Plants as Promising Safe Molluscicides for Control *Monacha Cartusiana* Snail

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Abstract: Glassy clover snail, *Monacha cartusiana* is the most destructive invasive snail in Egypt. The cost of synthetic molluscicides and their toxicity to the non target organisms have propelled the research of plant derived molluscicides. Juices from three plants [*Citrus limon*, (*C. limon*) *Coriandrum sativum* and *Mentha spicata]* were evaluated individually and as binary mixtures against *M. cartusiana*. The joint action between the binary mixtures of botanical juices was also investigated. The results indicated that *C. limon* fruit and *C. limon* peel juices were the most effective under the laboratory and field conditions. Of the various combinations studied, *C. limon* fruit + *C. limon* peel, *C. limon* fruit + *M. spicata* and *M. spicata* + *C. limon* peel were recorded the highest molluscicidal activity against snails. They were recorded 80% mortality of snails after 3 days of the laboratory experiment. Moreover, the mixture *C. limon* fruit + *C. limon* peel cause 100% mortality of snails at the end of experiment. The same botanical mixtures also showed the highest reduction of snail individuals under field conditions. There is a clear potentiation effect of the mixture *M. spicata* + *C. sativum* with co-toxicity factor of 53.26% under laboratory conditions. On the other hand, an additive effect was recorded in the combination of *C. limon* peel with each of *C. limon* fruit and *M. spicata* juice with co-toxicity factors of 7.92 and 9.43%, respectively. While, the other tested botanical mixtures showed an antagonistic effect. The findings of this study demonstrate that the binary combinations of the potent molluscicidal botanical juices are more effective than its use individually. They are effective for management of *M. cartusiana* under laboratory and field conditions.
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Keywords: Botanical juices, botanical mixtures, *Monacha cartusiana* snailjoint action.

1. Introduction

Land snails considered a destructive agricultural pest causing economic damage to a wide variety of plants as vegetables, cereal, fruit trees and ornamental plants **(Godan, 1983)**. The glassy clover snail, *Monacha cartusiana* is the most abundant snail in Egypt **(Ismail 1997, Arafa 1997 and Mahrous *et al.*, 2002)**. Different methods are used for control this pest such as chemical treatments by using pesticides. While these compounds caused dangerous environmental problems in addition to their toxic effects to the non target organisms **(Buchs *et al.*, 1989)**. Plants represent a promising alternative and effective method for control snails **(Salem *et al.*, 2017)**. It has a wide range of ideal properties such as high target toxicity, low cost, easy biodegradability and safety **(Singh *et al.*, 2000)**. Plants also richest with a renewable bioactive organic chemicals as terpenoides, alkaloids, glycosides, phenols and tannins that cause behavioural and physiological effects on pests **(Tantawy *et al.*, 2012)**. However, most of them may require other plant species with different mode of action to increase their potency **(Oparaeke, 2004)**. The botanical mixtures of plants could form a successful formulation of biopesticides play a key role in the control of agricultural pests **(Oparaeke *et al.*, 2005)**.

This study aimed to assess the molluscicidal activity of juices of different plants and their mixtures against *Monacha cartusiana* snails.

2. Materials and Methods

Tested plants

The plants selected for this study are listed in Table 1.

Preparation of the botanical juices and mixtures

The plants of *Citrus limon*, *Coriandrum sativum* and *Mentha spicata* were obtained from local market in Zagazig district, Egypt. These plants were specifically selected in this study because it is safe to the environment and also cheap in cost compared to many other plants. Leaves of each *C. sativum* and *M. spicata* were cut into small pieces and then chopped well by using an electric mill. The yield was transferred onto muslin cloth and squeeze to obtain clear crude juice. In concerning to *C. limon*, fruit was squeeze to obtain juice and the remaining peel was cut and chopped to obtain peel juice by the same way that described before. One rate of each crude juice was used **(Zaki, 2013)**.

Botanical mixtures were prepared by mixing rate of each plant juice with the other according to **Oparaeke *et al.* (2005)** with minor modifications. These juices and mixtures were prepared for

investigate their molluscicidal activity against snails in the following treatment.

Tested snails

Adult individuals of the glassy clover snail, *Monacha cartusiana* were collected from infested clover field at Malames village, Meniet El-Kamh district, Sharkia Governorate, Egypt. The snails were transferred in muslin bags to the laboratory and kept in a glass container containing moist clay soil and covered with muslin cloth for prevent the snails escaping. Snails were provided daily with fresh cabbage leaves for two weeks for acclimatization. Adult snails with the same shell diameter were selected and starved 24 hours before starting the treatment **(Abd El-Aal, 2001)**.

Table 1. Plants evaluated for molluscicidal activity

Family	Scientific name	Common name	Parts used	Image
Rutaceae	*Citrus limon*	Lemon	- Fruit - Peel	
Apiaceae	*Coriandrum sativum*	Coriander	Leaves	
Lamiaceae	*Mentha spicata*	Spearmint	Leaves	

Evaluation of the molluscicidal potency of botanical juices and their mixtures against *Monacha cartusiana* snail under laboratory conditions

One rate from each tested juice was chosen in this experiment. This rate was selected on the basis that it was the least in quantity to avoid the economic cost and in the same time it was represent the most effective rate of the juice against snails. Juices of *C. limon* fruit (20 ml), *C. limon* peel (7 ml), *C. sativum* (20 ml) and *M. spicata* (20 ml) were evaluated against the adults of *M. cartusiana* snail. Mixing each juice rate of *C. limon* fruit + *C. limon* peel, *C. limon* fruit + *M. spicata*, *C. limon* fruit + *C. sativum*, *M. spicata* + *C. sativum*, *M. spicata* + *C. limon* peel and *C. sativum* + *C. limon* peel were also tested against adults of the same snail species. Plastic boxes (3/4 kg capacity) were used in this experiment, each box contained ½ kg moistened clay soil. Ten adult snails and ten discs of cabbage leaves were put in each box. Each tested plant juice and each mixture were sprayed separately on the soil and cabbage discs. Three replicates were prepared for each plant juice and mixture and other three replicates were prepared by the same manner without any treatment as a control. All boxes covered with muslin cloth and secured with a rubber band for prevent the snails escaping. The mortality percentages of snails were recorded after 1, 3, 7, 14 and 21 days in all boxes. Mortality percentages were corrected according to **Abbott's formula (1925)**.

Joint action of the binary mixtures of botanical juices against *Monacha cartusiana* snails

The joint action of the botanical mixtures against *M. cartusiana* snails after 21 days of the experiment was expressed as the Co-Toxicity factor (C.F.) determined according to **Mansour *et al.* (1966)** as follows:

$$C.F. = \frac{\text{Observed mortality \% - Expected mortality \%}}{\text{Expected mortality \%}} \times 100$$

This factor was used to classify results into three categories. A positive factor ≥ 20 was considered potentiation, a negative factor ≤ 20 means antagonism and intermediate values between -20 and +20 indicate an additive effect.

Evaluation of the molluscicidal potency of botanical juices and their mixtures against *Monacha cartusiana* snail under field conditions

Field trial was conducted in March – 2018 at clover field heavy infested with *M. cartusiana* snails. This field located at Malames village, Meniet El-Kamh district, Sharkia Governorate, Egypt. Three infested samples with size 50 × 50 cm were selected for spraying by each crude plant juice at rates 100 ml for each *C. limon* fruit, *C. sativum*, *M. spicata* and 40 ml for *C. limon* peel juice. Three samples were also spraying by each botanical mixture. Before any treatment, live snails were counted in each sample. Control samples kept without any treatment. The number of living snails was counted in the same samples after 1, 3, 7, 14 and 21 days post treatment. Reduction percentages were calculated according to the formula of **Henderson and Tilton (1955)**.

Data analysis

Results were statistically analyzed and the treatment means were compared according to the method of **(Costat, 2005)** statical program analysis, computer program software.

3. Results

Molluscicidal activity of plant juices against *M. cartusiana* snail under laboratory conditions

The molluscicidal activity of the four plant juices against *M. cartusiana* snails was evaluated. As shown in Table 2. and Fig. 1. that is no mortality of snails observed in control groups with water, this indicating that no factor other than plant juices were responsible for the altered behavior and mortality of snails. It seems clearly from the obtains results that *Citrus limon* peel juice has the highest molluscicidal activity against snails by causing 63.33% mortality of snails after one day of the experiment. Clear furan has been observed in the shell aperture of the snails which treated with this juice before death (Photo 1A). It was followed by *Citrus limon* fruit, *Mentha spicata* and *Coriandrum sativum* juices which exhibited 40, 23.33 and 20% mortality of snails, respectively. The mortality which caused by the *C. limon* fruit (Photo 1B) and *M. spicata* (Photo 1C) juices increased to 80 and 26.66% after 14 and 7 days of the treatment with the two plant juices, respectively. While, the mortality which achieved by *C. sativum* (Photo 1D) and *C. limon* peel juices at the first day still stable till the end of the experiment. The means of snails mortality were significantly higher in comparison to the untreated snails.

Table 2. Molluscicidal activity of plant juices against *M. cartusiana* snail under laboratory conditions

Treatment	Mortality percentages after indicated days					General mean
	1	3	7	14	21	
C. limon fruit	40.00 ^{b}	40.00 ^{b}	63.33 ^{a}	80.00 ^{a}	80.00 ^{a}	60.66 ^{a}
C. *limon* peel	63.33 ^{a}	63.33 ^{a}	63.33 ^{a}	63.33 ^{b}	63.33 ^{b}	63.33 ^{a}
C. sativum	20.00 ^{c}	20.00 ^{c}	20.00 ^{b}	20.00 ^{c}	20.00 ^{c}	20.00 ^{b}
M. spicata	23.33 ^{c}	23.33 ^{c}	26.66 ^{b}	26.66 ^{c}	26.66 ^{c}	25.33 ^{b}
Control	0.00 ^{d}	0.00 ^{d}	0.00 ^{c}	0.00 ^{d}	0.00 ^{d}	0.00 ^{c}
P	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***
L.S.D. $_{0.05}$	1.56	1.56	1.82	1.56	1.56	15.35

The same letter in the same column means not significant at P < 0.05

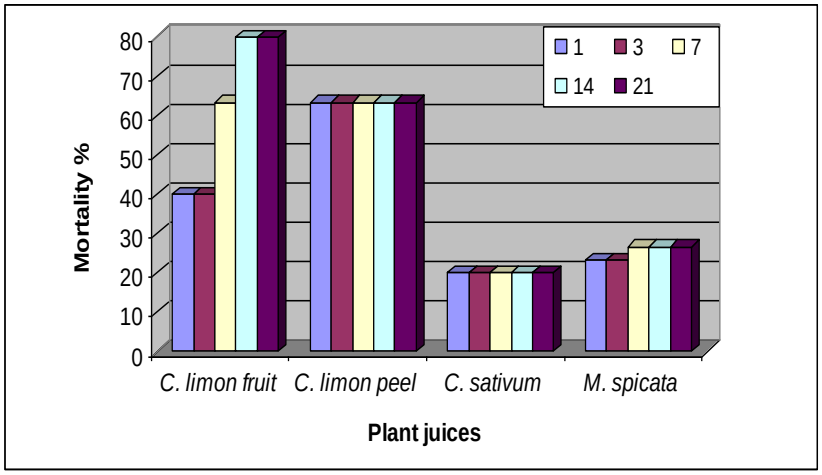

Fig. 1. Molluscicidal activity of plant juices against *M. cartusiana* snail under laboratory conditions.

Photo (1 A – D): Dead *Monacha cartusiana* snails after the treatment with plant juices. A, Clear furan in the shell aperture of snails treated with juice of *Citrus limon* peel. B, Breaking in the snails shell due to the treatment with *Citrus limon* fruit juice. C, Dead snails after the treatment with *M. spicata* juice. D, Dead snails after the treatment with juice of *C. sativa*.

Molluscicidal activity of botanical mixtures against *M. cartusiana* snail under laboratory conditions

Six binary mixtures of the plant juices were tested against *M. cartusiana* snails. Data in Table 3. and Fig. 2. indicated that *M. spicata* + *C. limon* peel was the most effective botanical mixture against snails. It was achieved 80% mortality of snails after only one day of the experiment followed by *C. limon* fruit + *C. limon* peel, *M. spicata* + *C. sativum*, *C. limon* fruit + *M. spicata*, *C. limon* fruit + *C. sativum* and *C. sativum* + *C. limon* peel which recorded 66.66, 63.33, 60, 50 and 46.66% mortality, respectively.

Table 3. Molluscicidal activity of botanical mixtures against *M. cartusiana* snail under laboratory conditions

Treatment	Mortality percentages after indicated days					General mean
	1	3	7	14	21	
C. limon fruit + *C. limon* peel	66.66 ^{ab}	80.00 ^{a}	80.00 ^{a}	100.00 ^{a}	100.00 ^{a}	85.33 ^{a}
C. *limon* fruit + *M. spicata*	60.00 ^{bc}	80.00 ^{a}	80.00 ^{a}	80.00 ^{ab}	80.00 ^{ab}	76.00 ^{ab}
C. limon fruit + *C. sativum*	50.00 ^{bc}	50.00 ^{b}	53.33 ^{b}	53.33 ^{c}	53.33 ^{c}	52.00 ^{c}
M. spicata + *C. sativum*	63.33 ^{abc}	63.33 ^{ab}	63.33 ^{ab}	63.33 ^{bc}	63.33 ^{bc}	63.33 ^{bc}
M. spicata + *C. limon* peel	80.00 ^{a}	80.00 ^{a}	80.00 ^{a}	80.00 ^{ab}	80.00 ^{ab}	80.00 ^{ab}
C. sativum + *C. limon* peel	46.66 ^{c}	46.66 ^{b}	60.00 ^{ab}	60.00 ^{bc}	60.00 ^{bc}	54.66 ^{c}
Control	0.00 ^{d}	0.00 ^{c}	0.00 ^{c}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
P	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***
L.S.D. $_{0.05}$	1.87	2.26	2.19	2.16	2.16	2.76

The same letter in the same column means not significant at $P < 0.05$

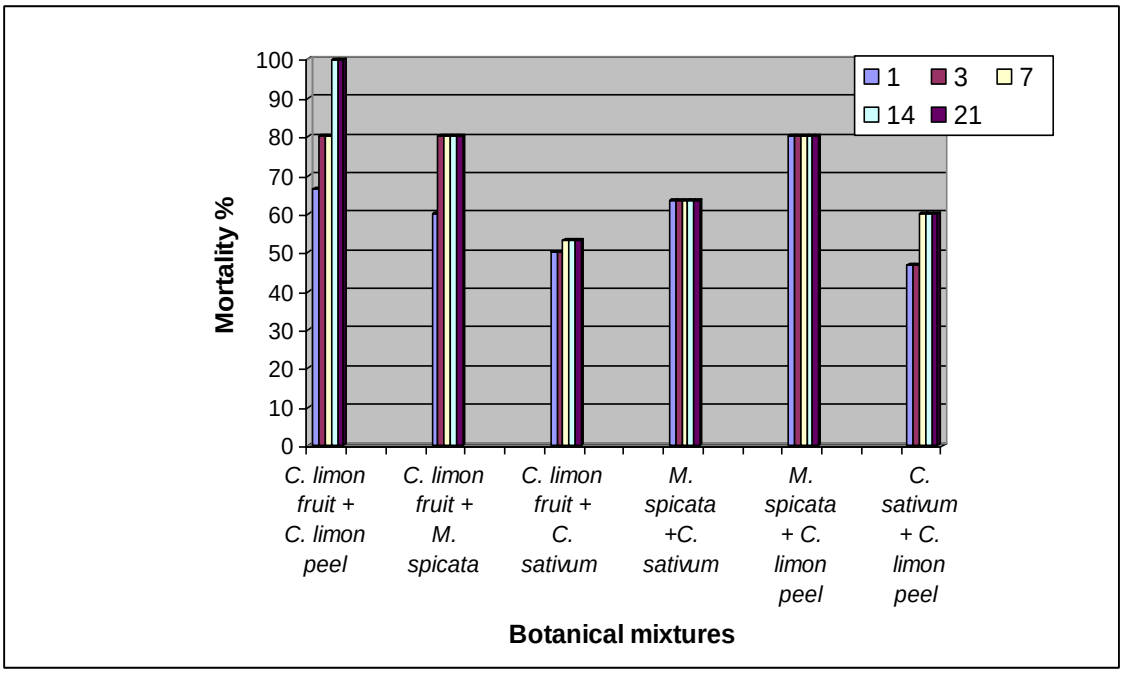

Fig. 2. Molluscicidal activity of botanical mixtures against *M. cartusiana* snail under laboratory conditions.

The mortality of snails which treated by *C. limon* fruit + *C. limon* peel and *C. limon* fruit + *M. spicata* mixtures highly increased to 100 and 80% after 14 and 3 days for the two mixtures, respectively. Moreover, *C. limon* fruit + *C. sativum* and *C. sativum* + *C. limon* peel mixtures also increased the mortality of snails to 53.33 and 60% after 7 days of the experiment, respectively. On the other hand, the other tested botanical mixtures were not showed any increase of snail's mortality till the end of the experiment period. All the botanical mixtures had significantly higher than the untreated control.

Table 4. Joint action of the binary mixtures of botanical juices against *M. cartusiana* snails

Treatment	Observed mortality %	Expected mortality %	Co-Toxicity factor
C. limon fruit + *C. limon* peel	100.00	92.66	7.92 d
C. *limon* fruit + *M. spicata*	80.00	85.33	6.24 a
C. limon fruit + *C. sativum*	53.33	84.00	36.51 a
M. spicata + *C. sativum*	63.33	41.32	53.26 p
M. spicata + *C. limon* peel	80.00	73.10	9.43 d
C. sativum + *C. limon* peel	60.00	70.66	15.08 a

a = antagonism p= potentiating (synergism) d = additive effect

The joint action between the binary mixtures of botanical juices against *M. cartusiana* snails was determined. The obtained results in Table 4. illustrated that the mixing of *C. limon* fruit juice with each of *M. spicata* and *C. sativum* juices and the mixture of *C. sativum* with *C. limon* peel juice showed an antagonistic effect with co-toxicity factors – 6.24, -36.51 and – 15.08%, respectively. While, the mixing of *C. limon* peel juice with each of *C. limon* fruit and *M. spicata* juices recorded an additive effect with co-toxicity factors 7.92 and 9.43%, respectively. The only potentiation effect was recorded by the combination of *M. spicata* with *C. sativum* with co-toxicity factor 53.26%.

Molluscicidal activity of plant juices against *M. cartusiana* snail under field conditions

The molluscicidal effect of plant juices against *M. cartusiana* snails was evaluated under field conditions (Table 5 and Fig. 3). As the juice of *C. limon* peel exhibited the highest effect against snails

in the laboratory, it was also recorded the highest initial effect 73.88% reduction under the field conditions. It was followed by *C. limon* fruit, *M. spicata* and *C. sativum* juices which recorded 54.91, 50.11 and 46.39% reduction, respectively. The effect of *C. limon* peel juice against snails increased with increase the experiment period. It was recorded also the highest residual effect 79.04% reduction. Comes in the second rank, *C. limon* fruit juice which gave residual effect 72.1%. The initial effect of *M. spicata* and *C. sativum* remained stable until the end of the experiment to give the same residual effects 50.11 and 46.39% reduction, respectively. That is a high significant difference in the reduction of snails treated with the different plant juices.

Table 5. Molluscicidal activity of plant juices against *M. cartusiana* snail under field conditions

Treatment	Reduction percentages after indicated days							General mean
	1	3	Initial effect	7	14	21	Residual effect	
C. limon fruit	52.33 ^{b}	57.50 ^{b}	54.91 ^{b}	72.1 ^{b}	72.1 ^{b}	72.1 ^{b}	72.1 ^{b}	65.22 ^{b}
C. *limon* peel	73.88 ^{a}	73.88 ^{a}	73.88 ^{a}	79.04 ^{a}	79.04 ^{a}	79.04 ^{a}	79.04 ^{a}	76.97 ^{a}
C. sativum	46.39 ^{b}	46.39 ^{c}	46.39 ^{b}	46.39 ^{c}	46.39 ^{c}	46.39 ^{c}	46.39 ^{c}	46.39 ^{c}
M. spicata	50.11 ^{b}	50.11 ^{bc}	50.11 ^{b}	50.11 ^{c}	50.11 ^{c}	50.11 ^{c}	50.11 ^{c}	50.11 ^{c}
P	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***
L.S.D. $_{0.05}$	8.64	10.23	9.43	6.46	6.46	6.46	6.46	7.09

The same letter in the same column means not significant at P < 0.05

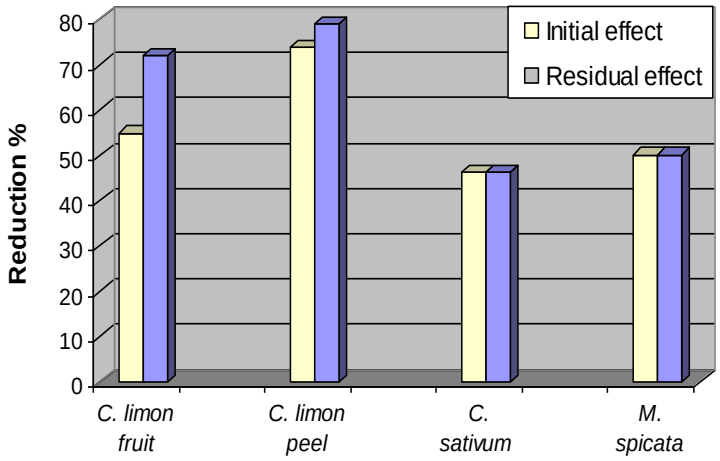

Fig. 3. Molluscicidal activity of plant juices against *M. cartusiana* snail under field conditions.

Molluscicidal activity of botanical mixtures against *M. cartusiana* snail under field conditions

As cleared in Table 6. and Fig. 4. most botanical mixtures achieved an excellent reduction of *M. cartusiana* snails under field conditions. The highest reduction of snails was recorded by *C. limon* fruit + *C. limon* peel which exhibited initial effect equal 88.12%. Next to this were *M. spicata* + *C. limon* peel, *M. spicata* + *C. sativum*, *C. limon* fruit + *M. spicata*, *C. sativum* + *C. limon* peel and *C. limon* fruit + *C. sativum* which caused 86.5, 77.92, 67.76, 59.34 and 58.08% reduction, respectively. The molluscicidal effect of all botanical mixtures was substantially increased with increase the experiment period. The highest residual effect 94.36% was also showed by the mixture *C. limon* fruit + *C. limon* peel. It was followed by M. *spicata* + *C. limon* peel, *M. spicata* + *C. sativum*, *C. limon* fruit + *M. spicata* and *C. sativum* + *C. limon* peel which achieved 91, 78.76, 77.75 and 71.58% reduction, respectively. While, the botanical mixture *C. limon* fruit + *C. sativum* was the least effective as it recorded the lowest residual effect 60.33% against tested snails. All the botanical mixtures showed high significant difference in the reduction of snails.

Table 6. Molluscicidal activity of botanical mixtures against *M. cartusiana* snail under field conditions

Treatment	Reduction percentages after indicated days							General mean
	1	3	Initial effect	7	14	21	Residual effect	
C. limon fruit + *C. limon* peel	84.70 [a]	91.54 [a]	88.12 [a]	91.54 [a]	91.54 [a]	100.00 [a]	94.36 [a]	91.86 [a]
C. *limon* fruit + *M. spicata*	62.51 [c]	73.02 [d]	67.76 [c]	73.02 [d]	80.12 [c]	80.12 [b]	77.75 [c]	73.75 [c]
C. limon fruit + *C. sativum*	58.08 [cd]	58.08 [f]	58.08 [d]	60.33 [f]	60.33 [e]	60.33 [d]	60.33 [e]	59.43 [e]
M. spicata + *C. sativum*	77.92 [b]	77.92 [c]	77.92 [b]	77.92 [c]	77.92 [cd]	80.45 [b]	78.76 [c]	78.42 [b]
M. spicata + *C. limon* peel	86.50 [a]	86.50 [b]	86.50 [a]	86.50 [b]	86.50 [b]	100.00 [a]	91.00 [b]	89.20 [a]
C. sativum + *C. limon* peel	53.66 [d]	65.02 [e]	59.34 [d]	65.02 [e]	74.86 [d]	74.86 [c]	71.58 [d]	66.68 [d]
P	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***
L.S.D. $_{0.05}$	4.69	4.66	4.10	4.64	4.22	2.69	3.31	3.20

The same letter in the same column means not significant at $P < 0.05$

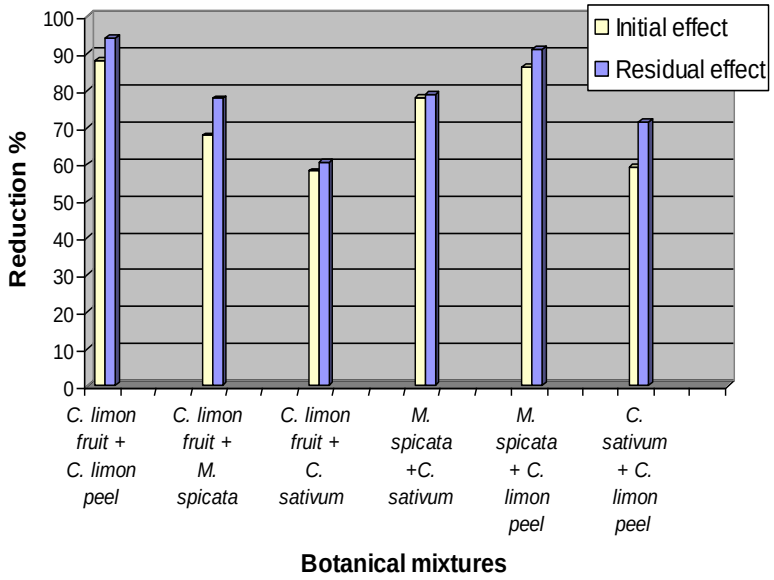

Fig. 4. Molluscicidal activity of botanical mixtures against *M. cartusiana* snail under field conditions.

4. Discussion

The use of natural crude plant products caused various degrees of reductions of the target pests and also have different levels of protection to the host. In the present study, the juice of *Citrus limon* fruit and *C. limon* peel were the most effective against *Monacha cartusiana* snails. They were recorded 80 and 63.33% mortality of the tested snails, respectively. These findings are in accordance with others obtained by **Benchawattanon and Boonkong (2006)** which reported that the extract of neem leaves and garlic (*Allium sativum*) showed more than 90% mortality of *Pomacea canaliculata* snail under laboratory conditions. The extract of *Derris elliptica* and *Cyperus rotundus* plants has also high toxic effect against the same snail species with LC_{50} of 23.68 mg / L and 133.20 mg / L, respectively **(Joshi *et al.*, 2005)**. In the same direction, **Radwan and El-Zemity (2007)** showed that thymol and eugenol have an observed molluscicidal effect against the white garden snail, *Theba pisana*. They cause rupture of the osmotic equilibrium of snail which lead to inflates and extension of the snail out of the shell. Our results indicated that the juice of *C. limon* peel which represent a plant waste exhibited the highest molluscicidal activity against snails under field conditions. At this trend, **Tangkoonboribun (2009)** consistent that the application of tobacco waste under field conditions proved its efficacy with 100% mortality of *P. canaliculata* snail. In the current study, *Mentha spicata* juice recorded 50.11% reduction of *M. cartusiana* snails under field conditions. A similar results were reported by **Salem *et al.* (2017)** stated that the crude extract of this plant achieved 44.1% reduction of *M. cartusiana* snails by spraying under field conditions. Added that, the chemical properties of the crude extract represent the main reason of the snail's death. Moreover, the variations in the molluscicidal effectiveness of plant extracts may be attributed to the species tolerance, concentration used and the phytochemical components **(Olofintoye, 2010)**.

As recorded in the present investigation, the binary mixtures of botanical juices showed an

excellent molluscicidal effect against *M. cartusiana* snails than its influence when used individually. The mixture of *C. limon* fruit + *C. limon* peel juice recorded 100% mortality of snails under laboratory and field conditions. The same effect was exhibited by the mixture *M. spicata* + *C. limon* peel juice against snails under field conditions. These results were strongly confirmed by **Oparaeke *et al.* (2005)** revealed that combining of two or more plant materials is more potent than when one plant material is used. Similarly, **Guruswamy *et al.* (2017)** reported that the combination of potent molluscicidal compounds from different plant extracts considered an effective alternative against land snails. The binary combination of nerium extract with each of tobacco, piper and neem extracts showed an observed synergistic effect against the *Pomacea maculata* snail. They are recorded 90, 93 and 100% mortality of the tested snails, respectively. The synergistic action of the binary combination of *Acalypha indica* and *Cedrus deodara* oil was more toxic to *Lymnaea acuminata* than the single treatment **(Rao and Singh, 2001)**. In the same aspect, **Chauhan and Singh (2011)** demonstrated that the binary combination of taraxerol from *Codiaeum variegatum* and the extract of *Euphorbia tirucalli* against the same snail species increased the toxicity by 9.51 times relative to the individual treatments. The activities of the botanical mixtures was vary according to the species combination and dosages **(Taguiling, 2015)**. In addition, the combination of plant materials was effective due to the synergistic effects. This synergism may be attributed to the phytochemical constituents of the single plant material **(Guruswamy *et al.*, 2017)**. These constituents when combined with each other they act synergistically to enhance the toxic substances in the botanical mixtures either by increasing or prolonging their effects **(Oparaeke *et al.*, 2005)**.

Conclusion

The results of this investigation shows that plant juices and their mixtures could form the basis for successful formulation and commercialization of Biopesticides. These plants are available in the local markets all the year, they are safe, cheap, easily biodegradable and environmentally friendly. It is important to throw the light especially on the juice of the plant waste, *Citrus limon* peel for control *M. cartusiana* snails. It has no cost and at the same time shows observed strong molluscicidal effect against the snail individuals. Moreover, it is represent a basic component in the most effective binary mixtures against the snail individuals. They could provide promising valuable alternatives to the chemical pesticides in the management of the harmful snail, *M. cartusiana*.

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