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The Utilization Of Biochemical Indicators To Assessing The Toxicity Of Some Pesticides Baits To The Glassy Clover Snail, *Monacha cartusiana* (Müller).

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ABSTRACT

Monacha cartusiana (Müller) snail was used to evaluate the impact of sub-lethal concentration (LC_{25}) of four pesticides on some vital enzymes such as Alanine transaminase (ALT), Aspartate transaminase (AST), Acetylcholinesterase (AChE), Phenol oxidase (PO), Acid phosphatase (ACP) and protease enzyme during two periods (activation and aestivation) by baits method. Inactive time, results expressed that, all pesticides elevated in the activities of ALT, AST, and PO enzymes. For, AChE enzyme, pestban was the most influential to inhibit AChE followed by methomyl, however, avaunt has been found to fluctuate in the level of AChE activity between rises and fall to record the lowest activity after 12hrs, whereas herbazed contribute to decreasing the inhibition at all time. Methomyl caused a high increase in the level of ACP which reached its maximum after 12hrs, followed by pestban and avaunt, but herbazed lead to decreasing at all active time. Meanwhile, all pesticides induced a depressing effect in the activities of protease enzyme. About aestivation periods is considering one of the most important characteristics that terrestrial snails possess, but the difference to be attributable to the months. In Egypt, the snails enter the summer hibernation phase from May to September. The activity of the pre-mentioned enzymes was evaluated for some treated snails during the aestivation period. Contrary, the results indicated that all pesticides had an effective role in reducing the level of those enzymes except the AChE enzyme, which remained elevated throughout this period as well as control compared to the active period.

Keywords: Gastropoda, bio indictors, pesticides.

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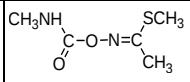
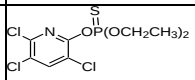
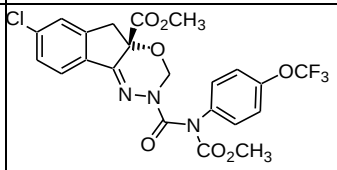
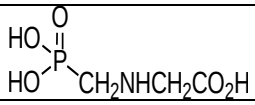
INTRODUCTION

The glassy clover snail, *Monacha cartusiana* (Müller) is considered as the most prevalent snail in various governorates in Egypt, the snail strafes agronomic, agriculture, and ornamental plants. Therefore, it was necessary to constrain its spread by chemical control which is still viewed as the best strategy, especially over enormous areas[29].

Conventional pesticides particularly carbamate and organophosphorus compounds are effective which is utilized in Egypt just as in numerous nations as spray or baits [18]. bait methods towards terrestrial gastropods and pestban as the organophosphorus compound was found to actuate oxidative stress and influence some biochemical targets in snails [31]. As regards, avaunt is a new pesticide that belongs to the oxidizing pesticides group which acts on the target organism as a sodium channel blocker [35]. Hence, linking the reactions caused by these pesticides with the progress of these compounds with the physiological system where biochemical indicators are known as biomarkers. The biochemical pointers know as biomarkers and can cut off as early notice be apportioning into three classes: exposed biomarkers, impact biomarkers, and sensitivity biomarkers [33]. Six enzymes were picked to conducting the present depending on their specific action. The enzymes were: Aminotransferase enzymes (ALT and AST) are generally circulated in creatures and normally estimated clinically as biomarkers for liver wellbeing [16]. Acetylcholinesterase (AChE) is found at essentially neuromuscular crisscrossed and within chemical synapses of the cholinergic, where its action interrupts to finish synaptic transmission [24]. An acid phosphate (ACP) is a hydrolyzed enzyme amenable for separate phosphate groups from numerous kinds of molecules including nucleotides, proteins, and alkaloids [6]. Protease additionally called a peptidase that catalyzes the breakdown of proteins, cleaves the peptide bond inside proteins by hydrolysis. .Protease is associated with many biological functions inside the animal's body [23]. Phenoloxidase (PO) completes the hydroxylation of monophenols to create diphenols and afterward oxidizes them to quinine [25], likewise it assumes a key job in melanin creation during cuticle sclerotization [9].The point of this study to assess the sub-lethal concentrations of Methomyl, Avaunt, Pestban, and Herbazed as pesticides which applied with baits technique against *M. cartusiana* and expose the changes on the vitality of six enzymes, AST, ALT, AChE, ACP, PO, and protease .

MATERALIS AND METHODS

Chemical used

Trade name	Common name	IUPAC	Structure formula
Methomyl 90% SP.	Lannate	S-methylN- (methylcarbamoxyloxy) thioacetimidate.	
Pestban 48% EC	Chlorpyrifos	O,O–dietheylo-3,5,6–trichloro-2-pyridyl phosphorothioate.	
Avaunt 15% EC	Indoxcarb	methyl(S)-N- (7-chloro-2,4,4a,5- tetrahydro-4a-(methoxycarbonyl) indeno[1,2-e][1,3,4] oxadiazia-2-ycarbonyl]-4- (trifluoromethoxy) carbanilate.	
Herbazed 48% WC	Glyphosate	N-(phosphonomethyl) glycine,isopropyl amminoum salt	

Preparation of samples

The LC_{25} of each pesticide was applied against adults of *M. cartusiana* snail using the poisonous baits method. The snails were randomly selected and the period of estimation was divided as activation and aestivation periods. Survival snails were collected after 4,8 and 12hours and then after 1,3,5 and 7 days for

activation periods. In course of aestivation samples of treated snails were kept pending their entry into the aestivation period. Then also, randomly selected after the onset of aestivation (June), mid of aestivation (July), and end of aestivation (August). Untreated control were collected and served as check samples. The shells of survival adult snails of *M. cartusiana* were expelled making consistently cut around the whorls beginning the aperture opening using bone scissors and the broken fragments were carefully removed and 1 g of the soft tissues were weighted, then homogenized in distilled water under cooling in a homogenizer for 3 minutes utilizing a Teflon homogenizer. The homogenates were centrifuged at (5000 r.p.m.) for 10 minutes at 5ºC. The supernatants were promptly held under cooling conditions in the refrigerator to quantify the activity enzymes according to [1].

Estimated enzymes

Determination of alanine (ALT) and aspartate aminotransferase (AST) activitie: Colorimetric determination of ALT & AST in the adult snail's homogenate was carried out according to [30].

Determination of acetylcholinesterase (AChE) activity: The obtained extraction of adult snails was probation for AChE activity in conformity with [14], method. The specific activity of AChE was expressed as mMoles of acetylthiocholine iodide hydrolyzed/mg protein/min.

Determination of Phenoloxidase enzyme (PO): Phenoloxidase was evaluated according to the method of [19] using catechol as a substrate.

Determination of Acid Phosphatase (ACP) activity: ACP was resolved utilizing sodium phenyl phosphate as a substrate. The method was depicted to [27].

Determination of Protein-hydrolyzing enzyme (Protease) activity: The activity of protease was carried out by the same method used by [15].

Statistical analysis

The obtained data were subjected to statistical analysis by one-way ANOVA and the least significant difference (LSD) at (P>0.05) Costat statically computer program [12].

RESULT AND DISCUSSION

Transaminases activities

Aspartate aminotransferase (AST, E.C. 2.6.1.1) and alanine aminotransferase (ALT, E.C. 2.6.1.2) are important enzymes that stimulate the interplay of amino acids and α –keto acids via switch the amino group as they have a crucial position in linking the metabolism of amino acids and carbohydrates, being an essential group of enzymes inside the gluconeogenesis pathway. These enzymes are not several located in hepatocytes, however also in many frame organs. In addition to being associated with an illness in features of the coronary heart [2]. The outcomes indicated that all tested material prompted significant augmented in the vivacity of ALT and AST enzymes when applied *M.cartusiana* to sub-lethal concentration. Data observed that methomyl pesticide due to piecemeal increase in the activity of AST whereas it is maximized thereupon 7^{th} day post-treatment with (132.89%), reverse to pestban pesticide which shows efficacy in ALT level through intervals of exposure whereas recorded (66.12%) post 1^{st} day, then diminished after 7^{th} day with (28.06%). Avaunt pesticide triggered to indecision in the level of transaminase activities, the highest increase was recorded after 12h. and 5^{th} day with (63.65%) & (56.72%) for ALT and AST enzymes, respectively than to control (Tables 1&2). The effects are comparable with [34] who reported that lannate, diazinon, and karate as molluscicides increased ALT and AST activities after treatment *Monacha cantiana*. Moreover, obtained data agree with [22], who revealed that, lannate (methomyl) due to an increase in the activities of AST and ALT enzymes of *Eobania vermiculata* snail with LD_{50} value through topical application method. Nevertheless, [13], provided the opposite effect whilst applied *M. cartusiana to* LC_{25} of biogard, biazed, and methomyl, records referred to that biogard caused massive increased in the level of ALT and AST followed by methomyl and bioazd. Also, results showed that there was a significant decrease in ALT activities ranged between (-17.16, -14.88, -6.30 & -20.68%)

thereupon 1, 3, 5, and7days post treatment with herbazed, respectively. As for AST activity caused a decrease after 1st day (-23.53%) then raised to reach its maximum after 3th day with (25.01%). The decline in AST and ALT may be attributed to the binding of the tested compounds with protein that leads to inhibition in aminotransferases activity which is known to be intimately associated with protein synthesis [17].

AChE activity

Acetylcholinesterase (AChE, E.C.3.1.1.7) is found in many types of conducting tissues as namely: nerve and muscles, central and peripheral tissues, motor and sensory fibers, and cholinergic and noncholinergic fibers. It is used as a marker of exposure to inhibit pesticides in mollusks. Data obvious that pestban was the most pesticide which caused high inhibition in the activity of AChE of a tested snail at all-time intervals and maximum inhibition was after 1st of treatment with (86.77%) followed by methomyl which due to minor inhibition through hours of exposure while the activity was recovered and exhibited to 52.67, 57.33 and 61.66 after 1,3, and 5 days of treatment then decline to 49.78 % inhibition on 7th day of treatment (Table 2). Carbamates are agonist of nicotinic ACh receptor (nAChRs) and do not cause a direct inhibition of AchE activity as shown for other pesticides such as organophosphorus [36]. However, avaunt has been found to fluctuate in the level of AchE activity between rises and falls to record the lowest activity after 12hrs. post treatment (13.01%) of inhibition. This is in harmony with [1] who reported that indoxacarb caused slight inhibition on AChE activity when treated *M. cartusiana* with LC_{50} & 0.5 LC_{50} after 1,3,5, and 7 days at laboratory conditions. The percent of inhibition remained constant were noticed, about 27.31, 27.08, and 27.74 % after 12 hrs, 1, and 3 days, respectively of herbazed treated snail then caused decreasing to inhibition of 14.93 &18.76% after 5and 7days, respectively. The decrease was not as distinctly as on the first day, this result could be ascribed to the accumulation of acetylcholine substrate (Ach) resulting from the inhibition of the enzyme during the first day [28].

Phenoloxidase activity (PO)

Phenoloxidase (E.C.1.14.18.1) is a significant safeguard framework in numerous invertebrates' creatures which eventually prompts melanization of microbes and harmed tissues. The process of melanization depends on activation of the enzyme phenoloxidase (PO) which is controlled by the prophenoloxidase (proPO) activation system [4]. Despite no clear characterization of *Monacha cartusiana* snail hemolymph was attempted. This is astounding because PO activity is viewed as a significant segment of the humoral reaction and an immunocompetent boundary in numerous arthropods [11]. The aim of this work to describe the particular PO movement in *Monacha cartusiana* hemolymph, this is basically essential for considers meaning to comprehend the job of PO action in snail biochemical.

So, the result in (Table 1) clarify that all tested materials caused increase in the activity of PO compared with control, as a defense response to their toxic effects tested after exposure, where methomyl was the most compounds which caused augmentation in the activity of PO post 4h. of treatment, the value was (19.53 O.D. unit/min/g.b.wt.) with 84.25 % of relative activity to reach the maximum increase in 5th day with 209.42 O.D. unit/min/g.b.wt. (106.96%) compared with control (Table 2). These results support the finding of [13] who noticed increasing in the activity of PO after treatment of *M. cartusiana* with methomyl, biogard, and biozd pesticides. [7] recognized that phenoloxidase was higher in hemolymph of M. cartusiana than those in the hemolymph of E. vermiculata when contrasted between biochemical constituents' substance,and elements concentrations. As a result, avaunt caused increase in the PO activity post 3rd day with (63.45%) in comparison with pestban. Herbazed due to a slight increased, PO enzyme levels were 36.05, 23.86, 20.51&19.88 % (Table 2).The diminished in some treatments occurred in some defensive mechanisms of the snail as a result to the high stress of the tested molluscicides.

Acid phosphatase (ACP)

Acid phosphatase (E.C.3.1.3.2) is an enzyme used to free annexed phosphoryl groups from other molecules during digestion Information demonstrated that all pesticides had the same impact on the activity of ACP; they caused an increase in the activity of the enzyme. Results revealed that methomyl treatment was the most pesticide which caused a high increase in the level of enzyme progressively, 241.51, 274.92, 184.56, 165.32 and 132, 69 % post 12h ,1,3,5, and 7 days respectively, (Table1&2) followed with pestban and avaunt

pesticides, This is not in concurrence with [21] who marked that methomyl caused a reduction in the activity of acid phosphatse when treatment *E. vermiculata* and *M. cantiana* with methomyl. While herbazed was the least one, which caused slight boosting in the level of ACP through the exposure time. This is expected to compounds may cause destabilization of the lysosomal membrane and the consequent release of the enzyme into the hemolymph or can trigger hyper synthesis of acid phosphatse which is subsequently released into the hemolymph [8].

Protease activity

Protease enzyme (E.C.3.4.21.211.) Motivates proteolysis, the disrupted of protein into more modest polypeptides, or single amino acids. Proteases are engaged with numerous organic capacities, including the processing of ingested proteins, protein catabolism proteins, and cell flagging [23]. (Table 1), elucidated the imparity in the value protease enzyme. It showed that avaunt treatment caused increase in the activity of protease enzyme after 4hr. with 84.40% then marked a decreased during 1, 3, 5, and 7 days post exposure with (-13.80,-14.50,-13.13 & -15.78 %). [32] found that a decline in protease activity at 24, 48 and, 72 h in every single substantial metal is measurably huge and Hg Cl2 stress indicated more reduction in protease action than Cu SO4 and Cd Cl2 after chronic exposure. Also, other treatments had efficacy in reducing the activity of the protease. Herbazed attained to a high reduction in the activity of protease after 5 and 7 days with (-9.91& -37.28 %), variance in protease action might become about because of physiological disturbance influence happened inside the snail body after the exposure to these molluscicides. [26] announced that protease action radically diminished at higher concentrations of endosulfan and profenophos, treated soils than the untreated controls, recommending that the protein is somewhat touchy to pesticides. Whereas, Hg Cl2 was seen as more potent in inhibiting protease activity.

Aestivation periods

In Egypt, snails aestivate during the summer months (June to September). In that period, the mouth gap is incidentally closed by a whitish calcium material called epiphragm. Snails have been accounted for household wastes which were changed over and used for their development and egg creation [3]. Table 3 showed the monthly changes in tissue biochemical of *M. cartusiana*, adult during the aestivation period. AST and ALT were seen as low all through the aestivation months in control snails contrasted with activity periods (previous data). Likewise, all materials which utilized lead to a significantly decreased in both enzymes, avaunt treatment was credited with causing a severe reduction in the level of AST activity through mid and end of aestivation with (-35.84 &- 59.41%), respectively compared with other materials, where, in ALT activity the values were almost close. [10] observed that ALT and AST enzymes depleted during aestivation months when studied eco-physiological adaption of the land snail, *Achatina achatina*. Additionally, they included that decrease demonstrates that the snails may have created potential cell injury because of oxidativetress and thermal heat. With regard to AChE activity, no significant differences were observed between values in methomyl, avaunt, pestban and herbazed, the activities remained stable in high proportions throughout the aestivation periods, as well, untreated snails compared with activity period. [20] noticed that AChE activity were higher in May compared to October when studied a package of PO activity, all pesticides lead to a high decrease in the activity of PO during aestivation periods, noteworthy avaunt induced to the highest decline with (-55.83, -35.20 & -4.78) at the time of onset, mid and end of aestivation, respectively. Control had the same impact in comparison with the activation period. [5] revealed that the activity of phenoloxidase in the hemocyte was significantly decreased in the aestivated *Pila globosa*, snail.

About acid phosphatase and protease enzymes, data cleared that all tested materials also, caused a decline in the activity of both enzymes during aestivation period. Methomyl lead to in the activity of ACP with (-66.61,-65.28 &-50.11%) for onset, mid, and end of aestivation, respectively. Also, it caused decreasing in the activity of protease enzyme in onset aestivation (-77.15) while, herbazed marked the lowest one which decreasing the activity of protease enzyme with (-21.15, - 40.96 & - 47.12%) in comparison with other tested materials. Contrary to control, the activities of both enzymes were low in summer months through about activity periods. [37] marked that, acid phosphatase were higher in summer seasons while protease enzymes were decreased in monsoon when assayed some enzymes activity in *Cryptozona bistrialis* snail. Diminished in the activity of enzymes due to the mouth gap during snails aestivation, that were eating and drinking consistently. Secretion of enzymes is in direct reaction to the presence of nearness supplements and when

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there was no substrate to act upon, it resulted in low enzyme activities as saw in this work. For higher enzymatic activities recorded probably won't be detached to the closeness of the two organs (stomach and hepaticpancreas) and subsequently conceivable in reverse ciliary and muscular movement of enzymes into the stomach, [3]. These finding on enzymes activity appears as concurrence with the present.

Table 1. Vitality of some biochemical parameters of *M.cartusiana* adult post exposed toLC_{25} of indicated pesticides through 4,8,and 12 hours by bait technique.

Tested materials	Time /hours	AST	% R.A.	ALT	% R.A.	AChE	% R.A.	PO	% R.A.	ACP	% R.A.	Protease	% R.A.
Methomyl	4h	114.12	24.45	98.36	30.71	321.44	39.22	19.53	84.24	103.91	53.82	74.91	20.00
	8h	118.63	28.68	113.69	50.66	302.24	44.34	16.35	32.71	107.83	72.97	84.25	34.80
	12h	121.04	29.19	129.55	68.55	253.48	28.89	27.53	57.50	213.00	241.51	101.67	62.67
Avunt	4h	101.20	10.59	85.46	13.56	290.90	26.00	15.89	50.00	95.67	41.62	105.25	84.40
	8h	105.25	14.16	106.97	41.66	297.59	42.12	17.85	44.98	123.65	98.34	96.83	54.92
	12h	112.26	19.82	125.78	63.74	222.24	13.01	25.41	44.56	152.79	145.07	92.91	48.65
Pastane	4h	100.01	9.19	108.58	44.29	360.11	56.07	17.96	50.56	118.88	76.08	79.33	26.92
	8h	107.57	17.00	126.05	67.00	364.68	74.17	16.93	37.41	129.75	108.13	109.66	75.45
	12h	109.62	17.00	135.19	76.90	270.96	37.78	22.09	26.68	200.01	220.68	103.91	66.26
Herbazed	4h	70.03	-23.53	82.23	9.27	267.05	15.76	13.61	28.39	91.45	35.48	93.5	49.60
	8h	95.61	3.70	83.31	10.32	254.54	21.56	13.91	12.90	91.63	47.00	99.16	58.65
	12h	97.45	4.01	90.30	17.48	250.35	27.30	19.77	12.39	111.17	78.24	78.91	26.25
Control	4h	91.59	-	75.25	-	230.88	-	10.60	-	67.55	-	62.50	-
	8h	92.19	-	75.51	-	209.38	-	12.32	-	62.34	-	62.50	-
	12h	93.69	-	76.86	-	196.65	-	17.59	-	62.37	-	62.50	-
P		***		***		***		***		***		***	
$L.S.D._{0.05}$		5.17		5.01		6.61		3.70		4.22		4.56	

SA= Specific activity. RA%=Relative activity, increase or decrease than control= (treatment – control) ÷ control x100; AST expressed as µg oxaloacetate/g., ALT as µg pyruvate/g.; AChE as O.D. unit /min/ g.b.wt.; PO as µg /g.b.wt.; ACP as µg/ g.b.wt.;Protaese as µg tyrosine/ /g.b.wt.

*P<0.05, **P <0.01, ***P <0.001 vs. control.

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Table 2:Vitality of some biochemical parameters of *M.cartusiana* adult post exposed to LC_{25} of indicated pesticides through 1,3,5,and 7 days by bait technique

Tested materials	Time /days	AST	% R.A	ALT	% R.A.	AChE	% R.A.	PO	% R.A.	ACP	% R.A.	Protease	% R.A.
	1	206.07	53.14	160.19	24.69	609.70	52.67	160.00	57.20	308.86	274.92	140.50	32.12
Methomyl	3	261.66	94.46	190.03	48.23	627.20	57.33	153.51	51.81	229.90	184.56	134.50	25.12
	5	258.66	92.23	156.97	22.70	644.45	61.66	209.42	106.96	217.19	165.32	123.03	10.09
	7	313.79	132.89	153.20	20.25	598.15	49.78	129.78	27.59	187.94	132.69	122.42	11.87
Avaunt	1	191.65	42.43	191.64	49.17	483.35	21.03	145.16	42.62	209.07	152.79	91.67	-13.80
	3	202.47	50.47	169.06	31.87	563.15	41.26	165.28	63.45	204.83	153.53	91.91	-14.50
	5	210.88	56.72	147.29	15.13	662.55	66.20	131.77	30.22	172.28	110.46	97.08	-13.13
	7	163.10	21.05	136.27	6.96	474.95	18.93	120.90	18.86	149.38	84.94	93.00	-15.78
Pestban	1	200.06	48.68	213.42	66.12	745.85	86.77	142.00	39.52	239.89	191.20	143.34	34.79
	3	238.88	77.53	197.83	54.31	701.75	76.03	136.00	34.49	211.66	161.99	157.42	46.44
	5	243.63	81.06	194.60	52.11	618.1	55.05	123.50	22.05	201.54	146.20	173.50	55.26
	7	274.28	103.56	163.15	28.06	631.75	58.19	119.64	17.62	184.61	128.56	184.42	67.02
Herbazed	1	150.78	12.05	106.43	-17.16	507.50	27.08	138.47	36.05	151.43	83.82	137.58	29.38
	3	168.21	25.01	109.12	-14.88	509.25	27.74	125.25	23.86	144.09	78.35	132.66	23.40
	5	164.42	22.19	119.87	-6.30	458.15	14.93	121.94	20.51	141.85	73.28	100.67	- 9.91
	7	157.92	17.20	101.05	-20.68	474.25	18.76	121.94	19.88	113.42	41.42	69.25	-37.28
	1	134.56	-	128.47	-	399.35	-	101.78	-	82.38	-	106.34	-
Control	3	134.56	-	128.20	-	398.65	-	101.12	-	80.79	-	107.50	-
	5	134.56	-	127.93	-	398.65	-	101.19	-	81.86	-	111.75	-
	7	134.74	-	127.40	-	399.35	-	101.72	-	80.77	-	110.42	-
P		***		***		***		***		***		***	
LSD 0.005		8.39		19.70		4.72		7.33		13.51		8.01	

SA= Specific activity. RA%=Relative activity, increase or decrease than control= (treatment – control) ÷ control x100; AST expressed as µg oxaloacetate/g., ALT as µg pyruvate/g.; AChE as O.D. unit /min/ g.b.wt.; PO as µg /g.b.wt.; ACP as µg/ g.b.wt.;Protease as µg tyrosine/ /g.b.wt.

*P<0.05, **P <0.01, ***P <0.001 vs. control.

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Table 3: Vitality of some biochemical parameters of *M.cartusian* adult post exposed to LC_{25} of indicated pesticides through aestivation periods by bait technique

Tested materials	Time /months	AST	% R.A.	ALT	% R.A.	AChE	% R.A.	PO	% R.A.	ACP	% R.A	Protease	% R.A.
Methomyl	Onset of aestivation	13.69	22.34	154.41	-7.42	654.15	94.69	33.78	-45.04	25.08	-66.61	21.25	-77.15
	Mid of aestivation	12.94	10.69	144.46	-16.67	621.00	91.81	70.34	23.18	25.78	-65.28	60.58	-34.97
	End of aestivation	6.50	-40.59	134.12	-24.59	619.01	91.20	69.00	14.87	35.48	-50.11	61.66	-23.88
Avaunt	Onset of aestivation	16.04	43.34	147.82	-11.37	649.31	93.25	27.15	-55.83	42.37	-43.60	57.25	-38.44
	Mid of aestivation	7.50	-35.84	145.92	-15.83	627.02	93.68	37.00	-35.20	57.58	-22.46	48.75	-47.68
	End of aestivation	4.44	-59.41	145.42	-18.24	614.18	89.71	57.80	-4.78	66.80	-6.06	26.50	-67.28
Pestban	Onset of aestivation	8.00	-28.51	153.28	-8.09	647.95	92.84	34.12	-44.59	49.40	-34.24	46.08	-50.45
	Mid of aestivation	9.29	-20.53	157.90	-8.92	607.77	87.73	37.86	-33.69	68.53	-7.71	34.50	-62.97
	End of aestivation	9.54	-12.80	153.61	-13.63	603.61	86.44	39.15	-34.82	80.66	13.42	25.00	-69.14
Herbazed	Onset of aestivation	12.94	15.63	146.75	-12.01	641.51	90.93	49.35	-19.71	62.18	-17.23	73.33	-21.15
	Mid of aestivation	13.44	15.00	145.78	-15.91	616.84	90.53	46.18	-19.12	72.49	-2.38	55.00	-40.96
	End of aestivation	12.00	9.68	138.82	-21.95	611.24	89.80	64.92	8.07	56.15	-21.03	42.83	-47.12
Control	Onset of aestivation	11.19	-	166.78	-	336.00	-	61.47	-	75.12	-	93.00	-
	Mid of aestivation	11.69	-	173.36	-	323.75	-	57.10	-	74.26	-	93.16	-
	End of aestivation	10.94	-	177.86	-	323.75	-	60.07	-	71.11	-	81.00	-
P		***		***		***		***		***		***	
LSD(0.05)		4.43		4.85		8.32		11.85		6.40		4.97	

SA= Specific activity. RA%=Relative activity, increase or decrease than control= (treatment – control) ÷ control x100; AST expressed as µg oxaloacetate/g., ALT as µg pyruvate/g.; AChE as O.D. unit /min/ g.b.wt.; PO as µg /g.b.wt.; ACP as µg/ g.b.wt.;Protaese as µg tyrosine/ /g.b.wt.
*P<0.05, **P <0.01, ***P <0.001 vs. control.

CONCLUSION

Generally, biochemical indicators are known to give early cautioning indications of environmental changes or stress conditions to the organism by measuring an organism's cellular or molecular responses of the target organism to xenobiotic agents. These vital signs have a role in understanding the mode of action of those pesticides in *Monacha cartusiana* and the extent of its impact during active and aestivation periods. Finally, data showed a high significance between four pesticides (methomyl, avaunt, pestban and herbazed) by time elapsing during active and aestivation periods in activities of six enzymes (AST, ALT, AChE, ACP, PO, and protease) in *M. cartusiana* snail.

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