



Impact of some plant seeds and kernels against adults of the white garden snail *Theba pisana* and the brown garden snail *Eobania vermiculata* (Gastropoda: Helicidae) and their effects on activity of amylase and invertase enzymes

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Abstract:

The molluscicidal impact of apple seeds *Malus domestica* L., Sinai peach kernels *Prunus persica* L. and amar apricot kernels *Prunus armeniaca* L. were assessed against adults of the white garden snail *Theba pisana* (Müller) and the brown garden snail *Eobania vermiculata* (Müller) (Gastropoda: Helicidae) using baits technique under laboratory conditions. Data indicated that mortality percentages of *T. pisana* and *E. vermiculata* snails were (0.0, 0.0, 6.67 %) and (0.0, 0.0, 0.0 %) for apple seeds powder at concentrations 3.75, 7.50 and 15 %, respectively. While, peach kernels powder mortalities were (6.67, 13.33, 33.33 %) and (0.0, 6.67, 13.33 %) at the same concentrations, respectively. Whereas, mortality percentages of apricot kernels powder were (20.00, 53.33, 100 %) and (6.67, 13.33, 40.00 %) at the same concentrations, respectively, after three weeks of treatment. Apricot kernels gave the highest molluscicidal effect, due to the presence of the amygdalin compound in apricot kernels more quantitative than peach kernels and apple seeds. So, amygdalin compound was used against adults of *T. pisana* and *E. vermiculata* snails. Results revealed that mortality percentages of adults of *T. pisana* and *E. vermiculata* snails using amygdalin compound separated from apricot kernels were (26.67, 40.00, 73.33, 100 %) and (0.00, 6.67, 13.33, 33.33 %) at concentrations 0.375, 0.750, 1.5 and 3 %, respectively after three weeks of treatment, due to *E. vermiculata* snail more weight than *T. pisana* snail. As regards, high performance liquid chromatography (HPLC) used for verification of amygdalin compound separated from apricot kernels. Finally, biochemical studies stated that decrease in the activity of amylase and invertase enzymes in adults of *T. pisana* and *E. vermiculata* snails treated with amygdalin separated from apricot kernels compared to control at concentrations 0.375, 0.750, 1.5 and 3 %.

Introduction

Land snails such as the white garden snail *Theba pisana* (Müller) and the brown garden snail *Eobania vermiculata* (Müller) (Gastropoda: Helicidae), feed on a wide variety of

plants, including cereals, vegetables, fruits, herbs, many ornamentals. They destroying seedlings, stunting growth, and reducing yields. Not only do they directly damage the plants they feed on

but the wounds they create allow plant pathogenic fungi to infect plants. The snails can also be vectors of various plant pathogens, and their mucus trails can contaminate grains, vegetables, fruits, and herbs. In large numbers, their bodies and shells can be contaminants of mechanically harvested crops. The importance of land snails as pest organisms has drastically increased in the past few decades (Godan, 1983; Garthwaite and Thomas, 1996 and Barker, 2002). Terrestrial gastropods are from the most significant threats to sustainable agriculture around the world (Barker, 2002). Furthermore, damage caused by snails is due mainly to feed and to contaminate with their bodies, faces or slime, leading to deterioration of the product quality besides and the financial loss (Lglesias *et al.*, 2003). The terrestrial snails were recorded to be harmful snails in many districts of Egypt attacking various plants (Eshra, 2013). Jeyaratnam (1990) found that about 25 million agricultural workers in developing countries are poisoned every year by pesticides. Wherefore, used of natural baits as plant seeds against land snails. Also, natural products from plant origin have received much attention as potentially useful bio-active compounds to develop alternatives to the conventional pesticides (Singh and Singh, 2004 and Gabr *et al.*, 2006).

The aim of this research is to study efficiency of plant seeds powder such as some the Rosaceae family seeds against the white garden snail *T. pisana* and the brown garden snail *E. vermiculata* adults and their effects on changes in amylase and invertase enzymes activities using poison bait technique of amygdalin compound separated from apricot kernels under laboratory condition.

Materials and methods

1. Tested animals:

Land snail, the white garden snail *T. pisana* and the brown garden snail *E. vermiculata* adults were collected from orchard cultivated with navel orange, *Citrus sinensis* L. at Menia El-Kamh district, Sharkia Governorate, Egypt. The collected snails were immediately transferred in white cloth bags to the laboratory. Healthy and similar individuals were chosen and kept in glass terrarium filled with moist clay soil adjusted at 75 % of water field capacity. Snails were fed daily with bran for two weeks before treatment for acclimatization.

2. Tested seeds and kernels :

Seeds and kernels used were anna apple seeds *Malus domestica* L. cultivated in Egypt, Sinai peach kernels *Prunus persica* L. cultivated in Sinai, Egypt and amar apricot kernels *Prunus armeniaca* L. cultivated in Egypt. All of seeds and kernels purchased from the local market. Seeds and kernels are members of the family Rosaceae.

3.Toxicity studies:

3.1. Preparation of tested seeds and kernels:

Apple seeds, peach kernels and apricot kernels pulled out fruit, and then dried at room temperature for 6 days. After the outer shells of kernel have been cracked out. Seeds and kernels dried previously are pulverized by means of a blender.

3.2. Poisonous baits technique:

Three concentrations 3.75, 7.50, and 15 % for tested seeds and kernels powder and four concentrations 0.375, 0.750, 1.5 and 3 % for amygdalin compound separated from apricot kernels were prepared by incorporating the appropriate amount of each compound with bran bait. Three plastic boxes (3/4 kg capacity) were used for each concentration. Five grams of baits were spread into each box. Control treatment was prepared using bran bait. Five adult individuals of *T. pisana* and *E. vermiculata* snails were put into each

plastic box, then covered with muslin cloth and secured with rubber band. Dead individuals were counted using stainless steel needle according to **El-Okda (1980)**. Mortality percentages were calculated after 1, 3, 7, 14 and 21 days and corrected by **Abbott's formula (1925)**.

4. Chemical analysis:

4.1. Sampling and extraction procedure for apricot fruits:

Kernels are pulled out fruits, and then dried at room temperature for 6 days to achieve standard drying and subsequently kept in electrical oven at 30 °C for 24 hrs. After the outer shells of the apricot kernel have been cracked out, the extracted kernels dried previously are pulverized by means of a blender. 5 gram of each sample is extracted three times with 100 mL iso-propanol using soxhlet technique for 3 h at 90 °C. The contents are passed through a filter paper (Wattmann 42), and then the extract is set aside for 72 hrs. until amygdalin compound precipitate settle down. The product is washed out three times with ethylic ether to obtain a dry powder of amygdalin compound (Muhammad *et al.*, 2013).

4.2. High performance liquid chromatography (HPLC) analysis of amygdalin compound separated from apricot kernels using an amygdalin compound standard was purchased from sigma-Aldrich (USA):

Amygdalin compound separated from apricot kernels analyzed by HPLC Chromatograph YL-9100 system with LC-18 (250 mm × 4.6 mm × 5µm). The mobile phase used was methanol: acetonitrile : Water 30 : 30 : 40 (v/v), at a 1 ml / min. The detection was made at wave length of 220 nm. Analyzes were performed at Micro Analytical Center, Cairo University, Giza Governorate, Egypt.

5. Biochemical studies:

5.1.Preparation of snails for biochemical assay:

The mollusca shells of adults of *T. pisana* and *E. vermiculata* snails were removed and the soft tissues were weighed, pooled, and homogenized as 1:10 (w/v) in distilled water. The homogenates were centrifuged at 5000 r.p.m for 20 minutes at 5 °C according to Abd El-Haleim *et al.* (2006). The supernatants were immediately assayed to determine the activities of amylase and invertase enzymes by the method of Ishaaya and Swiriski (1976).

6. Statistical analysis:

The statistical analysis was determined by using one-way test, (ANOVA), **Cohort Software (2005)**.

Results and discussion

1. Impact of apple seeds, peach kernels and apricot kernels powder against adults of *Theba pisana* and *Eobania vermiculata* snails:

Data presented in Tables (1 and 2) indicated that after three weeks, mortalities of adults of *T. pisana* and *E. vermiculata* snails were (0.0, 0.0, 6.67 %) and (0.0, 0.0, 0.0 %) with apple seeds powder at concentrations 3.75, 7.50 and 15 %, respectively. While, peach kernels powder mortalities were (6.67, 13.33, 33.33 %) and (0.0, 6.67, 13.33 % %) at the same concentrations, respectively. In the case of mortality percentages of apricot kernels powder were (20.00, 53.33, 100 %) and (6.67, 13.33, 40.00 %) at the same concentrations, respectively. Results revealed that apricot kernels powder exhibited the highest mortality action followed by peach kernels powder while, apple seeds powder gave the lowest effect, due to the presence of the amygdalin in apricot kernels more percentage than peach kernels or apple seeds. Moreover, results revealed a highly significance between the three concentrations of tested snails by time elapsing except one day of *T. pisana* snail and one day and three days of *E.*

vermiculata snail compared control. The results agreed with those obtained by Vickery *et al.* (1987) found that amygdalin compound is considered a main component of apricot kernel. However, its toxic cyanogenic glycosides. Geller *et al.* (2006) showed that consumption of cyanogenic plants, such as apricot kernels in humans, have been reported to cause both acute and sub-acute health problems (Depending on dose) such as headache, nausea, vomiting, abdominal cramps, dizziness, weakness, mental confusion, convulsions, cardiac arrest, respiratory failure, coma and in extreme causes death. Silem *et al.* (2006) mentioned that amygdalin compound contain high

amount of cyanogenetic glycoside might cause acute or chronic toxicity in animals. Donald (2009) explained that amygdalin compound is a cyanogenic glycoside present in kernels and seeds of fruits such as apples, apricots, almonds, cherries, plums and peaches. Halenar *et al.* (2013) indicated that amygdalin compound is a major component of the seeds of Prunasin family plants such as apricots, almonds, peaches, apples and other rosaceous plants. Bolarinwa *et al.* (2014) reported that amygdalin compound content of seeds and kernels from Rosaceae species were 14.37 mg/g of apricot kernels, 6.81 mg/g of peach kernels and 2.96 mg/g of apple seeds.

Table (1): Impact of apple seeds, peach kernels and apricot kernels powder on adults of *Theba pisana* snail using baits technique under laboratory conditions.

Days Seeds and kernels	Concentrations	Mortality percentages				
		One day	Three days	One week	Two weeks	Three weeks
Apple powder seeds	3.75 %	0.00 ^b	0.00 ^d	0.00 ^d	0.00 ^e	0.00 ^f
	7.50 %	0.00 ^b	0.00 ^d	0.00 ^d	0.00 ^e	0.00 ^f
	15 %	0.00 ^b	0.00 ^d	0.00 ^d	6.67 ^{de}	6.67 ^{ef}
Peach powder kernels	3.75 %	0.00 ^b	0.00 ^d	0.00 ^d	0.00 ^e	6.67 ^{ef}
	7.50 %	0.00 ^b	0.00 ^d	6.67 ^{cd}	13.33 ^{cd}	13.33 ^{de}
	15 %	0.00 ^b	6.67 ^c	13.33 ^c	20.00 ^c	33.33 ^c
Apricot powder kernels	3.75 %	0.00 ^b	0.00 ^d	6.67 ^{cd}	13.33 ^{cd}	20.00 ^d
	7.50 %	0.00 ^b	13.33 ^b	26.67 ^b	40.00 ^b	53.33 ^b
	15 %	13.33 ^a	40.00 ^a	60.00 ^a	86.67 ^a	100 ^a
Control		0.00 ^b	0.00 ^d	0.00 ^d	0.00 ^e	0.00 ^f
LSD _{0.05}		3.59*	6.22***	8.03***	8.81***	*** 8.78

Table (2): Impact of apple seeds, peach kernels and apricot kernels powder on adults of *Eobania vermiculata* snail using baits technique under laboratory conditions.

Days Seeds and kernels	Concentrations	Mortality percentages				
		One day	Three days	One week	Two weeks	Three weeks
Apple powder seeds	3.75 %	0.00 ^b	0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^c
	7.50 %	0.00 ^b	0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^c
	15 %	0.00 ^b	0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^c
Peach powder kernels	3.75 %	0.00 ^b	0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^c
	7.50 %	0.00 ^b	0.00 ^b	0.00 ^c	0.00 ^c	6.67 ^{bc}
	15 %	0.00 ^b	0.00 ^b	0.00 ^c	6.67 ^{bc}	13.33 ^b
Apricot powder kernels	3.75 %	0.00 ^b	0.00 ^b	0.00 ^c	0.00 ^c	6.67 ^{bc}
	7.50 %	0.00 ^b	0.00 ^b	6.67 ^b	13.33 ^b	13.33 ^b
	15 %	6.67 ^a	6.67 ^a	20.00 ^a	33.33 ^a	40.00 ^a
Control		0.00 ^b	0.00 ^b	0.00 ^e	0.00 ^c	0.00 ^c
LSD _{0.05}		3.58*	3.58*	5.08***	6.22***	8.03***

2. High performance liquid chromatography (HPLC) study:

HPLC is used for proving of amygdalin compound separated from apricot kernels by the comparison the retention time of the standard amygdalin with that amygdalin separated from apricot kernels under conditions that specified in method at 2.02 min (Figures 1, 2). Similar observations were obtained by Viorica-Mirela *et al.* (2006) reported that amygdalin quantity analyses by HPLC

method. Yan *et al.* (2006) whom found that amygdalin was successfully separated from the crude extract of *Prunus armeniaca* L. using high-speed countercurrent chromatography. Muhammad *et al.* (2013) studied that amygdalin in Iraqi plant seeds was extracted and determined by high performance liquid chromatography. Bolarinwa *et al.* (2014) applied a high-performance liquid chromatographic procedure for amygdalin quantification to investigate extraction efficiency.

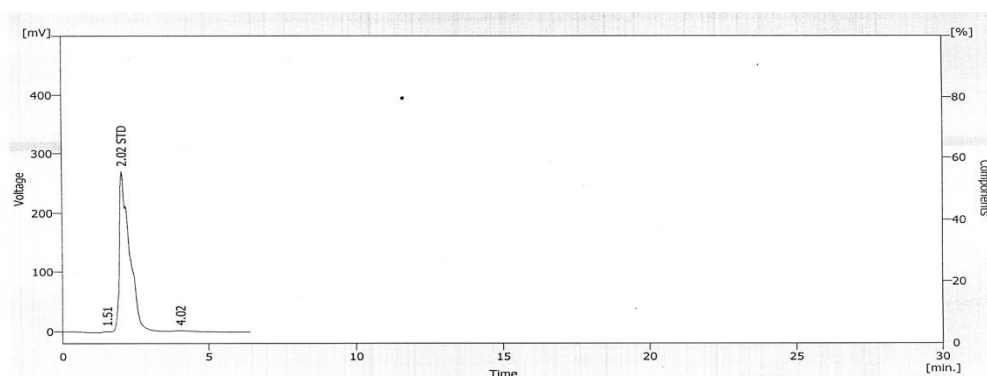


Figure (1): Chromatogram of the standard amygdalin compound.

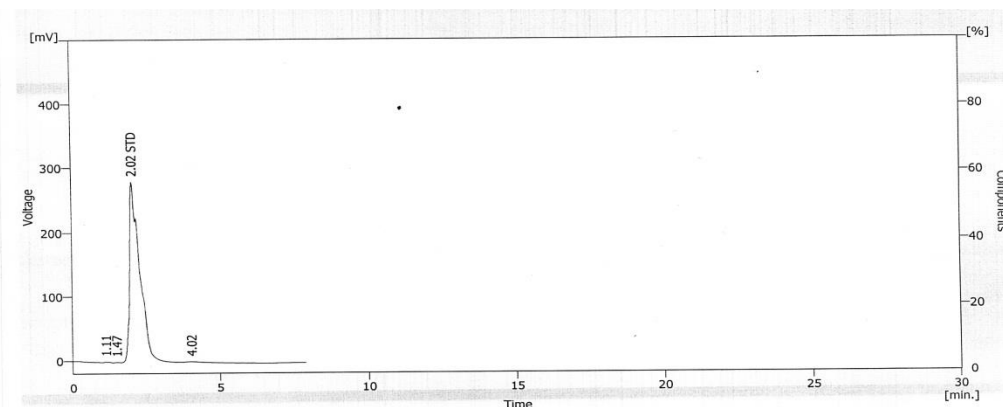


Figure (2): Chromatogram of amygdalin separated from apricot kernels.

3. Impact of amygdalin compound separated from apricot kernels on adults of *Theba pisana* and *Eobania vermiculata* snails:

Results in Tables (3 and 4) showed that, mortality percentages of *T. pisana* and *E. vermiculata* snail were (6.67, 40.00, 73.33, 100 %) and (0.00, 6.67, 13.33, 33.33 %) at concentrations 0.375, 0.750, 1.5 and 3 %, respectively after three weeks of treatment using

amygdalin compound separated from apricot kernels, due to *E. vermiculata* snail more weight than *T. pisana* snail. Otherwise, data found that a highly significance between the four concentrations in tested snails by time elapsing except one day and three days were no significance of *E. vermiculata* snail than control. Results agree with Walker and Kriebel (1990) reported that amygdalin compound is a toxic

cyanogenic glycoside. It is hydrolyzed by β -glucosidase into d-glucose, benzaldehyde and prussic acid (Hydrogen cyanide). Suchard *et al.* (1998) found that the ingestion of apricot kernels can thus cause cyanide poisoning. Chwalek and Ple. (2004) found that amygdalin compound is a cyanogenic glucoside initially isolated from the seeds of bitter almonds (*Prunus dulcis*). Viorica-Mirela *et al.*

(2006) reported that apricot kernels have considerable quantity of amygdalin compound is a toxic substance for human organism. Zhao (2012) explained that amygdalin compound is usually present in apricot kernels, bitter almonds and the seeds of other members of the genus *Prunus*. It is potentially dangerous because it can undergo hydrolysis to produce hydrogen cyanide (HCN).

Table (3): Impact of amygdalin compound separated from apricot kernels on adults of *Theba pisana* snail using baits technique under laboratory conditions.

Days Compound	Concentrations	Mortality percentages				
		One day	Three days	One week	Two weeks	Three weeks
Amygdalin	0.375 %	0.00 ^b	0.00 ^c	6.67 ^d	0.00 ^d	6.67 ^d
	0.750 %	0.00 ^b	6.67 ^{bc}	20.00 ^c	33.33 ^c	40.00 ^c
	1.50 %	0.00 ^b	13.33 ^b	33.33 ^b	53.33 ^b	73.33 ^b
	3.00 %	20.00 ^a	33.33 ^a	60.00 ^a	100 ^a	100 ^a
Control		0.00 ^b	0.00 ^c	0.00 ^d	0.00 ^e	0.00 ^e
LSD _{0.05}		5.43***	9.39***	10.85***	9.39***	9.40***

Table (4): Impact of amygdalin compound separated from apricot kernels on adults of *Eobania vermiculata* snail using baits technique under laboratory conditions.

Days Compound	Concentrations	Mortality percentages				
		One day	Three days	One week	Two weeks	Three weeks
Amygdalin	0.375 %	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^c
	0.750 %	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	6.67 ^{bc}
	1.50 %	0.00 ^b	0.00 ^b	6.67 ^{ab}	6.67 ^{ab}	13.33 ^b
	3.00 %	6.67 ^a	6.67 ^a	13.33 ^a	26.67 ^a	33.33 ^a
Control		0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^c
LSD _{0.05}		5.42 ^{ns}	5.42 ^{ns}	7.67**	7.67**	9.39***

4. Biochemical studies:

Results in Tables (5 and 6) found remarked decrease in the activity of amylase and invertase enzymes in adults of *T. pisana* and *E. vermiculata* snails treated with amygdalin compound separated from apricot kernels compared to control. The decreasing percentages reached its maximum level for tested snails by time elapsing were recorded (-19.33, - 29.48, - 52.32 and - 61.91) and (- 12.38, - 29.85, - 50.09 and - 60.70) of amylase and invertase enzymes for *T. pisana* snail, at concentrations 0.375, 0.750, 1.5 and 3 %, respectively after one week of treatment, (-9.16, - 10.04, - 42.22 and - 50.21) and (- 6.12, - 11.09,

- 40.93 and - 48.72) of amylase and invertase enzymes for *E. vermiculata* snail, at the same concentrations, respectively after one week of treatment. Results showed that a significance between the four concentrations in adults of *T. pisana* snail by time elapsing of amylase and invertase enzymes except three days of amylase enzyme than control. Otherwise, there were no significance between the same concentrations in adults of *E. vermiculata* snail by time elapsing except one week of the two enzymes. For instances, Farag (2012) recorded decrease in the activity of amylase and invertase enzymes in *Monacha cartusiana* (Müller)

(Gastropoda:Hygromiidae) and *E. vermiculata* snails treated with plant extracts such as castor oil compared to control. AS regards, when the route of amygdalin administration is oral then it is decomposed into prunasin by enzymes present in digestive tract after passing through the salivary and gastrointestinal stages. The major component of amygdalin in digestive fluids is prunasin which was incubated in a caco-2 cell culture system. Prunasin was decomposed into the mandelonitrile by β -glucosidase and hydroxylase through the small intestinal

wall producing hydroxymandelonitrile. Benzaldehyde is also component of amygdalin when it is decomposed by enzymes it can reduce pepsin activity and cause damage to digestive function (Heikkila and Cabbat, 1980; Shim and Kwon, 2010 and Song and Xu, 2014). Otherwise, Farag (2017) remarked increase in the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes in *M. cartusiana* snail treated with amygdalin compound compared to control.

Table (5): Changes in amylase and invertase enzymes activities in adults of *Theba pisana* snail treated with amygdalin compound separated from apricot kernels using baits technique.

SA = Specific activity as (ml glucose / ml)

Enzymes Compound	Conc. (%)		Amylase			Invertase		
			One day	Three days	One week	One day	Three days	One week
Amygdalin	0.375 %	SA	19.53 ^a	18.94 ^{ab}	17.24 ^{ab}	21.79 ^{ab}	20.77 ^a	20.31 ^{ab}
		RA%	- 6.78	- 10.19	- 19.33	- 1.54	- 2.99	- 12.38
	0.750 %	SA	17.39 ^{ab}	16.83 ^{ab}	15.07 ^b	18.92 ^{ab}	17.87 ^{ab}	16.26 ^{bc}
		RA%	- 16.99	- 20.20	- 29.48	- 14.51	- 16.46	-29.85
	1.50 %	SA	14.73 ^{bc}	12.63 ^{ab}	10.19 ^{bc}	16.87 ^{bc}	14.09 ^{bc}	11.57 ^{cd}
		RA%	- 29.69	- 40.11	- 52.32	- 23.77	- 34.13	- 50.09
3 %	SA	12.63 ^c	10.59 ^b	8.14 ^c	13.23 ^c	11.13 ^c	9.11 ^d	
	RA%	-39.71	- 49.79	- 61.91	- 40.22	- 47.97	- 60.70	
Control		SA	20.95 ^a	21.09 ^a	21.37 ^a	22.13 ^a	21.39 ^a	23.18 ^a
L.S.D _{0.05}			3.82**	9.28 ^{ns}	8.65*	4.95*	5.81*	5.21***

RA% = (Relative activity %) = [(Treatment – Control) / Control] × 100

Table (6): Changes in amylase and invertase enzymes activities in adults of *Eobania vermiculata* snail treated with amygdalin compound separated from apricot kernels using baits technique.

Enzymes Compound	Conc. (%)		Amylase			Invertase		
			One day	Three days	One week	One day	Three days	One week
Amygdalin	0.375 %	SA	16.76 ^a	15.92 ^{ab}	15.47 ^a	14.78 ^a	14.32 ^a	13.97 ^a
		RA%	- 0.65	-2.75	- 9.16	-3.21	- 4.34	- 6.12
	0.750 %	SA	16.01 ^a	15.33 ^{ab}	15.32 ^a	14.49 ^a	13.76 ^{ab}	13.23 ^a
		RA%	- 5.10	- 6.35	- 10.04	-5.11	- 8.08	-11.09
	1.50 %	SA	13.63 ^a	11.76 ^{ab}	9.84 ^b	11.35 ^{ab}	10.09 ^{ab}	8.79 ^b
		RA%	- 19.21	-28.16	- 42.22	- 25.67	- 32.60	- 40.93
3 %	SA	12.03 ^a	10.93 ^b	8.48 ^b	9.57 ^b	8.31 ^b	7.63 ^b	
	RA%	- 28.69	- 33.23	- 50.21	-37.33	- 44.49	- 48.72	
Control		SA	16.87 ^a	16.37 ^a	17.03 ^a	15.27 ^a	14.97 ^a	14.88 ^a
L.S.D _{0.05}			5.21 ^{ns}	5.02 ^{ns}	5.15*	6.99 ^{ns}	5.52 ^{ns}	3.04***

SA = Specific activity as (ml glucose / ml)

RA% = (Relative activity %) = [(Treatment – Control) / Control] × 100

The presented results studied especially those contained amygdalin using some plant seeds and kernels compound for controlling of *T. pisana*

and *E. vermiculata* snails. It suggests probability of using amygdalin compound that has the possibility to play an important role as molluscicidal for snails control. Results also found that decrease in the activity of amylase and invertase enzymes in snails treated with amygdalin compound separated from apricot kernels compared to control, so leading to death of snails.

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