Egyptian Journal of Plant
Protection Research Institute
www.ejppri.eg.net

Impact of some chemical pesticides against the glassy clover snail *Monacha cartusiana* (Gastropoda: Hygromiidae) adults and their effects on genotoxicity

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ARTICLE INFO
Article History
Received: 18/7/2022
Accepted: 8/ 9/2022

Keywords

Pesticides, *Monacha cartusiana* snail, Gastropoda, Hygromiidae and Genotoxicity.

Abstract

Toxicological studies disclosed that the molluscicidal activity of chemical pesticides, i.e., Methomyl, avaunt, pestban, and herbazed against the glassy clover snail, *Monacha cartusiana* (O. F. Müller) (Gastropoda: Hygromiidae) adults using the poisonous baits technique under laboratory conditions. Comparative toxicity indicated that methomyl was the most impact compound followed, with avaunt, pestban and herbazed pesticides. The mortality percentages were increasing with augmenting the concentrations of tested pesticides and times of exposure. Comparing the toxicity action of the four tested pesticides based on toxicity index, methomyl was considered as the standard pesticide recorded 100%, while a toxicity index values of avaunt, pestban, and herbazed for LC_{50} and LC_{90} were (70.00 ,57.19 and 53.00) and (65.26, 60.19 and 57.89), respectively. Finally, data indicated that all tested pesticides reasoned augmentation in DNA damage, tail length, DNA in tail, tail moment and olive tail moment. Methomyl was the most pesticide that caused increasing in DNA damage of target cells (17.58%), while avaunt caused 15.61% subordinate by pestban and herbazed with 11.60 and 10.59%, respectively compared with control (7.35%).

Introduction

Mollusks studied that diverse many distinguished lineages of terrestrial gastropods and belong to the second- biggest phylum next arthropods in terms of number of species. Terrestrial mollusks have been excessed greatly worldwide while forming about 6% of total species on earth and else showing economic importance (Clark and May, 2002). Land mollusks became one of the most dangerous pests attacking numerous plants (Shahawy, 2019). The devastating likelihood of these pests is far better today than in previous times as an effect of ever denser and faster transport and traffic (Godan, 1983; Ali and Suleman, 1992 and Barker, 2002).

Newly, in Egypt, land snails have been considered a dangerous pest that attaches several crops, i.e., Vegetables, cereals, fruit gardens, and decorative plants at the diverse growth stages causing a decrease in the production of these yields. Moreover, a land snail in some crops is now becoming evident with a reduction in the consequences of other pest groups

such as insects. Besides, the locomotion of snails causes unsolicited odor by secreting viscid liquids on the plants preventing men and field animals from feeding on this contaminated plant (Kassab and Daoud, 1964; El-Okda, 1984 and Sallam *et al*., 2009).

The glassy clover snail, *Monacha cartusiana* (O. F. Müller) (Gastropoda: Hygromiidae) is deemed the most predominant snail in several governorates in Egypt, and many authors reported a relatively high incidence and population density of *Monacha* spp. in Sharkia Governorate (Ismail,1997; Hegab *et al*., 1999 and Mahrous *et al*., 2002). Traditional pesticides especially carbamates and organophosphorus compounds are effectively used in Egypt just as in many nations as spray or baits (Heiba *et al*., 2002). So, both were applied, methomyl is one of the strong carbamates utilized especially in bait methods towards terrestrial gastropods and pestban as an organophosphorus compound was found to actuate oxidative stress and affect some biochemical targets in snails (Salama *et al*., 2005).

Regarding, Avaunt is a novel pesticide that belongs to the oxdiazine pesticides group which acts on the target organism as a sodium channel blocker (Shono *et al*., 2004). Finally, herbazed pesticide is used as herbicide in the laboratory using the baits technique. So, linking the reactions due to these pesticides with the progress of these compounds with the physiological system (Schlenk, 1999).

The main goal of the present work is to study the following points: Evaluation of the toxicity impact of some pesticides belonging to the different groups towards *M. cartusiana* adults under laboratory conditions and determined comet assay as geneotoxicity of tested pesticides.

Materials and methods

1. Examined animal:

Land snail, the glassy clover snail, *M. cartusiana* adults needed for the present trails were collected from a farm cultivated with Egyptian clover, *Trifolium alexandrinum* L. at Sharkiea Governorate, Egypt. Individuals were immediately transferred in white cloth bags to the laboratory. Healthy and similar snails were select and kept in glass terrarium filled with wet mud soil amended at 75% of water field capacity. Snails were fed daily with bran for two weeks before treatment for acclimation.

2. Examined chemical pesticides:

Pesticides were methomyl 90% S.P. (Trade name), lannate (common name), S-methyl-N- {(methyl carbamoyl) oxy} thioacetimidate (chemical name). Pestban 48% E.C. (Trade name), chlorpyrifos (common name), Chlorpyrifos (O, O-Diethyl O-3,5,6- trichloropyridin-2-pyridinyl) phosphorothioate (chemical name). Avaunt 15% E.C. (Trade name), indoxacarb (common name), S-methyl -7-chloro-2,4,4a,5-tetrahydro-4a-(methoxycarbonyl) indeno[1,2-e] [1,3,4] oxadiazia-2-carbonyl]-4-(trifluoromethoxy) carboxylate (chemical name). Finally, Herbazed 48% W.C. (Trade name), glyphosate (common name) N-(phosphonomethyl) glycine, isopropyl amminoum salt (chemical name).

3. Toxicity studies:

The present search was achieved at laboratory of Plant Protection Research Institute to research the influence of some pesticides which belonging to different chemical groups against the land snail, *M. cartusiana*, adults using baits technique.

4. Toxic baits technique:

Baits were designed by mixing five concentrations (2.00, 1.00, 0.5, 0.25 and 0.125%) of each tested compound merged with five parts of molasses then finished with wheat

bran to 100 parts and wetted with small amounts of water (Ghamry *et al.*, 1994). Three replicates of plastic boxes (3/4 kg capacity) were utilized for each concentration of pesticide; Five g of each toxic bait were spread into each box. Three plastic boxes (3/4 kg capacity) were utilized for each concentration. 5 g of each toxic bait was spread into each box.

Control treatment was designed using bran bait. Five adult individuals of snails were put into each plastic box, then enveloped with muslin cloth and secured with rubber band. Daily examination was carried out for treatments by utilization stainless steel needle (El-Okda, 1981).

The dead snails were secluded daily, and mortality percentages were registered until the 28 days after treatment. The average of mortality percentages of each component was corrected utilization Abbott's (1925) formula. LC_{50} and LC_{90} values were calculated utilization (BioStat, 2007) (Professional Build 3200).

Toxicity index (T.I) was determined utilization formula of Sun (1950) Neutralization as follows: Toxicity index = [LC_{50} or LC_{90} of the highest efficient compound / LC_{50} or LC_{90} of the other compound] ×100.

Relative potency (R.P) was determined utilization formula of Zidan and Abdel-Maged (1988) Neutralization as follows: Relative potency (R.P.) = [LC_{50} or LC_{90} of the other compound / LC_{50} or LC_{90} of the highest efficient compound].

5. Genotoxicity of pesticides:

Comet assay:

The tissues of *M. cartusiana*, adult processe d with LC_{25} of pesticides was utilized after three days of treatment for the alkaline comet assay according to **Singh *et al.* (1988)**. For each sample of pesticide, 100 incidentally opted cells were depicted and scanned for picture analysis. The cells with short heads and big fan-like tails were an exemption. The pictures were analyzed utilization the comet score analysis method for each cell, the length of DNA emigration (Tail length) was measured on Pixel from the center of the nucleus to the end of the tail, DNA percentage in the tail was measured wherein the total intensity (Fluorescence) in the cells, which was delimited as 100%, and the percentage of total intensity collimated to the intensity just in the tail was rimmed. The tail moment explaine d in arbitrary units was computable as (Tail length x% migrated DNA)/ 100.

6. Statistical analysis:

The statistical analysis was limited by utilizing one way test, (ANOVA), **Cohort Software (2005).**

Results and discussion

1. Toxicological studies under laboratory conditions:

1.1. Accumulation mortality percentage of *Monacha cartusiana* adults exposed to numerous chemical pesticides using baits technique:

Results in Table (1) reported that toxicity influence of tested pesticides, methomyl, avaunt, pestban, and herbazed against *M. cartusiana* adults. Data studied that methomyl (2%) due to mortality with percentages 100% after 28 days post-treatment, while at concentration 1% caused 40% mortality after the first day to reach its maximum effect than 14 days with that 100%. At a concentration of 0.5% remarked after one day with 20% to record 100% mortality percentage at 21st day. While, Avaunt initiated its initial impact after one week at concentration 2% with mortality percentage (86.67%) to achieve 100% mortality till the end of exposure snails, but at concentration of 1%, the mortality percentage rates acquired after 7, and 28 days were 53.33 and 100%. At concentration 0.25% mortality percentages and were (13.33,

20.00,40.00, 53.33, and 66.67%) post 3, 7, 14, 21 and 28 days post-treatment, inversion to concentration of 0.125% which have slightest impact and recording for 7 day without any impact and increased until 28 day with 33.33%. Regarded to pestban efficiency after 14 days of exposure gave 100% mortality of treatment at 2% concentration, to come with 40% mortality percentage on 7th day then killed all snails post 21st and 28th day when used to 1% of concentration.

Table (1): Accretive mortality percentage of *Monacha cartusiana* adults exposed to varied chemical compounds using baits technique.

Tested materials	Conc. %	Accretive mortality / day						
		1	3	7	14	21	28	Mean mortality
Methomyl	**0.125**	0.00	0.00	13.33	26.67	33.33	40.00	18.89^{kl}
	0.25	0.00	13.33	33.33	40.00	66.67	73.33	37.77^{hi}
	0.5	20.00	26.67	53.33	73.33	100.00	100.00	62.22^{de}
	1.0	40.00	53.33	86.67	100.00	100.00	100.00	80.00^{bc}
	2.0	73.33	86.67	100.00	100.00	100.00	100.00	93.33^{a}
Avaunt	**0.125**	0.00	0.00	0.00	13.33	26.67	33.33	12.22^{kl}
	0.25	0.00	13.33	20.00	40.00	53.33	66.67	32.22^{ij}
	0.5	13.33	26.67	33.33	73.33	93.33	93.33	55.55^{ef}
	1.0	26.67	40.00	53.33	100.00	100.00	100.00	70.00^{cd}
	2.0	53.33	73.33	86.67	100.00	100.00	100.00	85.56^{ab}
Pestban	**0.125**	0.00	0.00	0.00	13.33	26.67	33.33	12.22^{kl}
	0.25	0.00	6.67	13.33	33.33	46.67	53.33	25.55^{jk}
	0.5	6.67	13.33	26.67	53.33	73.33	86.67	43.33^{gh}
	1.0	13.33	26.67	40.00	86.67	100.00	100.00	61.11^{de}
	2.0	33.33	53.33	86.67	100.00	100.00	100.00	78.89^{bc}
Herbazed	**0.125**	0.00	0.00	0.00	6.67	13.33	13.33	5.55^{l}
	0.25	0.00	0.00	6.67	20.00	26.67	26.67	13.34^{kl}
	0.5	0.00	6.67	20.00	33.33	46.67	53.33	26.67^{jk}
	1.0	13.33	20.00	33.33	53.33	73.33	93.33	47.77^{fg}
	2.0	20.00	33.33	66.67	86.67	100.00	100.00	67.78^{cd}
Control		0.00	0.00	0.00	0.00	0.00	0.00	0.00^{l}
P		**0.0001*****	**0.0001*****	**0.0001*****	**0.0001*****	**0.0001*****	**0.0001*****	**0.0001*****
L.S.D.0.05		**12.46**	**9.28**	**9.59**	**9.29**	**8.30**	**8.31**	**19.55**

At 0.5% concentration the mortality percentages were (6.67, 13.33, 26.67, 53.33, 73.33 and 86.67%) after 1, 3, 7, 14, 21, and 28 days, respectively. For 0.25% concentration small effect happened begging from the third day, with percentage mortality of 6.67%, to be noticed after two weeks, by percentage mortality 33.33%, to reason its highest effectiveness after 28th the day of exposure with percentage 53.33%. While, Data not recorded any mortality until the 7th the day when processe d *M. cartusiana* adult was treated with 0.125%, to occur its impact, then 28 days with a percentage of 33.33 %. In connection with, herbazed, it existed constant at 66.67% for one week of treatment which was observed with 100% mortality percentage after 21and 28 days of exposure with 2% of concentration. At a concentration of 1% attained mortality percentages of 33.33% after 7 days, but after 21 and 28 days, it was reported with mortality percentages (73.33 and 93.33%), respectively. Regard to herbazd at a concentration of 0.5% gave mortality percentage with (20%) post 7day and after

28 days of treatment noticed with 53.33%. Herbazed at a concentration of 0.25%, noticed in constant death rates from 21 days until 28 days, also at a concentration of 0.125% with mortality with percentage (13.33%) for the same time of exposure. These outcomes were similar with Triebskorn and Ebert (1989) reported that increasing the concentration of methomyl pesticide and time elapsed due to increase the mortality percent of juveniles and adults. The carbamate, methomyl noticed with the highest toxic action against *M. cartusiana* snail followed by the marshal and dursban, respectively (Daoud, 2004). Ebenso *et al*. (2005) reported *Limicolaria aurora*, snail desistance feeding post-treatment with methomyl and furadan. At a concentration of 1%, methomyl was 92.5% mortality of *M. cartusiana* after 28 days of treatment El-Shafiey *et al*. (2010). Also, Shetaia *et al*. (2013), noticed that methomyl was the most potent compound in the laboratory for controlled *M. cartusiana*. Ismail *et. al*. (2022) studied that the impression of methomyl pesticide was increased as the concentration was increased in all treatments against some land snails *M. cartusiana*, *Succinea putris* and *Eobania vermiculata*. Also, it is clear from the results that *M. cartusiana* was the most sensibility for tested pesticide using the baits technique.

1.2. Comparative toxicity of methomyl, avaunt, pestban and herbazed as pesticides against adult of *Monacha cartusiana* snail using baits technique:

The tested pesticides could be arranged according to their ability against snail were (1436.40 ±14.12 and 3013.60 ±39.90), (2061± 24.60 and 4617.58 ±10.72), (2511.20 ± 28.15 and 5006.00 ±11.03) and (2710.60 ±25.13 and 5205.50±14.12) µg/ml, for methomyl, avaunt, pestban and herbazad, respectively. Comparing the toxicity action of the four tested toxicants based on toxicity index, methomyl was considered as the standard insecticide and registering 100%, while a toxicity index value of avaunt, pestban, and herbazed for LC_{50} and LC_{90} were (70.00 ,57.19 and 53.00%) and (65.26, 60.19 and 57.89%), respectively. The LC_{50}, LC_{90}, relative potency and toxicity index values were presented in Table (2) and Figure (1) whereas, methomyl was the most efficient compound followed by avaunt, pestban, while herbazed was the least once. Relative potency level can also be utilized as the easy method in comparing the degree of toxicity of different pesticides to any pest, where potency level was acquired by dividing the LC_{50} and LC_{90} of herbzed, which rated as the standard compound at LC_{50} and LC_{90} levels, by the corresponding Figure (1) of each tested pesticide. At LC_{50} level the relative potency levels expressed as the number of fold mention that methomyl, avaunt, and pestban were impact against tested snail which recorded (1.98, 1.31 and 1.07 fold) than herbazed (1.00), respectively while, at LC_{90} the relative potency level was (1.72, 1.13, and 1.04 fold) for methomyl, avaunt, and pestban, respectively. The previously mentioned data concurred with **Aioub *et al*. (2000)** reported that the carbamate compounds reason the most amazing effect contrasted with organophosphours, while the herbicides were the least toxicant against two types of snail, *Eobania vermiculata* (O. F. Müller) (Gastropoda: Helicidae) and *M. cartusiana* treatment with some pesticides such as; oxamyl, malathion, pirimphs-methyl, profenofos, isoproturon and tralkoxydim under laboratory conditions. Bailey (2002) suggested

that carbamates are considered feeding inhibitors. On the other hand, the LC_{50} value of dursban pesticide was 3.69 and 4.73% for *M. cartusiana* and *E. vermiculata*, respectively (Zedan *et al*. 2006). Carbofuran pesticide was the most toxic compound against *M. obstructa* adults whereas its LC_{50} value was 0.18% followed by methomyl 0.39%, pyriproxyfen 0.49%, benthiocarb 0.90% and fenthion 1.54% while dicofol pesticide was the lowest effective one; its LC_{50} value was 4.05% (Gabr *et al*. 2007). Also, Ali (2017) studied that the newly pesticide which belongs to the carbamates group was the most potent one followed by thymol as monoterpenes or kafrothriul as pyrethroid and round up as an organophosphorus when control of *M. cartusiana* adult using baits and leaf dipping technique under laboratory.

Table (2): Comparative toxicity of chemical compounds against adult of *Monacha cartusiana*, snail using baits technique.

Treatments (µg/ml)	Adult of *Monacha cartusiana*						
				Toxicity index		Relative potency	
	LC50	LC90	Slope	LC50	LC90	LC50	LC90
Methomyl	1436.4±14.12	3013.6±39.9	1.4	100	100	1.98	1.72
Avaunt	2061±24.6	4617.58±10.72	1.6	70.00	65.26	1.31	1.13
Pestban	2511.2±28.15	5006.00±11.03	2.45	57.19	60.19	1.07	1.04
Herbazed	2710.6±25.13	5205.5±14.12	3.6	53.00	57.89	1.00	1.00

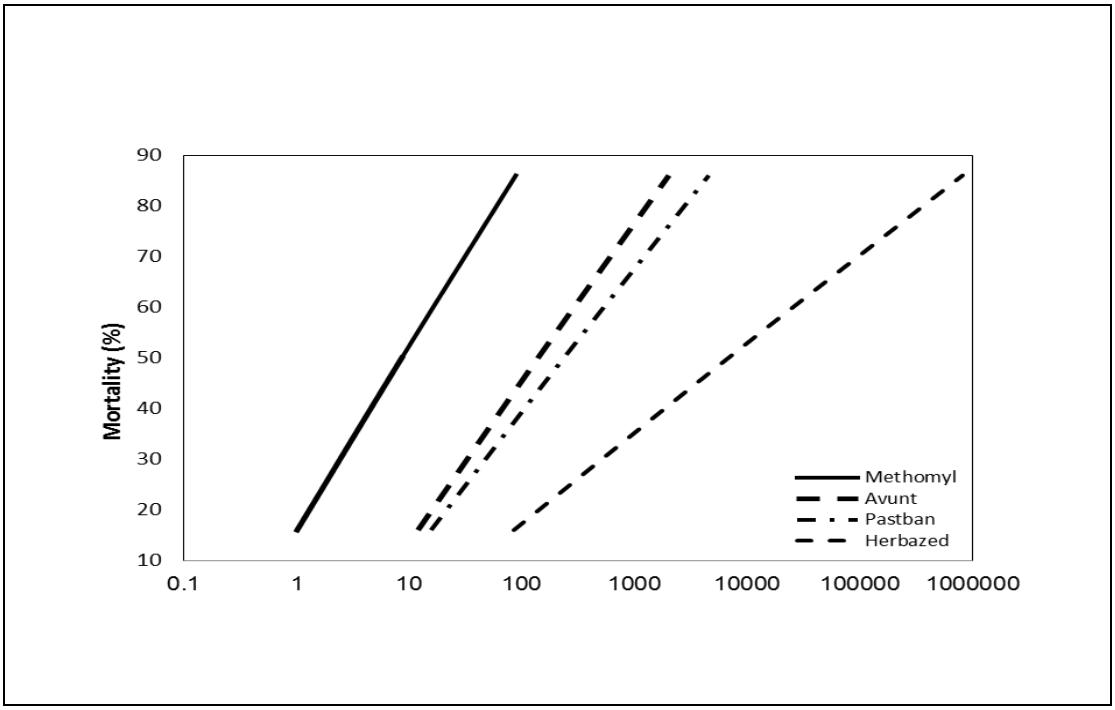

Figure (1): Toxicity regression line of some chemical compounds using baits technique against adult of *Monacha cartusiana*, snail.

2. Genotoxicology studies of *Monacha cartusiana*, adult:

Comet assay:

This research studied to identify potential geneotoxicty as comet assay, also to realize as the single-cell gel electrophoresis (SCGE) assay, is comprehensively applied as one of the standard ways to valuating DNA damage triggered through a range of DNA destructive agents in eukaryotic cells may be measured.

It depends on the quantification of the distortion and DNA fragments departing out of the cell nucleus during electrophoresis. This assay has obtained large-scale utilize in various areas including human bio monitoring, genotoxicology, ecological monitoring, and as an instrument for research into DNA damage or repair in different cell types.

Table (3) indicated that some standards checked in the hemolymph of *M. cartusiana* adult after three days of treatment with methomyl, avaunt, pestban and herbazed pesticides at (LC_{25}) by baits technique, where the data showed that all tested materials reasoned increasing in DNA damage, tail length, DNA in tail, tail moment and olive tail moment. Methomyl was the most pesticide which reasoned augmentation in DNA damage in target cells (17.58%), while avaunt caused 15.61% subordinate by pestban and herbazed with 11.60 and 10.59% compared with control (7.35%).

Also, a high increasing was recorded in tail length (TL) 14.39, 13.75, 12.77and 7.76 µm post exposure to methomyl, avaunt, pestban and herbazed, respectively compared control 6.80 µm. For DNA in tail, methomyl due to significant rising and observed with (12.70%) compared with herbazed which observed with least impact (6.92%). Moreover, the present data appeared augmentation in tail moment (TL) and olive tail moment (OMT) than control, and recorded with (1.05, 0.94, 0.86 and 0.77%) and (2.52, 1.95, 1.80, and 1.16%) for methomyl, avaunt, pestban and herbazed, respectively.

Comet were divided into four class (0-3) according to the extent of DNA immigration, comet with bright heads and no visibly tail was assigned as class 0, comet with very meager heads and long extendedly tails class 3, comets exhibited traits mediator between class 0 and 3 divide and earmarked to easily notable class 1 and 2 (Figure 2).

Also, DNA migration was valuation using visible scoring and pictures analysis is precise in the measurability of DNA damage, forgiveness to measure the fluorescence severity and distribution of DNA by the comet assay. In the visible scoring a numeric index is ordinarily user to symbolize the average DNA migration according to Burlinson *et al*. (2007). Also, similar observations obtained that Itziou *et al*. (2011) revealed that chlorpyrifos and parathion–methyl due to the highest damage in DNA of *E. vermiculata* snail digestive gland.

Table (3): Comet assay test for *M. cartusiana,* adult exposure to sub-lethal concentration (LC_{25}) of methomyl, Avaunt, Pestban and Herbazed.

Tested materials	DNA damage (%)	Tail length (µm)	DNA in tail (%)	Tail moment	Olive tail moment
Methomyl	17.58±0.31***	14.39±0.28***	12.70±0.22***	1.05±0.015***	2.52±0.062***
Avaunt	15.61±0.21***	13.75±0.20***	10.61±0.15***	0.94±0.008***	1.95±0.023***
Pestban	11.60±0.60***	12.77±0.11***	9.70±0.91***	0.86±0.014***	1.80±0.027***
Herbazed	10.59±0.12***	7.76±0.14*	6.92±0.47***	0.77±0.03***	1.16±0.045***
Control	7.35±0.18***	6.80±0.10***	3.56±0.18***	0.58±0.00***	0.41±0.056***

Data expressed as Mean ±Stander error; P >0.005.

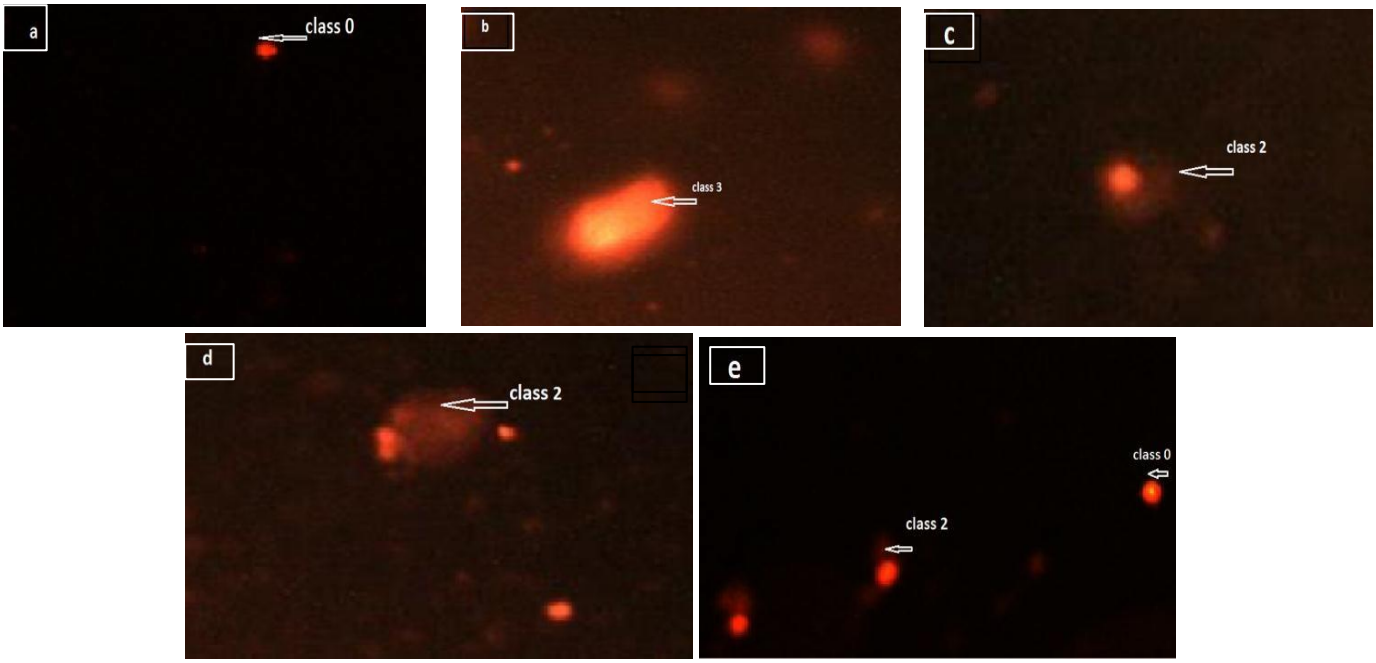

Figure (2): DNA content integrity via comet bioassay:
a) Control group showing undamaged DNA without comet tail, (Class 0).
b) Methomyl treatment showing an appearance of major damaged DNA (Class 3).
c, d) Avaunt and Pestban treatments showing an appearance of moderate damaged DNA (Class 2).
e) Herbazed treatment showing an appearance of heterogeneity of comet tail length (Class 0 and Class 2). (x.,400).

Mohamed *et al*. (2013) reported that atrazine and glyphosate as herbicides have a high impact on genotoxicants agents of *Biomphalaria glabrata* (Say) (Planorbidae: Gastropoda) snail. This proved the ability of these compounds to cause change inside *M. cartusiana* cells by using low concentrations with the baits technique. Guanggang *et al*. (2013) studied that methomyl at sub-lethal concentration has a significant influence on DNA damage, whereas reasoned increasing in the tail length, tail DNA and tail moment in vitro compared with control of *Drosophila* insect. Ibrahim *et al*. (2018) reported that DNA damage is a serious biomarker of toxicity in snails. Al-Fanharawi *et al*. (2019) indicated that CPF (Chlropyrifos) at concentrations 15, 22, and 45 mg/l due to an increase in comet length, tail length, and tail moment in the digestive gland of the snail *Viviparous bengalensis* (Lamarck) (Mesogastropoda : Viviparidae) . Finally, Gaber *et al*. (2022) reported the toxic impact of two sub-lethal concentrations of methomyl pesticide LC_{20} (0.075 g / L) and LC_{40} (0.180 g / L) on some biochemical parameters and histological for *M. cartusiana* snail.

The presented data reported that using some methomyl, avaunt, pestban and herbazed pesticides against the glassy clover snail, *M. cartusiana* adults. Results found that the accretive mortality percentage was increased progressively by raising the exposure time and concentration. Half lethal concentrations (LC_{50}) and toxicity index of different tested pesticides were evaluated; the compounds tested could be arranged to descend as methomyl, avaunt, pestban and herbazed, respectively according to their LC_{50} and toxicity index. Also, data was studied that all tested

pesticides have the ability for damaging DNA of *M. cartusiana*, treated with LC_{25} at three days compared with the control.

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