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Mona A Ali
Plant Protection Research Institute, Agricultural Research Center, Egypt

Doaa A Abou El Atta
Plant Protection Research Institute, Agricultural Research Center, Egypt

Mohamed A Ayyad
Plant Protection Research Institute, Agricultural Research Center, Egypt

Corresponding Author:
Mona A Ali
Plant Protection Research Institute, Agricultural Research Center, Egypt

Propolis, bee venom and *Beauveria bassiana* toxicity with field application; Controlling the terrestrial gastropod *Monacha cartusiana*

Mona A Ali, Doaa A Abou El Atta and Mohamed A Ayyad

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Abstract
Land snails in general, and the glass clover snail *Monacha cartusiana* (*M. cartusiana*) in particular, are pests that cause widespread crop damage. This research paper is an attempt to find effective biological alternatives to be applied within snail control operations. Propolis and honey bee venom, as well as Biossiana (*Beauveria bassiana*) fungicide, were applied using two laboratory application techniques, against the glassy clover snail *M. cartusiana* with different concentrations under laboratory and field conditions. Propolis showed the most toxicity with (1591.2) and (813.4) ppm LC_{50} values, followed by *B. bassiana*, while bee venom had the lowest potency effect with (2476.9) and (1480.1) ppm using leaf dipping and contact methods, respectively. The field experiment showed a considerable population diminution using propolis with (43.42%), followed by *B. bassiana* with (34.18%), while bee venom was only (20.09%) when compared with the recommended Agrinate (62.68%).

Keywords: Land snail, *monacha cartusiana*, bee propolis, bee venom, *beauveria bassiana*, field reduction experiment

Introduction
Terrestrial gastropods, particularly *Monacha cartusiana* (*M. cartusiana*) are a serious agricultural threat that can destroy a wide range of agricultural crops and causes a considerable damage in the agriculture sector in Egypt (Ali, 2017) [1], (Helmy *et al*, 2022) [2]. In temperate and humid regions around the world, they are regarded as major pests for a variety of agricultural and horticultural crops (Gazzy *et al*., 2019) [3]. The economic damage can be caused through feeding or with contaminating by their bodies, slime or faeces, causing financial loss and product quality degradation (Lokma, 2021) [4]. Searching for new, safe, non-traditional materials that can play an alternative role in control operations rather than chemical pesticides has become an urgent necessity. Propolis is one of the honey bee products used by bees for multiple functions; thermal insulation, seal hive cracks, and protecting bees from microorganisms and predators (Silva *et al*., 2018) [5]; resin acts as 50% of propolis' chemical composition, while, wax is 30%, essential oils 10%, pollen 5%, and other unidentified organic compounds 5% (Toreti *et al*., 2013) [6]. Flavonoids, different classes of terpenoids, steroids, aromatic aldehydes, phenolic compounds, esters, and alcohols all are identified as major ingredients in propolis (Huang *et al*., 2014) [7] Vitamins, minerals and enzymes are also identified in propolis (Mahdy and Abdel-Aal 2014) [8]. Venom is a complicated acidic mixture of proteins, enzymes, peptides, and a variety of simpler compounds (amino acids, catechol, amines, carbohydrates, and minerals) and has more than 60 different characteristics (Azzam *et al*., 2018) [9]. Melittin, the most abundant component in bee venom, has been investigated extensively for its anti-inflammatory, antibacterial, and anticancer activities (Lotfy, 2006) [10]. Propolis, pollen, bee venom and royal jelly all are products were applied against bacterial pathogens as promising materials that have therapeutic properties (Ghanem, 2011) [11]. Antimicrobial and antibiotic activities for honey bees and its constituents were thoroughly investigated (Esin Basim *et al*., 2006) [12]. Propolis has a variety of biological effects, including antiviral, antifungal, and antibacterial properties (Menezes *et al*., 1997) [13] (Cafarchia *et al*., 1999) [14] (Amoros *et al*., 1992) [15]. *Beauveria bassiana* has been produced and commercialised for pest control since it has been studied for usage against a wide variety of insect pests (Ezz *et al*., 2008) [16].

They are also environmentally safe and don't pose a health risk to people (Abdel-Wareth., 2019) [17]. *B. bassiana's* secondary metabolites have antifungal and antibacterial activity against some few pathogens (Parine *et al*., 2010) [18].

As a result, our recent study was conducted to characterize the potential impact of propolis, bee venom and *Beauveria bassiana* on the adult mortality of the glassy clover gastropod, *M. cartusiana*, in both laboratory and field application.

Materials and Methods

1. Collection and preparation

Adult *M. cartusiana* snails were collected from a cultivated field with clover in Mansoura area, Dakahlia, Egypt. Snails were delivered inside the lab and kept there, in glass boxes that contained sterilized sandy loamy soil with a height of about 10 cm at 25C°±2C° and 75%±5% soil moisture, feeding on (*Lactuca sativa* L.) leaves. For 14 days, the individuals were kept for adaption. Only healthy snails were utilized in the experiment; dead and sick ones were discarded. (Helmy *et al*., 2022) [2].

2. Tested Compounds

1. Propolis
2. Bee Venom
3. *Beauveria bassiana* (Biossiana 2.5% WP) 250 gm. /100 liter was obtained from Plant Protection Institute, A.R.C, Egypt.
4. Agrinate 24% SL. A carbamate molluscicide, chemical name: S-methyl N (Methylcarbamoyloxy) thioacetimidate.

2a. Colonies of honey bees and a study location

Collecting and producing bee's propolis and venom were conducted at a private apiary in Mansoura district, Dakahlia Governorate, Egypt.

2b. Collection of propolis (Resin) samples

Scraping propolis off of frame rests and edges, bottom boards, and the inside of hive boxes enabled the harvesting of propolis resin. Scrapings could contain propolis from multiple seasons (Bankova *et al*., 2006) [19].

2c. Extraction of propolis

Propolis was extracted from any impurities, such as bees wax, using an ethanol solvent in preparation for additional bio tests. After spending the night in a deep freezer (-20 °C), propolis was chopped into little pieces. After measuring a sample of propolis, 70% ethanol solvent (1:30 w: v) was added, and the mixture was left at room temperature for 24 hours. The propolis suspension was then subjected to a 20 °C ultrasonic bath for 20 minutes. The produced suspension was filtered at room temperature using filter paper, and the process was repeated with the portion that was trapped in the filter. The residue was then extracted once more under the same conditions (Popova, *et al*., 2004) [20]. The resulting extract will be evaporated to dryness for future experiments (Netíková, *et al*., 2013) [21].

2d. Bee venom collection

Bee venom was collected every 15 days for 20 minutes using the Bee Venom Collector Device. The device was put beside the third comb from the hive entrance. A sharp scraper was used to collect the dry venom. The fresh dried bee venom was carefully packed into a special container and stored in a dry and cool place until the experiment was done (Kosuge, 1969) [22].

3. Toxicity application techniques

3a. Leaf dipping technique

The toxicological activity of the investigated substances was assessed at 3 different concentrations (500, 1000, and 2000 ppm). The desired concentration was applied to fresh lettuce leaves for 60 seconds through dipping, and then the leaves were dried. The plastic containers (25 cm 10 cm 10 cm) holding 10 adults of *M. cartusiana* in clay soil were then supplied with the dried leaves (3–5 cm). Each box was covered with muslin fabric fixed with rubber band to prevent the gastropods from escaping. We evaluated three replicates of each concentration, with untreated lettuce discs serving as the control. The mortality % was noted after 1, 3, 5, and 7 days (Helmy *et al*., 2022) [2].

3b. Contact technique

The same pervious concentrations of tested compounds were prepared. On the bottom of a Petri dish (9 cm in diameter), two mL of each concentration were dropped and gently waved around in circles (Ascher and Eliyahu, 1981) [23]. Water was evaporated at room temperature, leaving a thin film with the indicated concentration of the tested substances. Ten adult test animals were exposed to various substances at various concentrations. The control treatment was performed purely with water. Daily counts of dead animals were recorded, followed by removal. Abbott's method was applied to adjust mortality percentages, and the statistical method of probit analysis was used to determine LC_{50} values (Abbott, 1925) [24].

4. Field experiment

The evaluated substances were applied to a clover field that was extensively invaded with *M. cartusiana* at Mansoura; Dakahlia Governorate. Each substance was utilized as a poisonous wheat bran bait so at concentration of 2%. The evaluated materials were combined with two parts toxin, five parts syrup of sugar cane, and ninety three parts wheat bran. All treatments were performed 3 times, and a control treatment using the same methodology but without any toxicants.

Each replicate was given in the form of 100 g squares of plastic. A live snail was observed before application in both the treatment and control, as well as at intervals of 1, 3, 7, 14 and 21 days until the experiment's termination. The percentages of *M. cartusiana* diminution were calculated using Henderson and Tilton method; (Henderson and Tilton, 1955) [25].

Henderson and Tilton formula

$$\text{Corrected \%} = \left(1 - \frac{\mathbf{n} \text{ in } \mathbf{Co} \text{ before treatment} * \mathbf{n} \text{ in } \mathbf{T} \text{ after treatment}}{\mathbf{n} \text{ in } \mathbf{Co} \text{ after treatment} * \mathbf{n} \text{ in } \mathbf{T} \text{ before treatment}}\right) * 100$$

Where: n is the number of living animals, T the number of living animals in treated plots after and before treatment, while Co is the number of live animals in control plots after and before treatment.

5. Analytical statistics

Probit analysis was used to determine the LC_{50} values, which are expressed in ppm units. One way ANOVA was applied to all statistics, which were all represented as mean ± SE (St *et*

al., 1989) [26]. Using Tukey's method, confidences with a 95% simultaneous confidence level were computed. A probability of 0.05 or less was regarded as significant. All statistical analysis was performed using Cohort Software (Cho *et al*., 2004) [27].

Results and Discussion

1. Toxicity tests of the tested compounds against *M. cartusiana* under laboratory conditions

Data presented in Table (1) and Fig. (1) Indicated that Agrinate was the most potent applied compound against *M. cartusiana* adult snails followed by Propolis and *Beauveria* then Bee venom using leaf dipping and contact techniques. LC_{50} values for leaf dipping were 1591.2, 1871.6, 2476.9and 701.2 ppm while, for contact were 813.4, 1074.2, 1480.1and 534.2 ppm for Propolis, *Beauveria*, Bee venom and Agrinate, respectively.

Studies in the field of applying venom and propolis to terrestrial snails are somewhat scarce, but there is some research's on these materials as an effective materials for controlling insects, including cotton leaf worm *(Spodoptera littoralis)*; bee venom toxicity through the LC_{50} values was investigated against the 4Th instar larvae of *S. littoralis* by different application techniques; 0.1 and 9.9 ppm using topical and injection methods, respectively (Sadek *et al*., 2022) [28]. *Beauveria bassiana* was evaluated as a bio-insecticide against the brown land snail *Eobania vermiculata*; the LD_{50} values were 0.479% and 0.216% ppm and while for Agrinate were 0.259% and 0.058% ppm; for 24 and 48 hours, respectively (Shaker *et al*., 2015) [29].

Table 1: LC_{50} and LC_{90} values of the tested compounds on *M. cartusiana* adult snails using leaf dipping and contact techniques under laboratory conditions.

Technique	Treatment	LC_{50} (ppm)	LC_{90} (ppm)	Slope± S.E.
Leaf dipping	Bee venom	2476.9	7839	2.56±0.75
	Propolis	1591.2	7695.9	1.87±0.58
	Beauveria	1871.6	7897.2	2.04±0.61
	Agrinate	701.2	3576.2	1.81±0.57
Contact	Bee venom	1480.1	7467.1	1.82±0.57
	Propolis	813.4	3325.8	2.09±0.58
	Beauveria	1074.2	4458.7	2.07±0.57
	Agrinate	534.2	1361.8	3.15±0.75

S.E. = Standard Error.

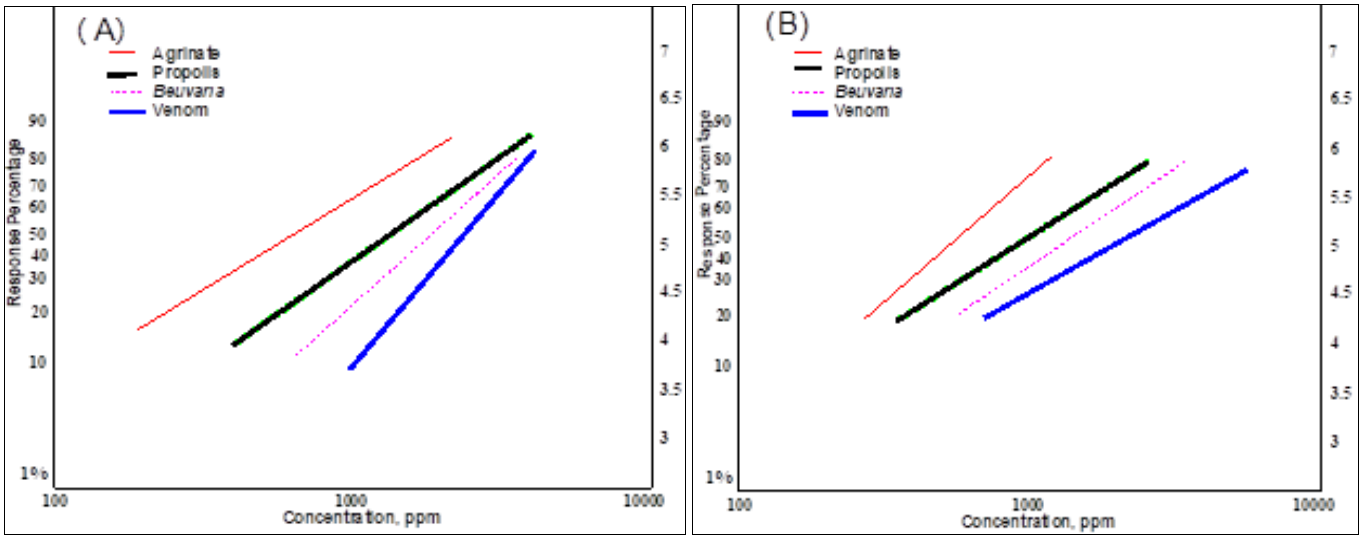

Fig 1: Con/probit regression line of tested compounds against *M. cartusiana* using leaf dipping (A) and contact (B) techniques

2. Field application of the tested compounds against *M. cartusiana*

Data presented in Table (2) showed the reduction percentage values of *M. cartusiana* snail exposed to Bee venom, Propolis, *Beauveria* and Agrinate using poisonous baits technique.

Table 2: Field reduction % of *M. cartusiana* snails following the application of the tested compounds

Treatment	Pre treatment	Reduction % after indicated days									Residual effect	Mean of Reduction
		1day		3days		Initial Kill	1 week		2 weeks			
		Mean (±SE)	Reduction %	Mean (±SE)	Reduction %		Mean (±SE)	Reduction %	Mean (±SE)	Reduction %		
Bee venom	54.0^{a}±7.51	51.17^{a}±5.2	10.66	48.0^{a}±5.77	21.26	15.96	47.5^{a}±7.22	23.62	50.0^{a}±1.73	24.79	24.21	20.09
Propolis	53.67^{a}±7.26	43.33^{a}±4.37	23.94	31.33^{ab}±1.86	48.29	36.12	31.67^{ab}±4.81	48.76	30.33^{b}±3.18	52.7	50.73	43.42
Beauveria	37.33^{a}±4.98	33.33^{a}±4.41	15.82	29.0^{ab}±0.57	31.18	23.5	25.0^{ab}±5.13	41.85	23.0^{b}±2.31	47.87	44.86	34.18
Agrinate	40.7^{a}±12.1	25.67^{a}±7.88	40.54	18.0^{b}±5.13	60.82	50.68	14.0^{b}±3.06	70.13	10.0^{c}±0.57	79.21	74.67	62.68
Control	44.0^{a}±4.58	46.67^{a}±6.49	—	49.67^{a}±6.12	—	—	50.67^{a}±9.77	—	52.0^{a}±1.73	—	—	—
P-value	0.47	0.06	—	0.002	—	—	0.012	—	0.00	—	—	—

Data revealed that Agrinate was the most effective substance for reducing the population density of *M. cartusiana* followed by Propolis and *Beauveria* then Bee venom. Data revealed that, after the first three days of treatment, the percentages of snail's reduction were 15.96, 36.12, 23.5 and 50.68% for Bee venom, Propolis, *Beauveria* and Agrinate, respectively. The residual effects of these compounds were 24.21, 50.73, 44.86 and 74.67% reduction, consequently with averages of 20.09, 43.42, 34.18 and 62.68% reduction for Bee venom, Propolis, *Beauveria* and Agrinate, respectively. Inside the hive; Propolis is utilized to kill snails by mummifying them (Stefan Bogdanov, 2016) [30]. Bee venom is easily destroyed and

denatured by the sun light and temperature and oxidation substances that may explain why venom is lower potency at field experiment; some bee venom types have more chemical constituents than another species that may lead to extra biological activity; *A. dorsata* venom has more elucidated pheromones than *A. mellifera* (Stefan Bogdanov, 2016) [31].

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