]

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الموجـــــــــز

أجريت هذه الدراسة لتوضيح تأثير إضافة مطحون القوقع الأرضى *Eobania vermiculata* لتحسين خصائص العلف المستخدم ضد أعمار مختلفة من *Rattus norvegicus* (ذكور وإناث غير ناضجة و ناضجة.، إناث حوامل ومرضعات). وقد أكدت النتائج أن إضافة القوقع الأرضي كان له تأثير فعال على جاذبية العلف لجميع الأعمار المختبرة حيث زادت عدد الزيارات والوقت الذي يقضيه بجانب الطعام. تم خلط تركيزات مختلفة (10 ، 15 و 20٪) من *E. vermiculata* مع علف *R. norvegicus*. وقد أظهرت النتائج زيادة الكمية المستهلكة منه, كما أتضح وجود علاقة ايجابية بين زيادة التركيز وكمية الطعام المستهلك لجميع الأعمار, وعند مقارنة تأثير مادة 4-ethyl phenol و مطحون *E. vermiculata* على خواص العلف, فقد سجل التأثير الأعلى على الجاذبية لمادة 4-ethyl phenol، ولكن الأستهلاك الأعلى من الطعام بواسطة ذكور وإناث *R. norvegicus* الناضجين فكانت لصالح العلف المصنع من *E. vermiculata*. ثم تم خلط علف مجهز بنسبة 20٪ من مطحون *E. vermiculata* مع 1 و 2,5٪ من 4-ethyl phenol، وكانت النتائج إيجابية حيث زادت كمية الطعام المستهلكة مقارنة بالكنترول، ولكن عند زيادة تركيز المادة إلى 5٪ انخفض استهلاك الطعام للذكور والإناث على حد سواء. تم اجراء تحليل كيميائى لجسم القوقع الأرضى *E. vermiculata*, وقد تبين احتواءه على كميات كبيرة من البروتين والكربوهيدرات والسكريات والكالسيوم.