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CLOVE: IS IT HAS A MOLLUSCICIDAL ACTIVITY AGAINST LAND SNAILS (*Monacha cartusiana*) ?

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ABSTRACT

The present study was carried out to evaluate the potential of the flower-bud powder and commercially available eugenol of *Syzygium aromaticum* against juveniles and adults of *Monacha cartusiana* using, baiting technique at three concentrations (10, 20 and 40 %) for bud-flower and (1, 2 and 4 %) for eugenol under laboratory and field conditions. The activity of both baits found to be time and concentration dependent, at the lowest clove-bud powder and eugenol concentration of 10 % and 1 %, mortalities were 67.5, 70, 75 and 47.5 %, respectively. However, the high mortality percentages (99, 100 and 100 and 97.5%) were obtained at concentrations of 40% and 4% clove-bud powder and eugenol for juvenile and adult snails, respectively 21 days after treatment under laboratory conditions, while under field condition reduction percentages were 39.6,57.2 and 62.4 % for adults snails at the three tested concentrations of clove-bud powder, respectively 21 days after treatment.

The molluscicidal concentration of both poising baits proved to be effective in killing both juveniles and adults *M. cartusiana* snail according to the period of exposure. Consequently, clove bud powder and essential oil (eugenol) of *S. aromaticum* appears to have a potential importance for land snails control in Egypt.

INTRODUCTION

Land snails are considerable one of an economic importance among pests attacks different types of plants. *Monacha cartusiana* is a well-known species in this category for its great damage to many vegetable crops in the Egyptian coastal areas (El-Okda 1983).

Several attempts have been paid to control its dispersal by using synthetic pesticides and different plant products against the snails (Ismail *et al.* 2005 and Genena *et al.* 2008). Natural products from plant origin have received much attention as potentially useful bio-active compounds in an effort to develop alternatives to the conventional pesticides. A large number of plant products which possess molluscicidal activity on both terrestrial and fresh water snails have been studied (Singh and Singh 2004; Gabr *et al.* 2006 and Shoaib *et al.* 2009). Clove is the dried flower bud of *Syzygium aromaticum* (L.) Merr. and Perry (Family Myrtaceae), grown naturally in Indonesia and cultivated in many parts of the world including Brazil (Costa, 1994 ; Correa *et al*. 1998 ; Agra *et al.* 2008). Through the phytochemical studies, clove contains free eugenol, eugenol acetate, caryophyllene, sesquetrepene ester (Rastogi and Mehrotra, 1984), phenyl propanoid (Miyazaw and Hisama, 2003), β caryophyllene (Ghelardini, *et al.* 2001), eugenol and acetyl eugenol (Srivastava, 1993). The highest concentration was eugenol (85-92%) (Dorman and Deans 2000). A kilogram of dried buds yields approximately 150 ml of eugenol (Ryman 2009).

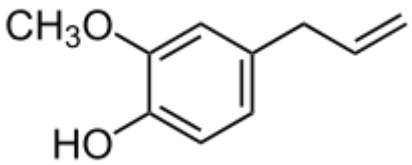
Clove is well known as food additive and it has a number of medicinal properties as anticarcinogenic (Zheng *et al.* 1992), as antibacterial, antiviral and as antifungal (Chaieb *et al.* 2007a). Besides, the reported anti-oxidant, anti-spasmodic, anti-helminthic activity (Poolman *et al.* 1995; Hirotaka *et al.* 2003 and Fatehi *et al.* 2004). The essential oil of *S. aromaticum* shows insecticidal activity (Park *et al.* 2000), act as potent antifeedants, antioviposition, fecundity reduction, repellenty and have acaricidal and termicidal activity (Brown and Minott, 1998; Ajayi and Nes 2001; Zhu *et al.* 2001; Akhtar and Yeoung 2008; Knio and Usta 2008).

Concerning the molluscicidal activity of the clove flower buds and the essential oil of *S. aromaticum*, few numbers of studies have been carried out only in controlling aquatic snails, which are vectors for many diseases. Most commonly are those for schistomiasis and fascioliasis. For instance, El-Din (2006) reported that the clove oil of *S. aromaticum* possessed a toxic effect against *Biomphalaria alexandrina* and *Bulinus truncatus*. Another study carried out by Kumar and Singh (2006) who found that the toxicity of *S. aromaticum* flower bud powder against the snail *Lymnaea acuminate*, they found that (96 h LC_{50}: 51.98 mg/ L) value was more pronounced than that other plants. However, until the present time, no study has been conducted to reveal the molluscicidal activity of *S. aromaticum* flower bud powder against terrestrial snails. Therefore, the objectives of this study were to evaluate the molluscicidal activity effects of dried clove bud and eugenol against *M. cartusiana* under laboratory and field conditions to provide information about the direct toxic effects of this plant as an alternative to synthetic chemicals.

MATERIALS AND METHODS

Plant materials:
Scientific name: Clove.
Biological name: *Syzygium aromaticum* / *Caryophyllus aromaticus* / *Eugenia caryophyllata*.
Other names: Clove, clovos, caryophyllus.
Family*: Myrtaceae*.
Parts used: flower buds and ready made (commercial) eugenol
Active compounds: The major compounds are eugenol (88, 58%), which is a member of the phenyl propanoids class of chemicals compounds (Chaieb *et al.* 2007a).

Active compounds



(C10H12O2)
IUPAC name: 4- Allyl-2- mthoxyphenol

The eugenol compound is responsible for most of the characteristic aroma of cloves Clove dried buds were purchased from super market in Zagazig district while the compound of ready made eugenol was purchased from Al-Gomhouria company for chemicals. The clove bud was grounded in a milling machine (mixer grinder) to obtain a fine dry powder, then sieved through 100 mm mesh size.

Tested animals:

Adults and juveniles of *M. cartusiana* snails were collected from heavily infested fields cultivated with Egyptian clover, from Malams locality, Menia-Elkamah, county, Sharkia Governorate and then transferred in plastic bag to the laboratory, snails fed on lettuce leaves for 4 days to acclimatization (El-Okda 1981).

Laboratory experiments:

For preparation of baits, three concentrations of each powder of clove and eugenol were prepared as poisonous baits by incorporating it with wheat bran to give 100 parts of poisonous baits.

Two groups of adults and juveniles snails were set for each concentration of clove powder (10, 20 and 40% gm.) and eugenol (1, 2 and 4%).

Ten experimental animals were introduced in each box. About 10 gm of each poison bait were spread into each box which that was covered with cloth netting secured with rubber bands to prevent snails from escaping. Each treatment was replicated 4 times. Another control groups were kept under similar conditions containing only wheat bran baits. Few milliliters of water were added daily into each box to provide suitable humidity for snail activity. The tested boxes were checked after 1, 3,7,14 and 21 days post treatment to recorded and removed dead animals. Snail mortality was established by the contraction of the body within the shell; no response to a needle probe was taken as evidence of snail death. Mortality percentages were calculated and corrected using Abbots formula (1925).

Field experiments:

To evaluate reduction percentages of *M. cartusiana* snails after 1, 7, 14 and 21days post treatment, the experiment was carried out in vegetable fields (cabbage and lettuce) at Malames locality, Menia-El-kamah district, Sharkia Governorate during April 2010. Three concentrations consisting of 10, 20 and 40 %, of *S. aromaticum* flower bud powder were tested as poisonous baits. Baits were prepared in the laboratory by incorporating the appropriate amount of each concentration with wheat bran (Ghamry 1997) and mixed with black sugarcane syrup 5% as attractant substances. About 100 grams of bait was offered on plastic pieces and spread along the edge of fields in the morning. Control group was done without clove powder and four replicates were used for every concentration. Number of dead and alive snails were counted in check and treatment area before application and after 1, 3, 7 and 21 days. Reduction percentages were calculated according to formula of Henderson and Tiliton (1955).

RESULTS

Laboratory toxicity of clove flower- bud power and the essential oil of *S. aromaticum* (eugenol) at three concentrations (10, 20 and 40 %) after 1, 3, 7, 14 and 21 days are given in table (1) and (2). The results indicated that both clove buds and eugenol had a toxic activities against adults & juveniles of *M. cartusiana* with all three tested concentrations at 21days after treatment.

A positive correlation was noticed between mortality percentage of tested snails and exposure time to the tested baits.

From Table (1) it is evident that high concentration induced the maximum mortality for juveniles & adults *M. cartusiana* snails, after 21 days of treatment, whereas the highest mortality percentage was 99% and 100% for *M. cartusiana* juvenile and adult snails at a clove bud concentration of 40% , respectively.

Also, it can be seen clearly that a relatively high in the rate of mortality percentage (67.5 and 70% for juvenile and adult *M. cartusiana* snails) were achieved even at the lowest clove buds concentration of 10% .

Table (1):-Effect of clove bud powder of *S. aromaticum* as a poisonous baits against juveniles (J) and adults (A) of *Monacha cartusiana* snails under laboratory conditions.

Concentration %(gm)	Mortality percentages after indicated days									
	1		3		7		14		21	
	J.	A.	J.	A.	J.	A.	J.	A.	J.	A.
10	15	20	25	30	35	40	47.5	50	67.5	70
20	20	25	35	40	55	60	70	75	90	92.5
40	25	37.5	42.5	45	66.5	70	91.5	93.5	99	100

N.S :No significance.

From the results shown in Table (2) it was observed that the land snail *M. cartusiana* mortality increased with increasing eugenol concentration and exposure time. After one day, clove oil (eugenol) failed to cause any mortality against both juvenile and adults of *M. cartusiana* at all three tested concentrations. The longer exposure caused more greater death in juveniles and adults of *M. cartusiana*, i.e. at 21 days after treatment with 4% concentration, 100% and 97.5% mortality was achieved respectively.

A relatively high rate of mortality percentage was occurred (75 and 47.5 %) in juveniles and adults of *M. cartusiana* snails at the lower concentration of eugenol (1%). The results obtained from the two previous experiments indicated that adults and juveniles of *M. cartusiana* sensitive towards the flower bud powder and eugenol of *S. aromaticum* at all tested concentrations over the 21 days period.

Also, under laboratory conditions, an excessive mucous secretion of snail and complete withdrawal of the whole body inside the shell was observed. These morphological features frequently occurred due to direct contact of minute clove powder granules with locomotory organs of snail.

Table (2):- Effect of the essential oil of *S. aromaticum* (eugenol) as a poisonus baits against juveniles (J) and adults (A) of *M. cartusiana* snails under laboratory conditions.

Concentration %	Mortality percentages after indicated days									
	1		3		7		14		21	
	J.	A.	J.	A.	J.	A.	J.	A.	J.	A.
1	0	0	0	0	25	12.5	60	35	75	47.5
2	0	0	12.5	5	47.5	40	87.5	70	92.5	80
4	0	0	25	0	70	45	85	77.5	100	97.5

The effect of different concentration of clove powder in controlling *M. cartusiana* snails was determined under field condition. Results in Table (3) revealed that reduction percentages were increased by increasing concentration and time elapsing. Mean reduction percentages after the first three days (initial effect) reach to 4.52, 6.79 and 10.52% for the three tested concentrations (10, 20 and 40%), respectively.

Regarding the residual effect for the three treated concentrations, it noticed that reduction percentages reach its maximum values in the end of the experiment (21 day) to give 39.6, 57.2 and 62.4% reduction, respectively. Mean reduction percentage for the residual effect for the three tested concentrations recorded 26.13, 41.73 and 49.46% reduction, respectively. Generally, it could be reported that the highest concentration 40 gave the highest reduction percentages after 21 days (62.4%) compared with (39.6%) for the lowest concentrations (10%), respectively.

Table (3): Effect of clove bud powder of S. aromaticum in controlling the land snails of *M. cartusiana* as poisonous baits under field conditions.

Conc. Of clove bud powder %	Initial effect (%Reduction during the first three days)			Residual effect (%Reduction during the rest period)			
	1	3	Mean	7	14	21	Mean
10	5.83	3.21	4.52	13.7	25.1	39.6	26.13
20	6.99	6.59	6.79	25.49	42.5	57.2	41.73
40	10.84	10.2	10.52	30.9	55.1	62.4	49.46

DISCUSSION

The present results clearly indicate that the flower bud powder and the clove oil eugenol of *S. aromaticum* are important sources of botanical molluscicides, since all used tested concentrations of this plant was found to be toxic even at low concentrations against juveniles and adults of *M. cartusiana* and the effect was concentration and time dependent.

The molluscicidal activity of *S. aromaticum* may be attributed to the presence of several constituents, mainly eugenol (Kumar and Singh, 2006), eugenol acetate beta-caryophyllene,2- heptanone (Chaieb *et al.* 2007 b). Acetyl- eugenol, alpha-humulene, methyl salicylate, iso-eugenol, methyl-eugenol(Yang *et al.* 2003),phenylpropanoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid,

ellagic acid and oleanolic acid (Cai and Wu 1996). Eugenol is the major compound in the essential oil extracted from *S. aromaticum*, comprising 75 to 85% of the total (Bauer *et al.* 2001) and it consists of a member of the phenyl-propanoides class of chemicals compounds (Chaieb *et al.* 2007a). Studies by Juven *et al*. (1994), indicated that the toxicity of clove oil (eugenol) was primarily due to phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increase permeability, unavailability of vital intracellular constituents and/ or impairment of bacterial enzymes systems (Farag *et al*. 1989). A few other studies were focused only on the aquatic snails for the molluscicidal activity of *S. aromaticum* and eugenol , for instance, Kumar and Singh (2006) reported that the toxicity of *S. aromaticum* flower-bud powder (96h LC_{50} = 51.98 mg/ L) was more pronounced against the fresh water snail, *L. acuminate*, than that of root latex powder of *Ferula asafetida* (46h LC_{50} = 82.71 mg/L) and seed powder of *carum carvi* (96h LC_{50} = 140.58 mg/L).

They also reported that eugenol present in *S. aromaticum* was very toxic against *L. acuminata* (LC_{50} 96h = 1.41 mg/L). The molluscicidal concentration of eugenol possesses very high toxicity; LC_{50} in the schistosomiasis-carrying aquatic snail, *Biomphalaria alexandrina* was found to be 28 mg/L (Radwan and El-zemity 2007), for *Bulinus truncates* was 24mg/L and for *Lymneae natalensis* was 22mg/L (El-Din 2006). It can be concluded from the result of our study that the potential of flower-bud powder and the essential oil of *S. aromaticum* (eugenol) may be used as a potent molluscicides for controlling snails. However, further studies are necessary to elucidate the mechanism of action in snail body.

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القرنفل: هل للقرنفل نشاط إبادى ضد قوقع المونـاكا كارتوسيانا؟

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تم إجراء هذه الدراسة بغرض تقييم فعالية مسحوق براعم القرنفل وكذلك المادة الفعالة (اليوجنول) كطعوم سامة ضد قوقع المونـاكا كارتوسيانا تحت الظروف الحقلية والمعملية بمحافظة الشرقيةوذلك بثلاث تركيزات مختلفة وهى 10، 20، 40% بودر لمسحوق براعم القرنفل، 1، 2، 4% سائل لمادة اليوجنول.

اثبتت النتائج أن الطعوم السامة لكلاً من مسحوق البراعم والمادة الفعالة للقرنفل لهم نشاط أبادى ضد قوقع المونـاكا كارتوسيانا وهذا يعتمد على فترة التعرض وكذلك التركيز حيث أنه عند أقل تركيز (10 % بودر مسحوق براعم القرنفل ، 1% لسائل مادة اليوجنول) كانت نسبة الموت 67.5، 70، 75، 47.5 % بالسنبة للأفراد الغير يافعة واليافعة على التوالى لكل منهما بعد 21 يوم من المعاملة. بينما أعلى نسبة موت99، 100 و 100و 97.5%) سجلت عند تركيز 40% بودر ، 4% سائل لمسحوق براعم القرنفل ومادة اليوجنول لكل من الأفراد الغير اليافعة واليافعة على التوالى بعد 21 يوم من المعاملة. أما تحت الظروف الحقلية بلغت أعلى نسبة للخفض فى التعداد 62.4% عند أعلى تركيز 40% بينما بلغت 39.6% عند أقل تركيز 10% بودر لمسحوق براعم القرنفل بعد 21 يوم من المعاملةوعموما فهذة محاوله للاستغناء عن استخدام المبيدات الكيميائية وإستبدالها بالمواد الطبيعية الأمنة علي البيئة.

قام بتحكيم البحث

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