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## EFFECT OF CLODINAFOF-PROPARGYL COMPOUND AGAINST LAND SNAIL, *HELIX ASPERSA*: IMPLICATIONS FOR CONTROL

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### ABSTRACT

The effect of clodinafop –propargyl against the mucus of land snail, *Helix aspersa*, was studied under laboratory and field conditions during autumn 2020 season. Snails were exposed to serial concentrations of the clodinafop-propargyl for successive seven days using contact and bait techniques. The Median Lethal concentration (LC<sub>50</sub>) was determined and the effect of LC<sub>25</sub> of test compound was examined on the total protein and alkaline phosphatase after seven days of treatment. In the field, the effect of the test compound used poison bait was evaluated after 21 days against *H. aspersa* on mango nursery trees at a reclaimed land in Abu- Rwash, Giza Governorate, Egypt. The effect of clodinafop –propargyl (25.200 ppm) was compared with methomyl (4.000 ppm) a compound recommended by Ministry of Agriculture and Land Reclamation in the field trials. The effects were evaluated after 1, 3, 7, 15, and 21 days of treatment with both compounds. Based on the laboratory data for clodinafop –propargyl the LC<sub>50</sub> were 6.750 ppm for the contact and 8.250 ppm for the bait method after seven days of treatment. Moreover, the compound caused a reduction in the total protein and alkaline phosphatase compared with the control. In the field, the compound caused 90.7% reduction in snail population compared to 75.0% reduction caused by methomyl after 21 days of treatment. Therefore, it be concluded that clodinafop –propargyl compound can be used as poison bait in the control programs to combat land snails.

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### INTRODUCTION

Land gastropods comprise a highly diverse group of molluscs. Several species of terrestrial snails and slugs spread into new areas outside their original habitat range due to agricultural and human activities. These species become pests in these new areas by causing damage to major economical field crops and horticulture plants. Moreover, they transmit the parasitic diseases that affect humans and farm animals (Pokryszko, 2002). The brown garden snail, *Helix aspersa*, is a terrestrial

land snail, and one of the known pest species in the world. It is widespread in several countries living under different environmental conditions as a pest of field, ornamental, and horticulture crops (Rosenberg G. and Bouchet, 2014; Eshra, 2013).

The present study was conducted to reduce the secretion of mucous (the first lines of defence) by using clodinafop- propargyl. Davies and Hawkins (1998) mentioned that the mucous, in general, is used to protect cell surfaces exposed to the external environment and is

crucial in molluscan defence and locomotion. This mucous is made up of a range of lectins, charged mucopolysaccharides, glycoproteins, proteins, uronic acid, sialic acid, hexosamine and a variety of other molecules in an aqueous carrier medium (Furuta *et al.*, 1995; Kandil *et al.*, 2014).

Alkaline phosphatase (ALK) plays a vital role in spermatogenesis (Mobarak, 2016; Kandil *et al.*, 2014; Pavlikova and Repas, 1975). It is also associated with protein structure (Mobarak, 2008; Pilo *et al.*, 1972). On the other hand, it helps in identifying the different zones of adult shell forming tissue (Marxen *et al.*, 2005). It has been reported that mucous cells contain acid and alkaline phosphatases and the mucus released from mucus cells which had activity of both enzymes (Ning *et al.*, 2005). Proteins provide the source for the rapid replacement of tissue proteins during tissue depletion, such as lipids, vitamins, hormones, and certain enzymes (Warnick and Carter, 1972).

Controlling the population of land molluscs is not a simple task because the habitat requirements and behavior differ according to species (Godan, 1983). Chemical control is still the most common method especially in large areas to protect plant and the specific molluscicides are very limited number against land snails. For this reason, it was necessary to find new effective compounds Ogeleka *et al.* (2017) who observed that the herbicide Grassate caused slower movement of snails after treatment compared to their movement before exposure to the herbicide. Clodinafop - proprgyl is a selective herbicide used to control annual grassy weeds in wheat fields (Bibi *et al.*, 2008), therefore we can test the herbicide to control the snails that prefer to hide under herbs. This study was designed to examine the effect of clodinafop- proprgyl herbicide on the land snail, *H. aspersa*, under laboratory and field conditions.

## MATERIALS AND METHODS

### Experimental animals.

Individuals of the brown garden snail, *H. aspersa*, were collected from mango nursery trees *Mangifera indica* 1 (Ewies MangoType). in Abu-Rwash, Giza Governorate, Egypt coordinate (N30.8" E31. 5'26") and were transported to the standard laboratory of Plant Protection Research Institute, Agricultural Research Center, Giza Governorate Egypt (N30°2'44" E31°12'26"). The animals were placed in small glass boxes containing moist soil (1:1 mixture of clay and sand, 10cm high).

Each box was provided with fresh green lettuce leaves and was covered with a muslin cloth secured with a rubber band, to prevent snails from escaping and maintained under  $20 \pm 2^\circ\text{C}$  in the laboratory for 2 weeks for acclimatization. A total of 10 animals were used for each concentration of treatment and also for the control.

### Test compounds

Common name: Clodinafop- Propargyl.

Trade name: Topik (15% WP), is a selective herbicide used to control annual grassy weeds in wheat fields. The LD<sub>50</sub> value for rats is 4000 mg/kg, and it is effective at a rate of 140 g/fed. It was obtained from Syngenta Egypt Company.

Common name: Methomyl

Trade name: Newmyl (20% SL) is an insecticide a carbamate compound, Ministry of Agriculture and Land Reclamation (MALR) has recommended this compound for use against land snail infestation in Egyptian fields, at a rate of 8- 10 kg/ fed used as bait. The LD<sub>50</sub> (Half Lethal Dose) value for rats is 17- 24 mg/ kg. It was obtained from Kafr El-Zayat Company, Egypt.

### Laboratory Experiments

#### Contact (thin film layer) technique

The thin film layer method was used in this study as described previously (Ascher and Eliyahu, 1981). The tested concentrations were 3.750, 7.500, 11.250, 17.250 and 25.200 ppm (Part per million) of clodinafop-propargyl. On the surface of each Petri- dish 2 ml of each concentration was applied with water. The water was evaporated under room temperature, leaving a residual layer film of the test compound. The snails were exposed individually to each concentration of the test compound for seven days. After that the live snails were transferred to clean cages and feeding on lettuce with follow-up. A parallel control test was conducted using plain water. Mortality percentages were calculated with recorded the observation and LC<sub>50</sub> (Half Lethal Concentration) value was determined as described by Finney (1971) after seven days of treatment.

#### Baiting technique

Serial concentrations of clodinafop- propargyl at the rate of (3.750, 7.500, 11.250, 17.250 and 25.200 ppm were prepared by mixing the test compound with 5% molasses + 93% wheat bran+2% of compound. Then 5 g.

of poison bait was placed on a plastic sheet on the surface of the soil in each glass box. The snails were exposed individually to the candidate concentrations of the test compound for 7 days. Control snails were exposed to the bait without test compound. After 7 days of treatment, the live snails were transferred to cleaner cages and were fed on bait without poison. Mortality percentages were calculated after seven days of treatment and LC<sub>50</sub> value was determined.

### Biochemical studies

Total protein content and ALK (Alkaline phosphatase) activity were measured after seven days of treatment with LC<sub>25</sub> of clodinafop – propargyl compound in the contact technique to elucidate the physiological effect, compared with the control.

### Sample preparation

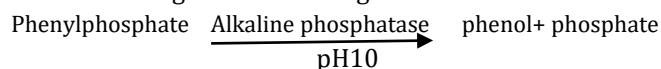
After removing the shell of treated snails, 1g of them was homogenized for 3 minutes under cooling in a homogenizer with 10 ml of sodium chloride 0.9N and then centrifuged (3000 rpm., for 15 min) to determine the total protein content according to Bergmeyer (1967) and modified according to Singh and Agarwal (1987). Another 1g was centrifuged (5000 rpm) for 20 min to determine (ALK) activity as described by Moss (2016). The extraction process takes not more than 24h under cooling conditions in the refrigerator. A Parallel control test was also conducted.

### Determination of total protein content.

Soluble protein was determined according to the method described by (Titez, 1994) using Biuret reagent. The developed color was measured at 546 nm spectrophotometrically.

### Determination of ALK activity.

ALK activity was evaluated spectrophotometrically according to Moss (1982). The developing of color was done according to the following reaction:



The liberated phenol was measured in the presence of amino – 4- antipyrine and potassium ferricyanide. The obtained results were statistically analyzed by one - way ANOVA (Analysis of Variance) and LSD (Least significant difference) at (P ≤ 0.05) using the Costat program (COHORT Software, 2005).

### Field Experiment

The most effective concentration in the laboratory was 25.200 ppm of clodinafop- propargyl against *H. aspersa* was evaluated as the poison bait. The compound was compared with methomyl 4.000 ppm (The compound recommended by MARL). The poison bait contained (93% wheat bran + 5% black honey+ 2% of tested compound concentration); 100gm of bait was placed on a plastic sheet under nursery trees. The two compounds were tested against, *H. aspersa* at a reclaimed land in Abu- Rawash, Giza Governorate on mango nursery trees. Nine plots were chosen (each of 60m<sup>2</sup>) three plot replicates for each compound and three plots for control, with a distance of 2m between each plot and the other (Randomized Blocks with Sub plot replicates). The bait was renewed each 4 days. Live snails were counted on randomized trees in each tested plot pre, and post treatment at 1, 3, 7, 15, and 21 days of treatment. The reduction in snail population was calculated after 21 days of treatment according to the formula described by Henderson and Tilton (1955).

Data Analysis, obtained data was analyzed as one way ANOVA, using Proc ANOVA in SAS software (Version 9.1; SAS Institute, Cary, NC, USA) (SAS Institute, 2008), and means were compared by Tukey's HSD (P= 0.05 level) in the same program.

## RESULTS AND DISCUSSION

### Laboratory studies

Table 1 shows the effect of clodinafop- prpargyl (herbicide) against land snail, *H. aspersa*, evaluated using contact and bait methods after two different periods of treatment. In the contact technique a gradual increase was observed in the mortality percentage with the increase in the concentrations of test compound. At 3.750, 7.500, 11.250, 17.250, and 25.200 ppm concentrations, the mortality rates were 10, 20, 40, 40, and 50% after 3 days, respectively, and 20, 60, 70, 90, and 100% after 1 week of treatment, respectively. In the bait technique, the mortality rates were 0, 10, 10, 50, and 60% after 3 days of treatment, and 30, 40, 50, 70, and 80% mortality after 1 week of treatment, respectively.

Hence, the herbicide compound, clodinafop- propargyl might be more toxic when used as contact than when used as poison bait and the LC<sub>50</sub> values were 6250 and 8250 ppm for contact and bait techniques, respectively after 1 week of treatment. These results were consistent with those reported by Mobarak (2016) who found that

the contact method was more toxic than the bait method against *Eobania vermiculata* treated with chlorofluazuron. However, contrasting results were reported by Gabr *et al.* (2006) who showed that vertemic was more toxic when used as poison bait than as contact against land snails, *Eobania vermiculata* and *Monacha obstructa*. It was also obvious that clodinafop-propargyl exerted a high toxic effect after long time of treatment with both tested methods. This may be due to the time that the compound takes after being consumed by the snail to arrive at the site of action and exert its

effect.

Moreover, the observed mortality might be due to the compound affected on protein of the snail's body. Ogeleka *et al.* (2017) investigated the effect of the herbicide compound Grassate on the land snail, *Achachatina marginata* and it decreased the growth and biomass of the land snail with increasing concentrations. Ali (2017) and Godan (1983) mentioned that herbicides not only kill weeds but also mollusks either through the animal skin or by ingestion through the intestine.

Table 1. Effect of clodinafop- propargyl herbicide on the land snail, *Helix aspersa* using contact and bait techniques, after two different periods of treatment.

Techniques	Concentration (ppm)	Mortality %		LC <sub>50</sub> (ppm) after 1 week	Upper (ppm)	Lower (ppm)
		72h	1 week			
Contact	3.750	0	20	6.750	10.200	4.200
	7.500	20	60			
	11.250	40	70			
	17.250	40	90			
	25.200	50	100			
Bait	3.750	0	30	8.250	15.000	1.500
	7.500	10	40			
	11.250	10	50			
	17.250	50	70			
	25.200	60	80			

### Clinical symptoms

Table 2 shows the clinical symptoms caused by clodinafop- propargyl on *H. aspersa* during the treatment periods. The compound caused decreasing in the weight of the treated snails (0.5 and 1.0 g) comparing with untreated snails (4 and 3 g). This may have occurred due to the loss of a percentage of the body water contents. The colors of mucus changed from creamy in control animals to brown in treated animals which may be due to the effect of the compound on the structure and cells of mucus. Furthermore, after treatment the mucus secretion contained bubbles and then its decision and caused the death of the snails. This might be attributed to the effect of the compound on mucus gland and its physiology.

The dehydration of mucus may be due to the effect of the compound on the enzymes which responsible for secreting mucus. The movement of the all- treated snails was also slower than that of untreated snails during the period of experiment which may be due to the toxic

effect of the compound on the locomotive cells. These results were consistent with those reported by Ogeleka *et al.* (2017) who observed that the herbicide Grassate caused slower movement of snails after treatment compared to their movement before exposure to the herbicide.

### Biochemical studies

Table 3 shows the results of the effect of the clodinafop-propargyl on total protein contents and ALK activity in *H. aspersa* after 7 days of treatment. The compound had a significant effect on the total protein content, reducing it from 1.8 g/dl in control to 0.186 g/dl in the treated animals indicating a-84% reduction. Moreover, the compound significantly inhibited the activity of ALK which was decreased to 202.6 U/l in treated animals from 304 U/l in control, animals indicating, a-33.4% reduction. These results, clearly indicate that the compound may has an effect on the amino acids that are essential for protein synthesis which ultimately the

death of treated snail. Ogeleka *et al.* (2017) reported that treatment with the herbicide glyphosate decreased the growth and biomass of land snail, *Achachina marginata* after treatment. It has also been reported that the application of herbicides to control weeds causes indirect effects on the growth and reproduction of land snails (Ogeleka *et al.*, 2017; Buia M. *et al.*, 2000). Beeby and Richmond (2002) also reported that glyphosate treatment decreased in the weight of the albumen gland, which is the source of protein for egg development inside the hermaphrodite snail gland. The compound clodinafop-propargyl also influenced the activity of ALK

which may explain the color change of mucus in treated animals. Moreover, the inhibition of ALK activity affected mucus production in mucus gland, which resulted in desiccation ultimately leading to death of treated snails. On the other hand, the defect in ALK activity affected the shell structure of snails. ALK helps in identifying the different zone of shell formation (Mobarak, 2014; Marxen *et al.*, 2005). Mobarak *et al.* (2015) reported the LC<sub>50</sub> of clove extract decreased ALK activity in the land slug *Limax flavus* after treatment. In addition, mucus cells contain acid and alkaline phosphatases that produce the mucus (Ning *et al.*, 2005).

Table (2) Clinical symptoms on the land snail, *Helix aspersa* during seven days of treatment with the herbicide clodinafop - propargyl.

Parameters	Untreated	Clinical symptoms on treated animals
Body weight	3 - 4 g	0.5 - 1 g
Color of mucus	Creamy	Brown
Viscosity of mucus	High viscosity	Low viscosity (liquid).
Mucus status	Normal	Bubbles and then dehydration.
Animal movement	Normal	Slower

Table 3. Effect of LC<sub>25</sub> (ppm) of clodinafop- propargyl on Total protein content and Alkaline phosphatase activity in the land snail, *Helix aspersa* after seven days of treatment.

Parameters	Control mean ± SE	Treatment mean ± SE	Differences%	LSD
Total protein content (g/dl)	1.18 ± 0.03a	0.186 ± 0.27b	-84.2	0.11
Alkaine phosphatase activity (U/L)	304 ± 40.5a	202.6 ± 81.4b	-33.4	75.2

$$\text{Difference\%} = \frac{\text{Mean of control} - \text{Mean of treatment}}{\text{Mean of control}}$$

a= Nonsignificant b=Significant

Table 4. Efficacy of clodinafop - propargyl herbicide comparing with methomyl compound as bait against *Helix aspersa* land snail after three weeks of treatment under field conditions.

Treatment	Rate of application (ppm)	No. of animals' pre- treatment		No. of live animals' post - treatment		Population reduction%	F value
		Total	mean± SD	Total	mean± SD		
control	-	345	21.3±11.2	603	34.5±14.4	-	
methomyl	4000	372	23.5±7.9	162	11.3 ± 6.1 <sup>b</sup>	75.0	9.59
Codinafop- prpargyl	25200	555	33.5±15.4	90	9.25±4.7 <sup>a</sup>	90.7	

P < 0.05, a = significant, b = non-significant.

### Field Performance

The effects of clodinafop- propargyl on land snail, *H. aspersa* in comparison with methomyl under field conditions are presented in Table 4. There was a

significant effect with 90.7% reduction in the snail's population after treatment with clodinafop- propargyl whereas methomyl treatment resulted in only 75.0% reduction. This implied that the herbicide compound clodinafop- propargyl produced good outcome and

higher efficacy against land snails under field conditions. It could reduce land snail populations and it could also be used as a herbicide when snails prefer to hide under weeds. Ali (2017) investigated the effect of roundup against *Monacha cartusiana* and reported 17.20% population reduction. Mobarak (2016) also recorded that 94%, and 78.7% population reductions in *Eobania vermiculata* treated with methomyl and chlorfluazuron treatments, respectively.

### CONCLUSION AND RECOMMENDATIONS

The compound clodinafop-propargyl achieved good results for reducing the population of land snail comparing with methomyl (the recommended compound). Therefore, this study recommends that it could be used in the control programs of land snails, in addition to its use as an herbicide, especially in wheat fields, to reduce the population of both pests' weeds and land snails.

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