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Molluscicidal effect of ammonium nitrate and some biopesticides on the brown garden snail *Eobania vermiculata* (Gastropoda: Helicidae)

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Abstract:

The molluscicidal activity of biopesticide formulations *Bacillus thuringiensis* and *Metarhizium anisopliae* at the under recommended, recommended and up to recommended concentrations was evaluated against adults of *Eobania vermiculata* (Müller) (Gastropoda: Helicidae) snail. Concentrations of both biopesticides were submitted to the ultraviolet radiation in different times of exposure and then assessed also against the same snail species. On the other hand, the molluscicidal effect of ammonium nitrate fertilizer individually and as post treatment against snails that pre treated with each of irradiated and non-irradiated biopesticides was investigated. The impact of all these treatments on the biological behavior and enzyme activity of snails were also studied. Ammonium nitrate caused the highest mortality 73.33% of snails at the highest concentration 2% after only 7 days of the experiment. On the other hand, the post treatment of snails with ammonium nitrate at the highest concentration 2% after treating them with each of the non-irradiated and irradiated up to recommended concentration 16×10^3 spores/ml of *B. thuringiensis* irradiated for 90 min., recorded the same highest mortality of snails, 70% after 7 and 14 days of the experiment, respectively. All of the tested treatments in this study completely prevented the egg laying of snails. In addition, *M. anisopliae* at 16×10^3 spores/ml irradiated for 60 min. achieved the highest reduction in the activity of alkaline phosphatase enzyme and decrease also the activity of the acid phosphatase enzyme compared with the control. On the contrary, the same treatment caused the highest increase in the activity of protease enzyme. The highest reduction in the level of amylase and lipase enzyme activity was recorded by *B. thuringiensis* at 4×10^3 spores/ml exposed to UV radiation for 60 min.

Introduction

Land snails are considered one of the most serious and destructive pest cause immense damage to a wide range of plants in Egypt (Gabr *et al*., 2006). *Eobania vermiculata* (Müller) (Gastropoda: Helicidae) represent one of the most harmful and abundant snails in different districts (Eshra, 2013). Due to the direct and latent hazards of the chemical pesticides and their toxic effect on the ecosystem, it is always advised to use safe biopesticides for controllling pests. In addition, the recent strategies of Integrated Pest Management (IPM) have mainly concentrated on the use of safe control method (El-Metwally *et al*., 2010 and Kares *et al*., 2012) for reaching to a minimum residue of pesticides in food (Vu *et al*., 2007).

Microbial agents (containing pathogenic microorganisms) represented one important component of biological control techniques (Moussa *et al*., 2014). It has low toxicity to the environment and ecosystem, low probability of target pests building up resistance, low cost of multiplication and registration, etc. (Rodrigues *et al*., 2016). Fungal biopesticides can be used as an alternative method to control the terrestrial gastropods (Hendawy *et al*., 2015). Entomopathogenic fungus *Metarhizium anisopliae* has pathogenic action against various agricultural pests (Marques *et al*., 2000 and Zappelini *et al*., 2010). Moreover, it can also produce some effective antimicrobial agents for control plant diseases (Butt *et al*., 2001). Bacteria considered also another microbial biocontrol method of land snails that received greater attention in the few years ago (Genena and Mostafa, 2010). *Bacillus thuringiensis* (Bt) is a gram negative bacterium which produces chemicals has a toxic effect against pests. The toxic activity of this bacterium against some land snails in Egypt has been investigated in several studies (Azzam and Belal, 2003 ; Genena *et al*., 2008 and Kramarz *et al*., 2007). Related to these aspects, fertilizer is a new molluscicide with different mode of action and improved effectiveness safety when applied at low rates. It is a successful molluscicide and at the same time improves plant growth and yield when added to the soil. Ammonium nitrate has a high toxicity against *E. vermiculata* snail at low concentrations (Hend, 2013).

Toxic effect of nitrate against pests may be related to *in vivo* reduction of nitrate to nitrites and a conversion of hemoglobin to methemoglobin (Scott and Crunkilton, 2000). Biopesticide formulations (Particles) contain the microorganisms encapsulated in some materials such as starch, cellulose and gelatin, etc. Each particle can contain hundreds of conidia and after the drying process, it is ready for agricultural use and can be applied directly on the target pest and even on soil and plants without the need for dispersion in an aqueous medium, facilitating the application (Burges, 1998). In the last few years, more attention has been paid to direct ultraviolet radiation effects on fungi and bacteria (Martyn *et al*., 2003). Ultraviolet radiation can reduce the viability of microorganisms conidia (Rangel *et al*., 2008). But it doseno´t have the same negative effect against the microorganisms when used as encapsulated formulated products. These products provide great benefits such as protecting conidia from radiation, increasing the shelf live, facilitating storage and transport (Batista *et al*., 1998).

In addition, when the biopesticides that exposed to radiation used to control pests it doesn't affect negatively on the ecosystem. Several studies confirmed that doses of ionizing radiation do not damage most fresh fruits and vegetables (Hallman, 2011). On the other hand, fungal species such as *Trichoderma viride*, *Saccharomyces cerevisiae*, *Coriolus versicolor* and *Phanerochaete chrysosporium* strongly

affected on the fertility of snails there were reduces the egg laying capacity of *B. alexandrina* snail (Ragab and Ismail, 2001). Radiation also reduced the reproduction capacity of terrestrial snails. It was decreased the number of eggs laid and prevent the hatching of those laid by the irradiated adults of the land snail, *Cornu aspersum* (Hallman, 2016). Biopesticides also negatively affected on the biochemical parameters of snails. It is decreased the level of total proteins and free amino acids of snails (El-Halim *et al*., 1990).

This study aimed to evaluate the effect of irradiated and non-irradiated biopesticides *B. thuringiensis* and *M. anisopliae* against the adults of *E. vermiculata* snail. The study also extends to assess the molluscicidal activity of ammonium nitrate alone and as post treatment to snails which pretreated with tested irradiated and non-irradiated biopesticides. The effect of all previous treatments on the biological and biochemical parameters of snails was also investigated.

Materials and methods

1. Collection and maintenance of *Eobania vermiculata* snails:

Adults of *E. vermiculata* were collected from infested navel orange field in Banadf village, Meniet El-Kamh district, Sharkia Governorate, Egypt. The snails were transferred in muslin bags to the laboratory and kept in a glass container (30 × 30 × 50 cm^3) containing moist clay soil and covered with muslin cloth for preventing snails escaping. Snails were supplied daily with fresh cabbage leaves for two weeks before any tests for acclimatization (Abd El-Aal, 2001).

2. Tested biopesticide formulations:

The two biopesticide formulations *Bacillus thuringiensis* (Berliner) and *Metarhizium anisopliae* (Bioranza) which used in this study were obtained from the Central Agricultural Pesticides Laboratory, Dokki, Egypt. Weights of 0.025, 0.05 and 0.1 gram of each biopesticide were diluted separately in 100 ml sterilized distilled water to obtain the under recommended, recommended and up to recommended concentrations of each biopesticide represents as 4×10^3, 8×10^3 and 16×10^3 spores/ml, consecutively.

3. Exposure of tested biopesticides to UV radiation:

Each biopesticide concentration was exposed to UV light; 254 nm (Philips, 20 W/4C) and at a distance of 50 cm for three times 30, 60 and 90 min. exposure.

4. Preparation of ammonium nitrate solution:

Pure ammonium nitrate (33% N) produced in pills form was purchased from El- Gomhouria Company, Egypt. Three concentrations 0.5, 1 and 2% of this fertilizer were prepared by dissolving the required amount in distilled water to obtain the appropriate concentration (Mahmoud, 1994).

5. Pathogenic activity of the biopesticide formulations exposed and not exposed to the UV radiation against *Eobania vermiculata* snails:

The pathogenic effect of *Bacillus thuringiensis* and *Metarhizium anisopliae* biopesticides at the under recommended, recommended and up to recommended concentration of each one represents as 4×10^3, 8×10^3 and 16×10^3 spores/ml was assessed separately against adults of *E. vermiculata* snails. Each tested concentration of each biocide was exposed to UV radiation at three exposure times (30 min., 60 min. and 90 min.).

Plastic boxes (3/4 kg capacity) were used, each containing 1/2 kg sterilized clay soil and ten adults of *E. vermiculata* snail. Water holding capacity of the soil was adjusted and ten discs of cabbage leaves were introduced in each box. Each irradiated concentration of both biocides was sprayed separately on the soil and cabbage discs (Foster *et al*., 1991). Three replicates were

prepared for each concentration. Other adults of the same snail species were treated with the same tested biocides as explained previously exactly, but without exposing their concentrations to radiation. Control boxes were prepared by the same manner without any treatment with three repetitions. All treated and control boxes were tightly covered with muslin cloth and secured with a rubber band for prevent snails escaping. Mortality percentages were recorded for all boxes at intervals of three days for one month and corrected by Abbott's formula (1925). At every time the results are recorded, cabbage discs were changed with other fresh discs.

6. Molluscicidal effect of ammonium nitrate against *Eobania vermiculata* snails:

Ammonium nitrate was tested against the adults of *E. vermiculata* snail at the concentrations 0.5, 1 and 2%. Plastic boxes were used in this experiment, each box contained 1/2 kg moistened clay soil. Ten adults and ten discs of cabbage leaves were placed on the soil surface of each box. The tested concentrations were directly sprayed on the soil and cabbage discs. Three replicates were prepared for each concentration and other three replicates were prepared by the same manner without any treatment as control. All boxes were examined daily, and mortality percentages were recorded for one month.

7. Toxic effect of ammonium nitrate against *Eobania vermiculata* snails pre-treated with non-irradiated and irradiated biopesticides:

Adults of *E. vermiculata* were treated with each of *B. thuringiensis* and *M. anisopliae* at the tested concentrations 4×10^3, 8×10^3 and 16×10^3 spores/ml for each biocide, respectively. Other adults of the same snail species were treated with the same tested concentrations exposed to UV radiation at the exposure times referred to previously (30 min., 60 min. and 90 min.). Three replicates were prepared for each irradiated and non-irradiated concentration, each replicate containing ten adult snails. The treatments were carried out by using spray technique as mentioned before in the previous experiments according to Foster *et al*. (1991). After 10 days of the experiment, snails which treated with irradiated and non-irradiated biocides at the tested concentrations were sprayed individually with ammonium nitrate at 0.5, 1 and 2% concentrations, respectively. Mortality percentages were recorded daily for one month.

8. Impact of the tested biopesticides and ammonium nitrate on the biological behavior of *Eobania vermiculata* snail:

During following up the molluscicidal effect of the biopesticides *B. thuringiensis*, *M. anisopliae* (Irradiated and non-irradiated) and ammonium nitrate individually against *E. vermiculata* snails and the treatment of snails with ammonium nitrate after treated with the irradiated and non-irradiated biopesicides in the previous experiments, the egg laying and biological behavior of the snails were also observed. At all of these treatments, treated and control boxes checked every two days for the presence of egg clutches (Hend, 2007). Examination of boxes continued for this purpose for a year, started from the beginning of the study experiments at 15 November 2019 until 20 November 2020.

9. Effect of some tested irradiated biopesticides on the biochemical parameters of *Eobania vermiculata* snail:

Due to the observed effect of tested biopesticides on the biological behavior of *E. vermiculata* snail, the effect of some selected irradiated biopesticide treatments on certain enzymes which related to the reproduction of snails was studied. These treatments were *B. thuringiensis* at under recommended concentration (4×10^3 spores/ml) exposed to UV for 60 min., *M. anisopliae* at up to

recommended concentration (16×10^3 spores/ml) exposed to UV radiation for 60 min. and *M. anisopliae* at recommended concentration (8×10^3 spores/ml) exposed to UV radiation for 90 min. The effect of each of these treatments on the enzymes alkaline and acid phosphatase, amylase, protease and Lipase which strongly related to the reproduction process in snails as mentioned by Clelland *et al*. (2001) and Ademolu *et al*. (2013) was estimated.

Ten snail individuals at 1 g weight were randomly taken from replicates of each of these treatments after 96 hrs. of the treatment. The snails were dissected according to the method of Segun (1975). Tissues of snails were homogenized and centrifuged at 4000 r.p.m. for 30 min. The sediment was discarded while the supernatants were kept in a deep freezer till it's using to determine the levels of alkaline and acid phosphatase, amylase, protease and lipase in tissues of tested and control snails.

The activity of alkaline and acid phosphatase (ALP and ACP) was determined according to the method of Kind and King (1954). While, amylase (AMY), protease (PRO) and lipase (LIP) activities were determined by the methods described by Adedire *et al*. (1999) and Ademolu *et al*. (2009).

10. Statistical analysis

All data from the above experiments were analyzed and the difference between means was tested by Costat (2005) statically program analysis, computer program software. The least significant difference (L.S.D.) at 0.05 level was also calculated.

Results and discussion

1. Pathogenic effect of irradiated and non-irradiated biopesticides against *Eobania vermiculata* snail:

In the treatment of *E. vermiculata* snails with each of *B. thuringiensis* and *M. anisopliae* with and without exposing to the UV radiation, there is no mortality was found in all boxes till the end of experiment which continued for 21 days. This result was strongly supported by Genena *et al*. (2008) reported that in the treatment of *E. vermiculata* and *Monacha cantiana* snails with eight strains of *B. thuringiensis*, the all strains did not show any adverse effect or mortality against both snail species.

The same results were also confirmed by Kramarz *et al*. (2007) indicated that *B. thuringiensis* had no negative effect against *Helix aspersa* snail under the laboratory conditions. This bacterium also not have observed effect against *Oncomelania hupensis* snail, it was recorded only 1.1% mortality of snails (Gao *et al*., 2008). While, Zedan (2004) revealed that *M. anisopliae* at the concentrations 0.5×10^8, 1×10^8, 2×10^8 and 4×10^8 spores/ml exhibited 28.75, 50, 67.86 and 85.71% mortality of *E. vermiculata* snails after 7 days of treatment, respectively.

The other biopesticide, Biovar (*Beauveria bassiana*) caused 40, 60 and 80% mortality of *Monacha obstructa* snails at the concentrations of 2×10^5, 4×10^5 and 8×10^5 spores/ml after 6 days of experiment, respectively (Bahy El-Din *et al*., 2016). At the same trend, Khidr (2015) showed that the double dose from the recommended dose of spinetoram 12% SC, Emamectin benzoate 0.5% EC and Emamectin benzoate 1.92% EC biopesticides recorded high mortality of *E. vermiculata* and *M. obstructa* snails than the using of recommended doses. *M. obstructa* was more sensitive and affected by these bioagents than *E. vermiculata* snails.

On the other hand, Khorramvatan *et al*. (2014) demonstrated that exposing of *B. thuringiensis* to UV radiation not increase its ability to control pests, this may be a return to the destroying effect of radiation against the bacterial conidia which reduce its potency against pests. The same effect was also recorded against the conidia of fungi, leads to decrease its pathogenic activity (Rodrigues *et*

al., 2016). On the other direction, Cortesao *et al*. (2019) showed that UV radiation cause mutation to *Bacillus subtilis* bacterium. The mutagenesis of this bacterium was strongly depending on the function of the structural components, coat layers and the dipicolinic acid as key protectants against the DNA damage. The mutation of bacteria by radiation can makes a severe form of it. This new form had strong pathogenic activity against host (Kong *et al*., 2010). α / β- type SASP group in the bacterial spores saturate DNA and protect it from damage by the UV radiation (Hathout *et al*., 2003).

2.Molluscicidal effect of ammonium nitrate against *Eobania vermiculata* snails:

The toxic effect of ammonium nitrate against *E. vermiculata* snails was investigated. As shown in Table (1), snails mortality increased with increasing the ammonium nitrate concentrations and exposure times. After one day of treatment, ammonium nitrate recorded its highest effect at the highest concentration 2.0% by record 56.66% mortality of snails. It was followed by the concentrations 1 and 0.5% which achieved 13.33 and 10% mortality, respectively. The mortality percentage reached to its maximum value 73.33% at the highest concentration 2% after 7 days of the experiment and then it still stable until the end of experiment. While, the other concentrations 1.0 and 0.5% recorded their highest molluscicidal effect at the end of experiment with 50 and 43.33% mortality after 21 days of the experiment, respectively. No death of snails was found in the control replicates. The obtained results also showed a high significant difference in the mortality of snails treated with the different concentrations in comparing with the control.

Table (1): Molluscicidal effect of ammonium nitrate against *Eobania vermiculata* snails.

Concentrations (%)	Mean of mortality % after indicated days					General mean
	1	3	7	14	21	
0.5	10.00 ^{b}	10.00 ^{b}	16.66 ^{b}	40.00 ^{b}	43.33 ^{b}	24.00 ^{b}
1.0	13.33 ^{b}	13.33 ^{b}	20.00 ^{b}	43.33 ^{b}	50.00 ^{b}	28.00 ^{b}
2.0	56.66 ^{a}	56.66 ^{a}	73.33 ^{a}	73.33 ^{a}	73.33 ^{a}	66.66 ^{a}
Control	0.00 ^{b}	0.00 ^{b}	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}
P	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***
L.S.D. $_{0.05}$	1.80	1.80	0.76	2.24	1.53	13.08

The same letter in the same column means not significant at P < 0.05.

These results were confirmed by Hend (2013) reported that mortality of *E. vermiculata* snails increases with increasing concentrations of ammonium nitrate and the time elapsed. This fertilizer recorded 56.66% mortality of snails at the highest concentration 2% after one day of experiment. While, the lowest tested concentration 0.5% showed only 20% mortality after the same period of treatment. At the same trend, urea caused 67 and 86% mortality of *E. vermiculata* and *Theba pisana* snails at the concentration 800 × 10^2 ppm after two days of treatment, respectively. The

same concentration showed 80 and 100% mortality of each snail species after three days of treatment, respectively (Eshra, 2014). El-Wakil (2009) had earlier observed that copper sulfate has a high toxic effect against *T. pisana* snail. While, potassium sulfate recorded a slight effect against the same snail species. Additionally, the using of ammonium bicarbonate has an advantage for being molluscicidal. But, the using of it has declined with the advent of urea and diammonium phosphate. These fertilizers causes agriolimacides and arionides to emit large amounts of mucus, which strongly leads to the dehydration and death of gastropod (Barker, 2002). Consequently, fertilizers considered a newer successful alternative molluscicide instead of pesticides. Moreover, it is safer than pesticides for use around pest and vertebrate wild life (Speiser and Kistler, 2002).

3. Molluscicidal effect of ammonium nitrate against *Eobania vermiculata* snails pre-treated with non irradiated and irradiated biopesticides:

The molluscicidal impact of ammonium nitrate at the concentrations 0.5, 1.0 and 2% against *E. vermiculata* snails which pre treated with the under recommended, recommended and up to recommended concentrations (4×10^3, 8×10^3 and 16×10^3 spores/ml) of each biocide separately was evaluated, respectively.

As cleared in Table (2), by increasing the concentrations of biopesticides and ammonium nitrate, the mortality percentages of snails increases. Snails which pre treated with *B. thuringiensis* at 4×10^3 spores/ml and then treated with the lowest concentration of ammonium nitrate 0.5% not affected at all until the 7th day of experiment. Low effect of this treatment was appeared at the 14th day of experiment with 13.33% mortality which remained constant until the end of experiment. *M. anisopliae* also recorded its lowest effect at the lowest tested concentration 4×10^3 spores/ml and post treatment with 0.5% of ammonium nitrate, caused only 10% mortality at the first day of experiment. This percentage remained as it is until the 14th day of experiment and then increased to 20% after 21 days. *B. thuringiensis* and *M. anisopliae* gave the same final mortality 43.33% at the recommended concentration 8×10^3 spores/ml and post treatment with ammonium nitrate at the concentration 1%. Moreover, both biopesticides recorded their highest impact at the up to recommended concentration 16×10^3 spores/ml and post treatment with ammonium nitrate at the highest concentration 2% by record 40 and 56.66% mortality after one day of experiment, respectively.

Table (2): Molluscicidal effect of ammonium nitrate against *Eobania vermiculata* snails pre-treated with non irradiated biopesticides.

Pre-treatment with biopesticides Conc. (spores/ml)	Post-treatment with ammonium nitrate Conc. (%)	Mean of mortality % after indicated days					General mean
		1	3	7	14	21	
Bacillus thuringiensis							
4×10^3	**0.5**	0.00 ^{e}	0.00 ^{d}	0.00 ^{c}	13.33 ^{c}	13.33 ^{d}	5.33 ^{d}
8×10^3	**1.0**	26.66 ^{bc}	26.66 ^{b}	26.66 ^{b}	43.33 ^{b}	43.33 ^{bc}	33.32 ^{b}
16×10^3	**2.0**	40.00 ^{b}	60.00 ^{a}	70.00 ^{a}	70.00 ^{a}	70.00 ^{a}	62.00 ^{a}
Metarhizium anisopliae							
4×10^3	**0.5**	10.00 ^{de}	10.00 ^{cd}	10.00 ^{bc}	10.00 ^{c}	20.00 ^{cd}	12.00 ^{cd}
8×10^3	**1.0**	20.00 ^{cd}	20.00 ^{bc}	20.00 ^{b}	20.00 ^{c}	43.33 ^{bc}	24.66 ^{bc}
16×10^3	**2.0**	56.66 ^{a}	56.66 ^{a}	56.66 ^{a}	56.66 ^{ab}	56.66 ^{ab}	56.66 ^{a}
Control		0.00 ^{e}	0.00 ^{d}	0.00 ^{c}	0.00 ^{c}	0.00 ^{d}	0.00 ^{d}
P		.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***
L.S.D. $_{0.05}$		1.42	1.42	1.70	2.19	2.41	16.01

The same letter in the same column means not significant at P < 0.05.

The mortality 56.66% which recorded by *M. anisopliae* as pre treatment and post treatment with 2% of ammonium nitrate remained constant until the end of experiment. While, the mortality of snails which pre treated with *B. thurigiensis* at the up to recommended concentration 16×10^3 spores/ml and post treated with 2% of ammonium nitrate increased to 70% at the 7th day of experiment and then stabilized till the end of experiment. All tested treatments had significantly higher than the control.

Table (3) showed the effect of ammonium nitrate at the concentrations 0.5,

1 and 2% against *E. vermiculata* snails that pre treated with each of 4×10^3, 8×10^3 and 16×10^3 spores/ml of *B. thuringiensis* and *M. anisopliae* separately, respectively. Each concentration of both biopesticides was initially exposed to the UV radiation for 30, 60 and 90 min. before the treatment of snails.

Table (3): Molluscicidal effect of ammonium nitrate against *Eobania vermiculata* snails pre-treated with irradiated biopesticides.

Pre-treatment with biopesticides Conc. (Spores/ml)	Exposure times to UV radiation (min.)	Post-treatment with ammonium nitrate Conc. (%)	Mean of mortality % after indicated days					General mean
			1	3	7	14	21	
Bacillus thuringiensis	**30**							
	60		0.00^{e}	0.00^{e}	13.33^{fg}	16.66^{ef}	16.66^{fg}	9.33^{fg}
4×10^3	**90**		13.33^{de}	13.33^{de}	13.33^{fg}	20.00^{e}	20.00^{f}	16.00^{efg}
		0.5	16.66^{cde}	16.66^{cde}	23.33^{def}	23.33^{de}	23.33^{ef}	20.66^{def}
	30							
	60		0.00^{e}	13.33^{de}	13.33^{fg}	20.00^{e}	20.00^{f}	13.33^{fg}
8×10^3	**90**	**1.0**	16.66^{cde}	16.66^{cde}	20.00^{ef}	33.33^{cde}	33.33^{def}	24.00^{def}
			20.00^{cd}	23.33^{cd}	46.66^{abc}	46.66^{bc}	46.66^{bcd}	36.66^{bcd}
	30							
	60		46.66^{ab}	46.66^{ab}	50.00^{abc}	50.00^{bc}	53.33^{abc}	49.33^{abc}
16×10^3	**90**	**2.0**	50.00^{ab}	50.00^{ab}	50.00^{abc}	56.66^{ab}	60.00^{ab}	53.33^{ab}
			56.66^{a}	56.66^{a}	63.33^{a}	70.00^{a}	70.00^{a}	63.33^{a}
Metarhizium anisopliae	**30**		0.00^{e}	10.00^{de}	16.66^{efg}	20.00^{e}	20.00^{f}	13.33^{fg}
	60		16.66^{cde}	16.66^{cde}	20.00^{ef}	26.66^{de}	26.66^{ef}	21.32^{def}
4×10^3	**90**	**0.5**	16.66^{cde}	20.00^{cd}	20.00^{ef}	26.66^{de}	30.00^{def}	22.66^{def}
	30		16.66^{cde}	23.33^{cd}	23.33^{def}	23.33^{de}	23.33^{ef}	22.00^{def}
	60	**1.0**	20.00^{cd}	20.00^{cd}	20.00^{ef}	20.00^{e}	30.00^{def}	22.00^{def}
8×10^3	**90**		23.33^{cd}	23.33^{cd}	40.00^{bcd}	40.00^{bcd}	40.00^{cde}	33.33^{cde}
	30		20.00^{cd}	23.33^{cd}	23.33^{def}	23.33^{de}	30.00^{def}	24.00^{def}
	60	**2.0**	33.33^{bc}	33.33^{bc}	33.33^{cde}	33.33^{cde}	33.33^{def}	33.33^{cde}
16×10^3	**90**		50.00^{ab}	50.00^{ab}	56.66^{ab}	56.66^{ab}	56.66^{abc}	54.00^{ab}
Control			0.00^{e}	0.00^{e}	0.00^{g}	0.00^{f}	0.00^{g}	0.00^{g}
P			.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***
L.S.D. $_{0.05}$			1.90	1.89	1.80	1.80	1.73	17.52

The same letter in the same column means not significant at P < 0.05.

It seems clear from the obtains results that by increasing the concentration of ammonium nitrate and biopesticides and the exposure times to UV; the mortality of snails increases. After one day of the experiment, the pre treatment with highest concentration 16×10^3 spores/ml of *B. thuringiensis* that irradiated for 90 min. by UV and post treatment with ammonium nitrate at the highest concentration 2% recorded the highest mortality of snails 56.66%. Over the same period of experiment, *B. thuringiensis* at the other concentrations 8×10^3 and 4×10^3 spores/ml and the same exposure time to UV 90 min. and post treatment with 1 and 0.5% of ammonium nitrate, the mortality of snails were 20 and 16.66%, respectively. The mortality of snails increased gradually by increasing the experiment period, it reached to its maximum value 70% at 16×10^3 spores/ml of the same biopesticide that irradiated with UV for 90 min. and post treatment with 2% of ammonium nitrate after 14 days of experiment. The other two concentrations 8×10^3 and 4×10^3 spores/ml gave their highest activity after 7 days of experiment with record 46.66 and 23.33% mortality at the same exposure time 90 min. and post treatment with 1 and 0.5% of ammonium nitrate, respectively. This activity of these treatments remained stable until the end of the experiment. On the other hand, the pre treatment of snails with *M. anisopliae* at the highest concentration 16×10^3 spores/ml which irradiated by UV for 90 min. and post treatment with 2% of ammonium nitrate record 50% mortality after one day of the experiment. While, the pre treatment of snails with the other concentrations 8×10^3 and 4×10^3 spores/ml that irradiated with UV for the same exposure time 90 min. and post treatment with ammonium nitrate at 1 and 0.5% achieved 23.33 and 16.66% mortality after the same period of experiment, respectively. These treatments gave their maximum effect against snails with record 40 and 30% mortality after 7 and 21 days of the experiment, respectively. While, the highest concentration 16×10^3 spores/ml that exposed to UV for 90 min. and post treatment with 2% of ammonium nitrate showed the highest mortality 56.66% after only 7 days of experiment. This mortality remained constant until the end of the experiment. It is also worth to mention that the maximum mortality which exhibited by the pre treatment with the lowest concentration 4×10^3 of each of *B. thuringiensis* and *M. anisopliae* that exposed to the UV for the lowest exposure time 30 min. and post treatment with the lowest concentration 0.5% of ammonium nitrate were only 16.66 and 20% after 14 days of the experiment, respectively.

Generally, the pre treatment of snails with each of *B. thuringiensis* and *M. anisopliae* at the highest concentration that exposed to UV radiation for 90 min. and then treated with ammonium nitrate at the highest concentration were the most effective treatments against snails. But, the treatment which contained *B. thuringiensis* was clearly outweighs the other which contains *M. anisopliae* during the experiment period. Moreover, the means of snails' mortality were significantly higher in comparison with the untreated snails.

Data in support to the results of Tables 2. and 3. are limited. While, in this trend Ragab and Shoukry (2006) reported that the pre- exposure of *Lymnaea natalensis* snails to each of ammonium nitrate and urea for 24 hr. caused an additive action to niclosamide against snails. In a similar report, Hussein *et al*. (2016) stated that the pre treatment of *Biomphalaria alexandrina* snails with the inorganic fertilizer NPK increased the infection severity by *Schistosoma mansoni* against treated snails. The pre-exposure of the same snail species to anilofos, butachlor and isoprothiolane showed a synergistic effect to uccmaluscide. While, the treatment with isoprothiolane and

butachlor gave additive effect to copper sulphate and niclosamide against snails (Zidan *et al*., 2002). The pr-exposure of the other snail species, *Theodoxus fluviatilis* to metals due to its originating from a metal contaminated habitat increased tolerance at the post-exposure with metals from antifouling paints compared to snails exposed to metals for one time (Maria *et al*., 2017). On the other hand, Drauzio *et al*. (2015) revealed that UV radiation induce the production of *Metarhizium robertsii* conidia. It causes a production of resistant mycillium used as formulation to control pests. In a related study, Ariel *et al*. (2017) reported that the blue and red light increased also the production speed and germination of conidia for the same fungus and killed the pests faster. Similarly, Henrique *et al*. (2015) confirmed that UV radiation increased the production of *Colletotrichum acutatum* fungus conidia. Colonies exposed to this radiation produced 1.7 times more conidia than colonies which not exposed and this contributed strongly to increasing the activity of fungus. On the contrary, Khorramvatan (2014) showed that UV radiation obviously reduced the potency of *B. thuringiensis* against the pests. While, Rodrigues *et al*. (2016) stated that preparation of biopesticides as formulation protect the microorganism structure from the damage by UV radiation. Effect of radiation on molds depend on the wave length of the photons which cells are exposed (Fuller *et al*., 2013). Moderate exposure to the visible light (400 – 700 nm) encourages the production of conidial spores and the harmful secondary metabolites as aflatoxin (Henrique *et al*., 2015). This toxin has a high lethal effect against animals and humans (Yan *et al*., 2008).

4. Effect of the biopesticides and ammonium nitrate on the biological behavior of *Eobania vermiculata* snail:

The effect of all study treatments including irradiated and non irradiated biopesticides (*B. thuringiensis* and *M. anisopliae*), ammonium nitrate individually and pre treatment with biopesticides and then treatment with ammonium nitrate on the biological behavior of *E. vermiculata* snails was observed for a year starting from 15 November 2019 to 20 November 2020. The results showed that all treatments had a noticeable and clear negative impact on the biological behavior of treated snails in comparing with the untreated individuals in the control. All study treatments completely prevented the egg laying of snails during the experiment period for a year, except one replicate in the treatment with the recommended concentration 8×10^3 spores/ml of *M. anisopliae* exposed to UV radiation for 90 min. At the end of January 2020, the snails in this replicate laid only one abnormal oval and transparent egg compared to the normal spherical white eggs that were laid by untreated snails in the control (Photo 1). At observing this egg, it is noticed that it completely burst after only five days of it's laying. A month after laying this egg, the snails in the same replicate laid two normal (non-transparent) eggs, but they were also burst after 11 days of their laying. In comparison with the control replicates, the untreated snails laid 18 egg clutches during two months from December 2019 to February 2020. The number of eggs per clutch ranged between 10 and 48 eggs. On the other hand, the hatchability ranged from 93.75 to 100%. While, the mean of incubation and hatching periods was 23.7 and 4.6 days, respectively.

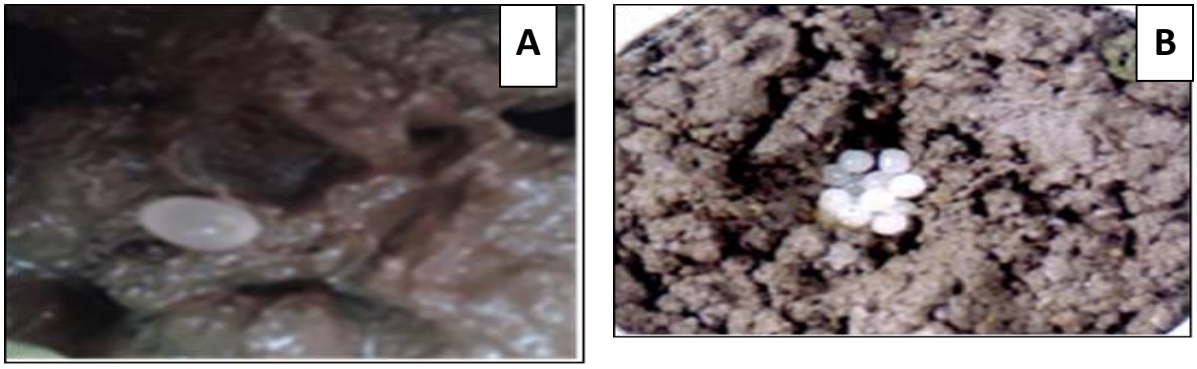

Photo (1 A , B): *Eobania vermiculata* eggs. A, view of abnormal oval and transparent egg laid by *E. vermiculata* snails treated with irradiated *M. anisopliae*. B, view of normal eggs laid by untreated *E. vermiculata* snails.

These results are in agreement with Ragab and Ismail (2001) indicated that the fungal strains; *Trichoderma viride*, *Saccharomyces cerevisiae*, *Coriolus versicolor* and *Phanerochaete chrysosporium* were highly reduced the egg laying capacity of *B. alexandrina* snail. Moreover, the fungus *Aspergillus fumigatus* completely prevent the eggs hatching of the same snail species (Gamalat *et al*., 2013). In the same trend, Hend (2007) confirmed that *Fusarium oxysporum* and *Fusarium semitectum* were strongly decreased the number of eggs laid by *Cochlicella acuta* snail. While, *Trichoderma harzianum* completely prevent the egg laying of both of *C. acuta* and *Theba pisana* snails. The same author added that *Aspergillus ochraceas* caused observed burst of *T. pisana* eggs after three days of their laying. On the other hand, the ultraviolet radiation reduced also the reproduction capacity of snails (Ragheb *et al*., 2018). Consequently, it is highly decreased the number of eggs laid by the land snail, *Cornu aspersum* and prevent the hatching of these eggs (Hallman, 2016). Moreover, it is rapidly killed the embryos of *Planorbarius* snail (Martin, 2008). It is worth noting that ammonium nitrate also has strong negative effect on the biological behavior of snails. It is prevented the egg hatching of *E. vermiculata* and *Monacha cartusiana* snails at the tested concentrations of 0.5,1, 1.5 and 2% (Hend, 2013). This finding also parallels the report of Gomaa *et al*. (2008) assured that ammonium nitrate has a potent toxic effect against the *E. vermiculata* eggs. Additionally, bayluscide and hydrothol 191 cause high reduction of the egg laying capacity of *B. alexandrina* snail at the sublethal concentration as earlier reported by Abd El-Latif *et al*. (1987).

5. Biochemical impact of some irradiated biopesticides on *Eobania vermiculata* snail:

Some treatments were chosen for this assay, which is *B. thuringiensis* at 4×10^3 spores/ml exposed to UV radiation for 60 min. as one from the *B. thuringiensis* treatments which prevented egg laying during the study period, *M. anisopliae* at 16×10^3 spores/ml exposed to UV for 60 min. as one from the *M. anisopliae* treatments which prevented egg laying also and *M. anisopliae* at 8×10^3 spores/ml exposed to UV for 90 min. as treatment including snails showed noticeable and strange phenomenon, that laid one transparent egg which burst after laying. The effect of these treatments on the activity of enzymes that strongly related with the biological behavior of snails is investigated.

As illustrated in Table (4), *B. thuringiensis* and *M. anisopliae* exposed to UV for 60 min. caused observed decrease in the activity of alkaline phosphatase enzyme to 684 and 662 U/g, respectively in comparing with 837 U/g in the control. Whereas, the treatment of snails with *M.*

anisopliae exposed to UV for 90 min. showed slightly increase in the activity of the same enzyme to 843 U/g. *B. thuringiensis* and *M. anisopliae* exposed to UV for 60 and 90 min. induced observed increase in the activity of acid phosphatase enzyme from 1095.66 U/g in the control to 1124.33 and 1215.33 U/g, respectively. Conversely, *M. anisopliae* exposed to UV for 60 min. reduced the activity of the same enzyme to 1043.66 U/g.

Table (4): Effect of some irradiated biopesticides on the enzymes activity of *Eobania vermiculata* snail.

Biopesticides Conc. (spores/ml)	Exposure times to UV radiation (min.)	ALP (wt.b.g/mU)	ACP (wt.b.g/mU)	AMY (wt.b.g/min/ glucose ug)	PRO (wt.b.g/min/ alanine-L,D ug)	LIP (wt.b.g/mU)
Bacillus thuringiensis **4×10^3**	**60**	684.00 ± 2.89	1124.33 ± 1.76	36.83± 1.30	63.60 ± 1.20	99.00 ± 2.51
Metarhizium anisopliae **16×10^3**	**60**	662.00 ± 2.51	1043.66 ± 3.28	43.33 ± 1.08	68.73 ± 1.56	131.00 ± 2.08
Metarhizium anisopliae **8×10^3**	**90**	843.00 ± 2.52	1215.33 ± 2.03	68.23 ± 1.42	49.33 ± 1.31	122.66 ± 1.20
Control		837.00 ± 1.15	1095.66 ± 2.96	84.73 ± 2.34	57.56 ± 1.62	125.00 ± 3.22

ALP = Alkaline phosphatase ACP = Acid phosphatase AMY = Amylase PRO = Protease LIP = Lipase

Regarding amylase enzyme, all treatments caused clear decline in the activity of it to 36.83, 43.33 and 68.23 U/g by *B. thuringiensis*, *M. anisopliae* exposed to UV for 60 min. and *M. anisopliae* exposed to UV for 90 min., respectively compared with 84.73 U/g in the control. While, *B. thuringiensis* and *M. anisopliae* exposed to UV for 60 min. increase the activity of protease enzyme from 57.56 U/g in the control to 63.60 and 68.73 U/g, respectively. On the other hand, *M. anisopliae* exposed to UV for 90 min. caused clear reduction in the activity of the same enzyme to 49.33 U/g. Highly decrease in the activity of lipase enzyme was achieved by *B. thuringiensis* exposed to UV for 60 min. which recorded 99 U/g followed by *M. anisopliae* exposed to UV for 90 min. which reduced it to 122.66 U/g in comparison with 125 U/g in the

control. Whereas, *M. anisopliae* exposed to UV for 60 min. increase the activity of the same enzyme to 131 U/g. These results are in harmony with those obtained by Kandil *et al*. (2014) showed that abamectin reduced the activity of alkaline and acid phosphatase enzymes in *E. vermiculata* snail from 768.3 and 3.37 U/L in the control to 373.3 and 1.05 U/L, respectively. Similarly, it is decreased also the total lipid of the same snail species to 2.16 g/dl in comparison with 7.03 g/dl in the control. Whereas, it is increased the total protein from 0.17 g/dl in the control to 2.83 g/dl. On the contrary, Emamectin benzoate 0.5% EC and 1.92% EC caused observed reduction in the total protein of *E. vermiculata* individuals but spinetoram 12% SC highly enhanced it. All these biopesticides induced an increasing in the level of alkaline and acid phosphatase enzymes of the same snail species after 48 hrs. of the treatment (Khidr, 2015). El-Halim *et al*. (1990) had earlier reported that most parasites reduced the level of total proteins and free amino acids of snails which may cleared that the parasite obtains its protein requirements and amino acid from the host. Disturbance in the functions of the internal organs of snails may lead to alterations in the protein functions and metabolic processes was strongly depending on the tested compound and concentration (Tolba *et al*., 1997). On the other hand, UV exposure caused also abnormal biochemical and physiological processes in invertebrates (Misra *et al*., 2005). The abnormal depression in the total lipid may be due to the decrease of lipid synthesis capacity or attribute to an increase in the hydrolysis of hepatic lipid due to the stress conditions (Saxena *et al*., 1989). Moreover, an increase in the total protein could be due to an increasing of biosynthesis process occurred by high enzyme stress (Khater *et al*., 1990).

In summary, this study supported the use of ammonium nitrate fertilizer at low concentrations as effective molluscicide against *E. vermiculata* snail. This was also the first report on the effect of UV radiation on the activity of *B. thuringiensis* and *M. anisopliae* biopesticides against this snail species. The post treatment of snails with ammonium nitrate at the highest concentration after the treatment with the highest concentration of each biopesticide exposed to UV for 90 min. has the most ability to kill snails. Moreover, all treatments of this study prevent the egg laying of snails and have observed negative impact on the enzymes activity of snails. So, these compounds could be developed as promising molluscicide candidates which additionally are much safer than existing molluscicides for control land snails. In addition, due to its ability to reduce the egg laying of snails it can be considered future compounds have a superior ability to reduce large numbers and many generations of *E. vermiculata* snails in the fields.

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