Effect of Clove Plant Extract on Land Slug and Their Reproductive System underLaboratory and Field Conditions.

Soha A. Mobarak; Randa A. Kandil and SamahM.AbdEl-Kader

Plant ProtectionResearch Institute, ARC., Giza Egypt.
Soha_snails@yahoo.com

Abstract: Effect of clove (*Syzygium aromaticum*) plant extract was studied against land slug, *Limax flavus*, under laboratory and field conditions. Slugs were treated with different concentrations of plant extract as a contact for one week and LC_{50} value was calculated. Result indicated that plant extracted by ethanol more toxic than plant extracted by acetone. The pathological changes of reproductive system were studied on the treated animals. Results revealed that the clove plant extracted by ethanol caused severe swelling in the size of the organs of the reproductive system more than plant extracted by acetone. Also, the effect of $LC_{50}$of each plant extract on the nervous tissue enzymes acetylcholine esterase (AchE) and alkaline phosphatase (ALP) of slugs was studied after 7 days of treatment. Results showed that AchE and ALP activity decreased after 3 days of treatment by acetone extract while they increased after 7 days post treatment. Vis-versa occurred in case of ethanol plant extract whereas it enhanced the level of ALP post 3 and 7 days of treatment. While it caused fluctuate effect in case of AchE after the both periods of treatment. On the other hand, the performance of ethanolic extract (which gave good results in laboratory) was evaluated under the field conditions. The plant extract was tested as a spray comparing with methomyl (the recommended compound). The results indicated that the crude plant extract gave satisfying results compared with methomyl whereas it gave 74.5% population reduction comparing with 94.4% for methomyl, respectively. Finally, we can conclude that clove plant extract by ethanol was achieved good results to control the slugs. It has high toxic effect against slugs in addition the teratogenic effects on reproductive system on the surviving individuals leading to prevent the slug to produce eggs.

[Soha A. Mobarak; Randa A. Kandil and Samah M.AbdEl-Kader. **Effect of Clove Plant Extract on Land Slug and Their Reproductive System Under Laboratory and Field Conditions.***J Am Sci*2015;11(12):250-255]. (ISSN: 1545-1003). http://www.jofamericanscience.org.32. doi:10.7537/marsjas111215.32.

Key words: Clove extract, (*Syzygium aromaticum*) - Land slug, (*Limax flavus*)- reproductive system - nervous tissue enzymes.

1. Introduction

Recently the land slugs have been injurious pests to agriculture and horticulture in Egypt (Soha Mobarak and Randa Kandil, 2014). They fed on seeds and damage different parts of plants (Briner and Frank, 1998). Land mollusca were controlled by chemical molluscicides. These chemical compounds may lead to problems of toxicity to non- target organisms and caused environmental pollution (Gabr *et al*., 2006). Thereby, the present work was conducted to study the molluscicidal effect of one natural compound clove oil (plant extract) when used as a contact against land slug, *Limax flavus*, one of the harmful species in Egypt under laboratory and field conditions. So the aim of this work is to study the following points:

1- The effect of clove plant extracted by ethanol and acetone on land slug *Limax flavous*.

2- Pathological changes on the reproductive system (hermaphrodite) of slugs after treatment by both extracts.

3- The impact of the tested extracts on nervous tissue enzymes AchE and ALP.

4- Evaluate the efficiency of crude plant extract against slugs under field conditions comparing with methomyl (the recommended compound).

2. MaterialandMethods

1. Tested Animals:

The individuals of slug *Limax flavus* were collected from Abu- Rawash area, Giza Governorate and transported to the laboratory. Animals were kept in a small plastic boxes containing 8-10 cm moist soil provided with fresh green lettuce leaves, covered with muslin secured with rubber band to prevent slugs from escaping and kept under 20±2 ˚c in laboratory (Maha Fouad, 2005).

2. Preparation of Crude Extract:

Dry flowers of clove (*Syzygium aromaticum*) were ground in grinder to obtain a fine powder. Then about 2kg of powder were extracted by 3 liters of ethyl alcohol 95% using homogenizer. The extract was collected through anhydrous sodium sulphate filter and evaporated to dryness under reduced pressure by a rotary vacuum evaporator of a water bath adjusted at 37 ˚c. The crude extracts were weighted and kept in deep freezer until use. This

process was repeated with acetone (Freedman *et al.*, 1979).

***4*.Bioassay.**

Contact technique (thin layer film) was used according to Asher and Mirian (1981). Different concentrations of the tested extract, two ml of each concentrations was spread on inner surface of Petri-dish by moving the dish gently in circles. Solvent was evaporated under room condition in a few minutes leaving a thin layer film of extract on surface of Petri dish. Animals were exposed to the candidate concentrations for one week in Petri dish. A parallel standard test was conducted using ethanol and acetone. Also, a parallel control test was conducted using plain water. The killed animals were daily counted and mortality percentage was calculated after one week LC_{50} was determined according to Finney (1971).

***5.* Anatomical studies:**

Slugs were treated with LC_{50} of each extract. The treated slugs were anaesthetelized in 1% solution of chloral hydrate for 12 hours (Shoieb, 1997). Reproductive system of the treated slugs were carefully removed with using binocular stereo microscope then photographed and compared with those of untreated.

***6.* Biochemical studies:**

Effect of $LC_{50}$of extract compound on nervous tissue enzymes were determined to clarify the physiological impact on *Limax flavus* slug. Acetylcholine esterase (AchE) and Alkaline phosphatase (ALP) were measured after 3 and 7 days of treatment. A parallel control test was conducted.

Sample preparation:

After 3 and 7 days of treatment, the treated animals of slug *Limax flavus* were washed with water and the nervous tissue was dissected from the buccal mass and then used for measurement of enzyme activity. Nearly 12- 15 slugs were dissected to get 50 mg of nervous tissue for 5 minutes with 1.0 µ phosphate buffer pH = 8 at 4 °c and centrifuged (1000 r.p.m.) for 30 min. The following procedure was used according to Bergmeyer (1963) modified by Singh and Agarwal (1989).The supernatant was used as an enzyme source.

***6.1.* Determination of acetylcholine esterase (AchE):**

AchE was determined according to Ellman *et al.*(1961) the principle of the test depends on the following reaction:

Acetylcholine $\xrightarrow{\text{Acetylcholinesterase}}$ thocholine + acetate

Thiocholine + dithiobisnitro benzoate $\longrightarrow$ 2- nitro- 5- mercaptobenzoate (yellow color).

Calculation: concentration of AchE U/L = 23460× ΔE/ 30 sec.

***6.2.* Determination of Alkaline phosphatase (ALP) activity:**

Tissue homogenate was prepared in ice cold 0.9% Nacl and centrifuged (5000 r. p. m.) for 15 min., at 4°c the supernatant was used as an enzyme source. ALP activity was determined using phenyl phosphate by the method described by king and kind (1954). The developing of color was done according to the following reaction:

Phenyl phosphate $\xrightarrow{\text{alkaline phosphatase}}$ phenyl + phosphatase

Calculation:

$$\text{Concentration of ALP U/L} = \frac{\text{Sample}}{\text{Standard}} \times 75.$$

Statistical Analysis:

The obtained results were statistically analyzed by one way ANOVA and Least significant difference (LSD) at ($P< 0.05$) Costat program (Cohort, 2005).

***7.* Field Experiments:**

Clove (*Syzygium aromaticum*)plant extracts and methomyl (the recommended compound) were tested against *Limax flavus* land slug under semi field conditions. The efficacy of each compound was based on the reduction of slug population after 7 days of treatment according to the formula of Henderson and Tilton (1952).

The field experiments were set up in small plots in an experimental area at Giza Governorate i.e., orange tree. Three plots (each of $4m^2$) were chosen. One of them for clove treatment, the second for methomyl compound (recommended) treatment and the other was left without treatment as a check control. Each plot was far from the other by 2m. The tested compounds were applied as a spray under plants which the places of slugs using hand sprayer (3litres).Alive slugs were counted daily pre and post treatment during 7 days.

3. Results and Discussion

***1.* Toxicity studies:**

Data in Table (1) presented the comparative response to clove plant extract by acetone or ethanol on land slug *Limax flavus* used as a contact treatment. Results showed that mortality percentage increased with increasing the concentration of both crude extracts. The last concentration 2% achieved complete mortality against slugs for both extracts.. LC_{50} values were 0.6% and 0.36% of clove acetone extract and clove ethanol extract on the slug, respectively. Result revealed that ethanol extract was more toxic than acetone extract. These differences in toxicity may be due to ethanol extracted toxic substances more than acetone. Kumar and Singh (2006) recorded that the LC_{50} of *Syzygium aromaticum* was 51.98 mg/l after 96h and ethanol extract was more toxic than other organic extract. El-Din (2006) reported that the clove

oil of *S. aromaticum*, possessed a toxic effect against *Biomphalaria alexandrina* and *Bulinus truncatus*. The molluscicidal concentration of eugenol possesses was very high toxicity as $LC_{50}$was 28 mg /L *Biomphalaria alexandrina* freshwater snails (Radwan and El-Zemity, 2007).

2. **Reproductive System Anatomy:**

The reproductive system in slugs is hermaphrodite in which the gametes formed in the ovotestis pass down the common hermaphrodite duct to the albumen gland, where the male and female systems separate. The spermatozoa are carried down the vas deferens to the penis and are introduced into the bursa copulatrix of the copulatory partner, while the eggs after fertilization pass through the oviduct, where they receive the mucous coverings which from the egg capsule. (Duncan, 1958). The normal reproductive system was described in Fig. (1). Effect of clove acetone extract on reproductive system was shown in Fig. (2) it can be seen slight swelling in the size of the organs of reproductive system. Fig., (3) showed the effect of clove ethanol extract on reproductive system it is cleared that severe swelling in the size of all organs of the reproductive system comparing with control. From the previous result it is clear that ethanol extract has a strong action more than acetone extract. These results may be due to ethanol being able to extract substances more toxic than substances which extracted from clove with acetone solvent. This action caused teratogenic effect on reproductive system so it caused inhibition in regeneration so it caused inhibition in regeneration so it caused decreasing in the population density of slugs. Kumar *et al.,* (2011) studied the effect of *Syzygium aromaticum* on biochemical changes in the ovotestis of snail *Lymnaea acuminata*. The treatment with LC_{50} caused reduction of free amino acid, protein and nucleic acid in the ovotestis of snail after 96h. Srivastava and Singh (2015) studied the effect of LC_{50} of *Syzygium aromaticum* on the biochemical changes in the ovotestis of snail *Lymnaea acuminata* after 24h. It caused maximum significant reduction in free amino acid, protein, DNA and RNA in the ovotestis.

3. **Effect on Enzymes in the Nervous Tissue.**

Acetylcholin esterase (AchE) and alkaline phosphatase (ALP) are important and critical in the nervous tissues (Srivastava and Singh, 2015).Table (2) indicates the effect of LC_{50} of *Syzygium aromaticum* crude plant extracted by acetone and ethanol on AchE and ALP enzymes. Results showed that AchE level decreased with significant after 3 days of treatment compared with control with 38.4% difference. While it increased again after 7 days of treatment with 62.2% difference for acetone crude plant extracted. The crude plant extracted by ethanol caused decreased significant with the prolongation periods of treatment at 3 and 7 days after treatment by ethanol extract with 39.9% and 21.3% difference with control, respectively. In case of ALP, the crude extracted by acetone caused significant decrease after 3 days of treatment while it was increase after 7 days of treatment with 32.3% and – 2.3% difference with control, respectively. While the crude plant extracted by ethanol caused highly increase in the level of enzyme after 3 and 7 days of treatment with –279.5% and -665.9% difference with control, respectively. These mean that crude plant extract by ethanol was more effective than extract by acetone. From the previously result it noticed that ethanol extract caused inhibition on AchE and sever increase on ALP. Kumar and Singh (2012) recorded that the snails fed on bait containing sublethal concentration of eugenol (*Syzygium aromaticum*) causing a significant inhibition 20% and 49.5% of control in ALP and AchE activity, respectively in the nervous tissue of snail *Lymnaea acuminata*. Also, Srivastava and Singh (2015) confirmed that the LC_{50} of *syzygium aromaticum* after 24h., caused maximum significant reduction in AchE activity in the ovotestis and nervous tissue of *Lymnaea acuminata*snails. In addition, Pradeep *et al*., (2009) indicated that eugenol (*Syzygium aromaticum*) inhibited the AchE and ALP activity *in vivo* and *in vitro* exposure of *Lymnaea acuminata* snails.

4. **Field experiments.**

The field performance of the tested compounds against *Limax flavus* was shown in Table (3). Results revealed that methomyl (the recommended compound) caused higher population reduction percentage for slug 94.4% than *Syzygium aromaticum* clove plant extract by ethanol 74.5%.

From the field results we found that the plant extract gave population reduction percentage less than methomyl but it achieved good results effect on slugs. This observation may be due to the weather factors or natural conditions (temperature, humidity and light ….etc.) may affect this compound inducing degradation or decomposition led to reduce its toxic action against slugs. Ismail and Samah AbdEl-Kader (2011) studied that clove powder bait 40% gave 62.4% population reduction after 21 day of treatment against *Monacha cartusiana* and snails under field condition.

Finally we can be concluded that the plant extract of *S. aromaticum* may be used as molluscicides for controlling land slugs. Whereas it killed the individual or causes teratogenic in the reproductive system leading to reduce in the product of eggs and reduce the population density of slugs.

Table (1).LC_{50} of clove extract against land slugs *Limax flavus* using contact technique after one week of treatment.

Clove extracted by	Concentrations of clove Extract	Mortality%	LC_{50} %
Acetone	0.25 0.5 1.0 2.0	20 40 60 100	0.60
Ethanol	0.25 0.5 1.0 2.0	40 60 80 100	0.36

Table (2). Effect of LC_{50} of clove extract on the nervous tissue enzymes in *Limax flavus* land slug after different periods.

Enzymes (U/l)	Clove extracted by	Control Mean ±SE	Period after treatment.				LSD
			3 days		7 days		
			Mean ±SE	Diff. %	Mean ±SE	Diff. %	
AchE	**Acetone**	25907.5±1028.8^{aA}	15960.6±582.3^{bB}	38.4	15968.4 ± 133.7^{bB}	62	3952.9
	Ethanol	25907.5±1028.8^{aA}	15561.7±582.3^{bB}	39.9	20378.9±1725.8^{bB}	21.3	5388.1
LSD			3386.9		5186.1		
ALP	**Acetone**	112.0 ±2.5^{bB}	75.8 ±1.4^{cB}	32.3	114.6 ±5.8^{cB}	-2.3	16.4
	Ethanol	112.0 ±2.5^{cB}	425.1 ±17.1^{bA}	-279.5	857.8 ±27.5^{aA}	-665.9	84.7
LSD			44.9		73.7		

abc= Values in row with different letters are significantly different at (p< 0.05). AchE= Acetylcholine esterase
^{AB}= Values in column with different letters are significantly different at (P< 0.05). ALP= Alkaline phosphatase.

Table (3). Field performance of clove extract and Methomyl against land slug *Limax flavus*

Treatment	Rate of applications	No. animals before treatment.	No. alive animals after treatment	% Population Reduction
Control	-	41	110	-
Methomyl	20.0 ml/l	33	5	94.4
Clove extract	20.0 ml/l	28	56	74.5

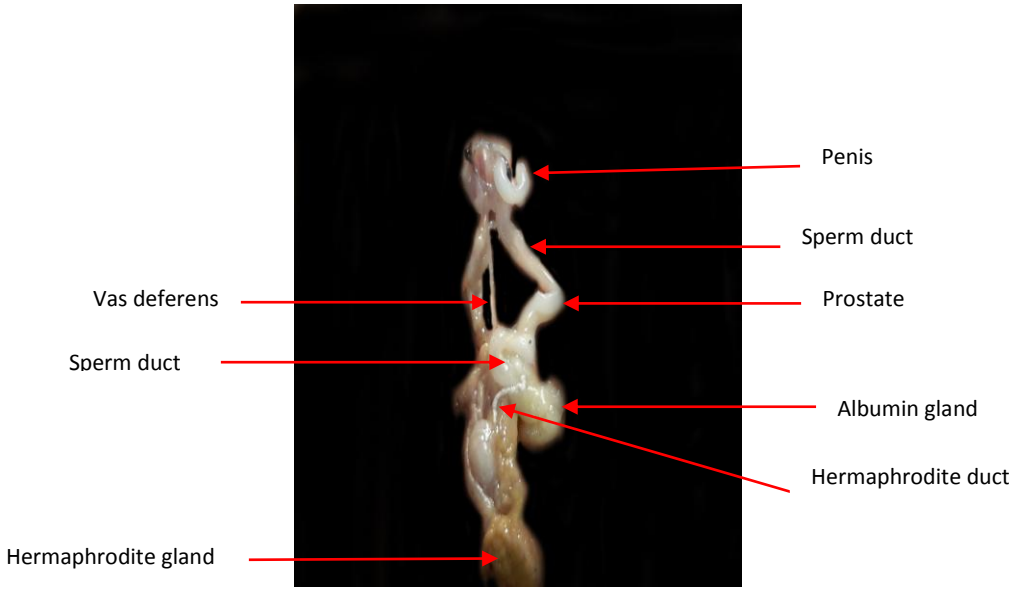

Fig.1.Untreated *Limax flavus* showing the normalsize of organs of reproductive system.

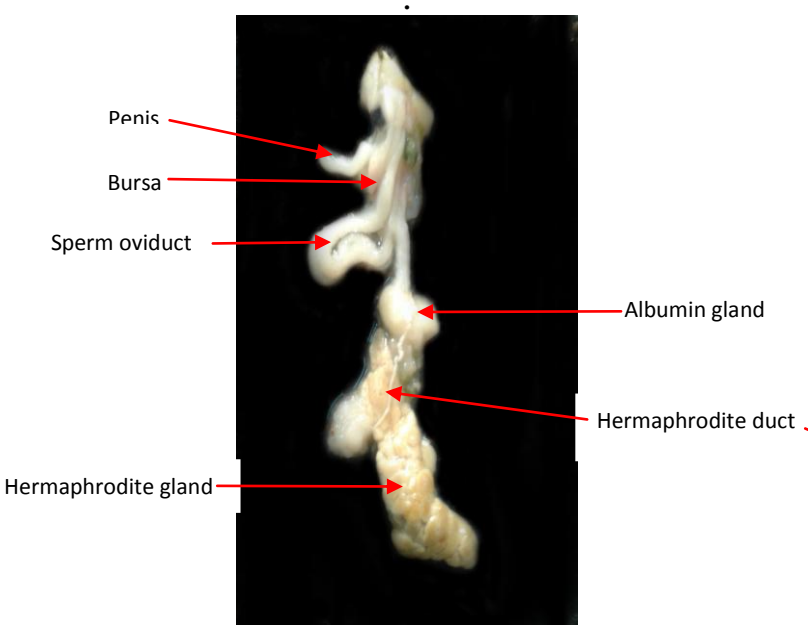

Fig. 2. *Limax flavus* treated with clove plant extracted by acetone. Showing slight swelling in the size of organs of reproductive system

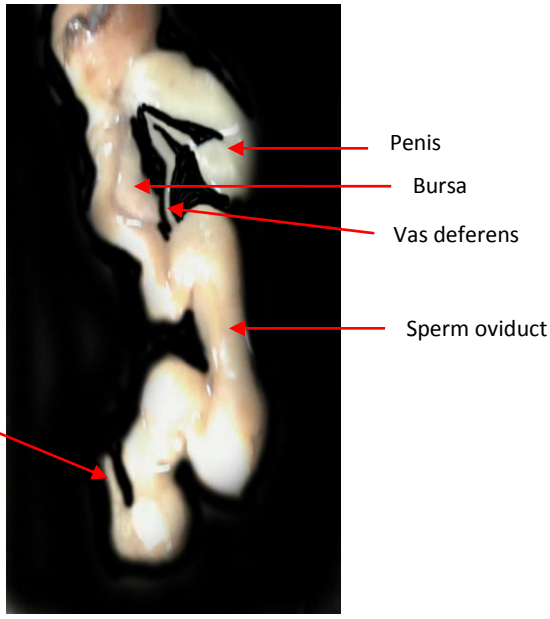

Fig. 3. *Limax flavus* treated with clove plant extracted by ethanol. Showing severe swelling in the size of organs of reproductive system.

References

1. Asher, R. S. and Marian, F. (1981). The residual contact toxicity of bay sir 8514 to *Spodoptera littoralis* larva. Phytoparasitica., 9(2): 133-137.
2. Bergmeyer, H. (1963). Methods of Enzymatic analysis Academic press in New York, USA, PP. 886.
3. Briner, T. and Frank, T. (1998). The palatability of 78 Wildflower strip plants to the slug *Arion lusitanticus* Ann. Appl. Biol., 133: 123-133.
4. Chort Software (2005). Costat Program V. 6.311. (780 lighthouse), Ave. PMB 320, Monterey, CA. USA.
5. Duncan, C. J. (1958). The anatomy and physiology of the reproductive system of freshwater snail *physa fontinalis* (L.). J. of Zoology 131 (1): 55- 84.
6. El-Din, A. T. S. (2006). Molluscicidal effect of three monoterpenes oils on schistosomiasis and fascioliasis vector snails in Egypt. J. of Egyptian Society of parasitology 36 (2): 599- 612.
7. Ellman, G. L.; Courtuery- Adersand, V. J. R. and Feat Featherstone, R. M. (1961). A new rapid colorimetric determination of acetylcholine esterase activity. Biochem. Pharmacol., 7: 88-95.
8. Freedman, B.; Nowak, J. and Kwolek, W. F. (1979). A bioassay for plant derived pest control agent using the European cornbrer. J. Econ. Entomol., 72: 45-54.
9. Finney, D. J. (1971). Probit analysis. A statistical treatment of the sigmoid response curve. Cambrige Univ.
10. Gabr, W. M.; Youssef, A. S. and Fatma Khidr, K. (2006). Molluscicidal effect of certain compounds against two land snail *Eobania vermiculata* under laboratory and field conditions. Egypt. J. Agric. Res. 84(1): 43-50.
11. Ismail, Sh. A. A. and Smah M. Abd El-Kader (2011). Clove, Is it has a molluscicidal activity against land snails *Monacha cartusiana*? J. Plant Protection and Pathology. El-Mansoura Univ., 2(5): 561-589.
12. Kind, P. R. N. and King, E. J. (1954). Estimation of plasma phosphatase by determination of hydrolysed phenol with amino antipyrine. J. Clin. Path., 7: 322- 326.
13. Kumar, P. Singh, D. K. (2006). Molluscicidal activity of Ferula asafetida, *Syzygium aromaticum* and *Carumcarvi* and their active components against the snail *Lymnaea acuminata*. Chemosphere, 63 (9): 1568- 74.
14. Kumar, P.; Singh, V. K.; Singh, D. K. (2011). Bait formulations of molluscicides and their effects on biochemical changes in the ovotestis

of snail *Lymnaea acuminata* (mollusca; Gastropoda; Lymnaeidae). Rev. Inst. Med. Trop. 53 (5): 271- 275.
15. Kumar, V. K. and Singh, D.K. (2012). Feeding of bait to snail *Lumnaea acuminata* and their effect on certain enzyme in the nervous tissue. Biochemistry. 6 pages.
16. Maha Fouad, M. (2005). Histopathological changes induced in the mucus glands of brown garden snail *Eobania vermiculata* treated with malathion and methomyl pesticides. Egypt. J. Agric. Res., 83 (1): 251-259.
17. Pradeep, K.; Singh, V. K. and Singh, D. K. (2009). Kinetics of enzyme inhibition by active molluscicidal agents ferulic acid, Umbeliferone, Eugenol and Limonene in nervous tissue of snail *Lymnaea acuminata*. Phytother. Res., 23: 172-177.
18. Radwan M. A. and El-Zimity, S. R. (2007). Naturally occurring compounds for control of harmful snail. Pakistan Journal of Zoology 39: 339-344.
19. Shoieb, M. A. (1997). Ecological studies on some common snail species and their control in Ismailia Governorate. M. Sc. Thesis, Fac. Agric. Suez Canal Univ.: 1-137. Egypt.
20. Singh, D. K. and Agarwal, R. A. (1989). Toxicity of piperonyl butoxide carbaryl synergism on the snail *Lymnaea acuminata*, Inter. Revue. Ges. Hydro. Biol., 74: 689- 699.
21. Singh, S. and Singh, D.K. (1998). Effect of active Molluscicidal agents of common species. On biochemical parameters in the ovotestis of *Lymnaea acuminata*. Malays Appl. Biol., 27: 45-49.
22. Srivastava, A. K. and Singh, V. K. (2015). Action of bait containing Eugenol (*Syzygium aromaticum*) on biochemical changes in freshwater snail *Lymnaea acuminata* Biochemistry and molecular Biology (BMB), 3(1): 1-6.

12/25/2015