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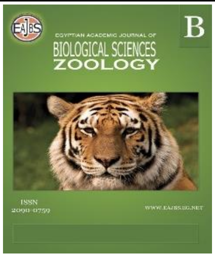

The Toxic Effect of Different Formulations of *Trichoderma* Isolates on the Hatchability of *Monacha cartusiana* and *Eobania vermiculata* Eggs

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ABSTRACT

The toxic effect of different formulations of six *Trichoderma* isolates on the hatchability of *Monacha cartusiana* and *Eobania vermiculata* eggs (*T. hamatum*, *T. viride*, *T. harzianum*, *T. koningii*, *T. asperellum* and *T. pseudokoningii*) were evaluated under laboratory conditions. The experiments were conducted at the Biological Laboratory of Econ. Entom. and Agric. Zoology Dept., Fac. Agric., Menoufia University. Statistical analysis of the data indicated that there were significant differences among the 3 concentrations of each *Trichoderma* germ isolates on the hatchability of *M. cartusiana* eggs. The toxicity was increased by increasing the concentrations of each isolate. Furthermore, there were significant differences among the six *Trichoderma* germ isolates in their toxic effect on the hatchability of *M. cartusiana* and *E. vermiculata* eggs. The lowest hatchability % of *M. cartusiana* eggs was recorded (0.0 %) after four weeks of exposure to *Trichoderma harzianum* and *T. asperellum* treatments at $6x10^6$ CFU / g, followed by *T. asperellum* treatment at $6x10^6$ CFU / g as 23.3%. As for *E. vermiculata* egg experiment, there were significant differences among the six *Trichoderma* germ isolates in their toxic effect on the hatchability of *E. vermiculata* eggs. The lowest hatchability percentages of *E. vermiculata* eggs were recorded as 3.3 and 6.7 %, after four weeks of *T. harzianum* and *T. asperellum* treatments at $6x10^6$ CFU/g, followed by *T. asperellum* treatment at $4x10^6$ CFU / g as 16.7 %. There were significant differences among the tested six *Trichoderma* extracts in their toxic effect on the hatchability of *M. cartusiana* eggs. The lowest hatchability % of *M. cartusiana* eggs were recorded (0.0 and 3.3 %) after four weeks of exposure to *T. harzianum* and *T. asperellum* extracts. In addition, the lowest hatchability of *E vermiculata* eggs was recorded (0.0 and 0.0 %) after four weeks of exposure to *T. harzianum* and *T. asperellum* extracts treatment, followed by *T. pseudokoningii* extract at 26.7 %. GC-Mass spectrometry analyses of *T. harzianum* isolates revealed the high contents of Glacial acetic acid, 2,3-butanediol, Trichodermaerin, Aspereline A, which may be responsible for toxic effects.

INTRODUCTION

Gastropods are a highly diverse group of animals, they have successfully colonized most of the world's terrestrial habitats pests (Smith and Kershaw 1979). snails have a high impact on many field crops, vegetables and horticultural (Godan 1983, Feldkamp 2002, Iglesias *et al*. 2003) such as feeding on leaves, stems, and fruits. This feeding activity can reduce crop yields and affect agricultural productivity. In Egypt, the two land snails, *Monacha cantiana* (Montague) and *Eobania vermiculata* (Muller) became the most important agricultural pests causing substantial damage to different crops (El-Deeb *et al*., 1999, Mahrous, 2002 and Khidr *et al*.,2005). Chemical control measures, such as snail baits or molluscicides, can be effective (Geasa *et al*. 2013; Castle *et al*. ,2017) but should be used with caution. It is essential to follow the instructions carefully and consider any potential impacts on non-target organisms and the whole environment (Gabr *et al*. 2006; Moustafa *et al*., 2016). there is a growing interest in discovering acceptable biological, eco-friendly molluscicides as a natural and environmentally safe alternative to synthetic chemicals (Ahmed *et al*., 2023). On the other hand, fungi represent the best microbial agent used to control land snails. Due to it being cheap in cost, easy to use and applied as a spray or germs powder. In addition, it can produce toxins and enzymes which have a great effect in the control of plant diseases (Geasa *et al*., 2013). *Trichoderma* is one of genera that present everywhere in the environment. *Trichoderma* are closely related with their ability to produce a wide range of lysing enzymes, to degrade substrates and to possess high resistance to microbial inhibitors. *Trichoderma* sp. consists of few numbers of fungal strains that are used as biocontrol agents due to their abilities to antagonize a wide range of phytopathogenic fungi, bacteria and oomycetes, through several mechanisms that are activated in *Trichoderma* by the pathogens. In addition, *Trichoderma* sp. stimulates plant growth and development by means of the production of plant growth-promoting molecules (Eziashi, *et al*., 2007 and Singh *et al*., 2014).

Therefore, the present study was conducted to evaluate the molluscicidal activity of six fungal isolates of *Trichoderma* spp, on the hatching rate of two land snail eggs, *M. cartusiana* and *E. vermiculata*.

MATERIAS AND METHODS

Tested Snails:

Fifty adults of tested snails, the glassy clover snail *M. cartusiana* and the brown garden snail *E. vermiculata* were hand collected from certain highly infested nursery host plants during their active period in November 2022 for rearing during season 2022/ 2023 they were observed daily. Members of each species were kept in glassy boxes 70x40 x 40 cm containing moist clay soil with 85-95% R.H. to a depth of 8-10 cm and fed with fresh lettuce leaves, *Lactuca sativa* L. as a main source of food; then covered with muslin cloth fixed with rubber bands to avoid snails from escaping. These cultural boxes were examined daily; fresh food and moisture were supplied as required, and the soil was searched for new clutches of eggs. Newly deposited clutches were singly removed, then 10 eggs were arranged in a wet culture dish (culture dish of 9 cm diameter on wet filter paper on a sponge slice as a transparent plastic cup). They were daily observed till hatching to determine the hatchability percentage.

Identification of *Trichoderma* Isolates:

Six fungal isolates i.e.: *Trichoderma hamatum* (T1), *Trichoderma viride* (T2), *Trichoderma harzianum* (T3), *Trichoderma koningii* (T4), *Trichoderma asperellum* (T5) and *Trichoderma pseudokoningii* (T6) were cultivated on potato dextrose agar. The isolated *Trichoderma* fungi were cultured onto 20% malt extract agar, incubated for 4 days at 25°C, then, identified at the Plant Pathology Department, Faculty of Agriculture, Menoufia University, Egypt according to Bissett (1991) and Ramirez (1982).

Liquid Filtrations and Spores Culture Production:

Preparation of *Trichoderma* spp. culture filtrates: *Trichoderma* culture filtrates were prepared by inoculating the disk of the fungus onto liquid potato dextrose medium in flasks (100/200 ml), then incubated by shaking at 25°C for 3 days then incubated for 7 days. Trichoderma culture filtrates were prepared by eliminating the mycelial mates, then the filtrates were centrifuged at 8000g for 10 min. and filtered through a Hydrophobic filter (type A/E, Gelman Sciences, Ann Arbor, M1) (Harman et al, 1992).

The spores were taken by rinsing in 10 ml sterilized distilled water and then it was filtered using muslin cloth. The number of spores in the suspension was determined using a haemocytometer slide and adjusted to the three concentrations; 2×10^6, 4×10^6 and 6×10^6 spores/ml by adding the amount of sterilized distilled water to the fungal spores (Hend, 2007).

Laboratory Experiments:

Three recommended concentrations (2×10^6, 4×10^6 and 6×10^6) of all *Trichoderma* germs were used against the eggs of *Eobania vermiculata* snail. and *Monacha cartusiana.* Each concentration has three replicates. Each replicate contains 10 snail eggs put on filter papers. The eggs were sprayed with the tested concentrations in the Petri dish. The other three replicates were sprayed with water as a control. All Petri dishes were kept at ambient temperature. The percent of hatchability was observed daily for 4 weeks. The percentage of hatchability was calculated as follows:

The percentage of hatchability = no. hatched eggs/no. of treated eggs× 100

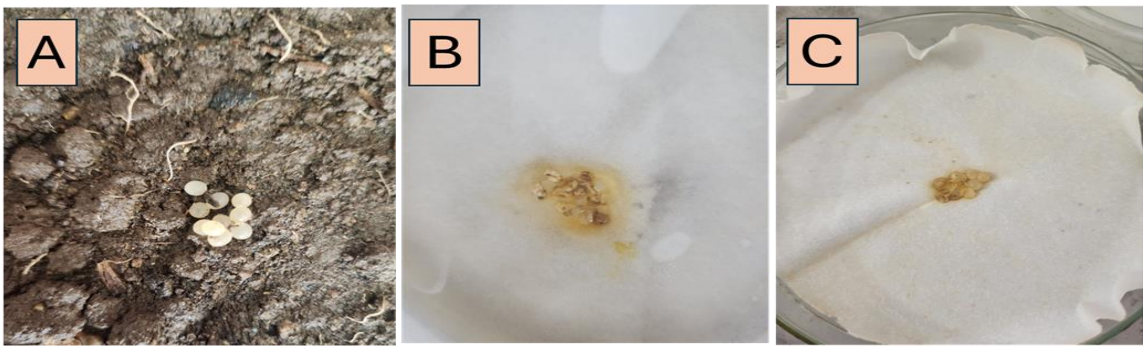

Fig. 1 A: Healthy eggs, **B & C**: Treated eggs with *Trichoderma* culture filtrates

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:

The volatile components of *Trichoderma* spp. were examined using a Trace Ultra Gas Chromatographer paired with a DSQ II Mass Spectrometer (Thermo Scientific). Thermo Scientific's TR-5MS (30 m 0.25 mm 0.25 m) capillary column was used for the chromatographic separation of the components according to the method of Adams (1995). The compounds were identified from relevant data stored in databases from literature and equipment (Adams Book 07, Nist 98, Xcalibur). The Relative Retention Index was calculated using a range of n-alkanes (C8–C24). Relative % of the compounds have been obtained.

RESULTS AND DISCUSSION

The obtained results in Table (1), show the toxic effect of different formulations of six *Trichoderma* germ powders on the hatchability of *Monacha cartusiana* eggs under laboratory conditions.

Analysis of the obtained data indicated that there were significant variations among the three concentrations of each *Trichoderma* germ isolates in their toxic effect on the hatchability of *Monacha cartusiana* eggs. The toxicity was increased by increasing the concentrations of each isolate. The highest toxicity was recorded with the highest concentration 6x10^6 CFU per g followed by 6x10^4, while the lowest effect was observed with the lowest concentration6x10^2.

Furthermore, statistical analysis of the data in Table (1) indicated that there were significant variations among the six *Trichoderma* germ isolates in their toxic effect on the hatchability of *Monacha cartusiana* eggs.

The lowest hatchability percentages of *Monacha cartusiana* eggs were recorded (0.0 %) after four weeks of *Trichoderma harzianum* and *Trichoderma asperellum* treatments at 6x10^6 CFU / g, followed by *T. asperellum* treatment at 6x10^4 CFU / g as 23.3%.

Table 1: The toxic effect of *Trichoderma* germ powders on the hatchability of *Monacha cartusiana* eggs

Trichoderma isolates	conc.	Number of hatched eggs/30eggs				Total	H%
		one week	two weeks	three weeks	four weeks		
T1 *Trichoderma hamatum*	2x10^6	0	1.3	6.0	7.33	22.0 b	73.3
	4x10^6	0	0.7	5.7	6.0	18.0 c	60.0
	6x10^6	0	0.7	3.3	5.33	16.0 cd	53.3
T2 *Trichoderma viride*	2x10^6	0	0.7	6.0	7.33	22.0 b	73.3
	4x10^6	0	0.3	3.7	5.33	16.0cd	53.3
	6x10^6	0	1.0	2.0	4.33	13.0 e	43.3
T3 *Trichoderma harzianum*	2x10^6	0	2.0	7.0	7.0	18.0 c	60.0
	4x10^6	0	1.7	2.0	3.33	12.0 e	40.0
	6x10^6	0	0.0	0.0	0.0	0.0 g	0.0
T4 *Trichoderma koningii*	2x10^6	0	1.3	6.0	9.0	27.0 a	90.0
	4x10^6	0	1.3	3.3	6.0	18.0 c	60.0
	6x10^6	0	0.7	1.3	5.33	16.0 cd	53.3
T5 *Trichoderma asperellum*	2x10^6	0	1.3	3.0	4.33	13.0 e	43.3
	4x10^6	0	1.0	1.7	2.33	7.0 f	23.3
	6x10^6	0	0.0	0.0	0.0	0.0 g	0.0
T6 *Trichoderma pseudokoningii*	2x10^6	0	2.0	3.3	7.0	21.0 b	70.0
	4x10^6	0	1.0	2.0	5.67	17.0 c	56.7
	6x10^6	0	0.3	1.7	4.67	14.0 de	46.7
Control		0	2.0	7.0	9.0	27.0 a	90.0
LSD 5%	-	-	-	-	-	2.4	

The different letters for the column mean significant differences at 5% level

The obtained results in Table (2), show the toxic effect of different formulations of six *Trichoderma* germ powders on the hatchability of *Eobania vermiculata* eggs under laboratory conditions.

Statistical analysis of the data indicated that there were significant differences among the three concentrations of *Trichoderma* germ isolates in their toxic effect on the hatchability of *Monacha cartusiana* eggs. The toxicity was increased by increasing the concentrations of each isolate. The highest toxicity was recorded with the highest concentration 6x10^6 CFU per g followed by 6x10^4, while the lowest effect was observed with the lowest concentration 6x10^2.

Furthermore, statistical analysis of the data in Table (2) indicated that there were significant differences among the six *Trichoderma* germ isolates in their toxic effect on the hatchability of *Eobania vermiculata* eggs.

The lowest hatchability percentages of *E. vermiculata* eggs were recorded (3.3 and 6.7 %) after four weeks of *Trichoderma harzianum* and *Trichoderma asperellum* treatments at $6x10^6$ CFU/g, followed by *T. asperellum* treatment at $6x10^4$ CFU / g as 16.7 %.

Table 2: The toxic effect of *Trichoderma* germ powders on the hatchability of *Eobania vermiculata* eggs

Trichoderma isolates	conc.	Number of hatched eggs /30 eggs				Total	H%
		one week	two weeks	three weeks	four weeks		
T1 *Trichoderma hamatum*	$2x10^6$	0	3.0	3.7	6.3	19.0 de	63.3
	$4x10^6$	0	1.3	2.0	4.7	14.0 fg	46.7
	$6x10^6$	0	1.0	2.7	4.7	14.0 fg	46.7
T2 *Trichoderma viride*	$2x10^6$	0	3.7	4.7	7.3	22.0 c	73.3
	$4x10^6$	0	0.7	2.0	5.0	15.0 f	50.0
	$6x10^6$	0	1.3	1.7	4.3	13.0 fg	43.3
T3 *Trichoderma harzianum*	$2x10^6$	0	0.3	1.7	4.7	14.0 fg	46.7
	$4x10^6$	0	2.0	3.3	4.0	12.0 g	40.0
	$6x10^6$	0	0.7	0.7	0.7	2.0 i	6.7
T4 *Trichoderma koningii*	$2x10^6$	0	3.3	4.7	7.0	21.0 cd	70.0
	$4x10^6$	0	3.0	4.3	6.0	18.0 e	60.0
	$6x10^6$	0	1.7	3.3	4.3	13.0 fg	43.3
T5 *Trichoderma asperellum*	$2x10^6$	0	3.0	3.0	4.7	14.0 fg	46.7
	$4x10^6$	0	0.7	1.0	1.7	5.0 h	16.7
	$6x10^6$	0	0.0	0.3	0.3	1.0 i	3.3
T6 *Trichoderma pseudokoningii*	$2x10^6$	0	4.3	7.3	8.3	25.0 b	83.3
	$4x10^6$	0	2.0	3.7	5.0	15.0 f	50.0
	$6x10^6$	0	3.0	4.7	4.7	14.0 fg	46.7
Control		0	7.7	9.3	10.0	30.0 a	100.0
LSD 5%		-	-	-	-	2.0	

The different letters for the column mean significant differences at 5% level

The obtained results on the potential effect of six *Trichoderma* extracts on the hatchability of *Monacha cartusiana* eggs 1, 2, 3, and 4 weeks of treatment are shown in Table (3). Statistical analysis of the data indicated that there were significant variations among the six *Trichoderma* extracts in their toxic effect on the hatchability of *Monacha cartusiana* eggs.

The lowest hatchability percentages of *Monacha cartusiana* eggs were recorded (0.0 and 3.3 %) at four weeks of exposure to *Trichoderma harzianum* and *Trichoderma asperellum* extracts treatment, followed by *T. hamatum extract* at 36.7 %.

Table 3: The potential effect of six *Trichoderma* extracts on the hatchability of *Monacha cartusiana* eggs

Trichoderma extracts	Number of hatched eggs/30 eggs				Total	H%
	One week	Two weeks	Three weeks	Four weeks		
Trichoderma hamatum	0	2.7	3.7	3.7	11.0 d	36.7
Trichoderma viride	0	4.3	5.7	5.7	17.0 b	56.7
Trichoderma harzianum	0	0.0	0.0	0.0	0.0 e	0.0
Trichoderma koningii	0	3.7	4.3	4.3	13.0 cd	43.3
Trichoderma asperellum	0	0.0	0.0	0.3	1.0 e	3.3
Trichoderma pseudokoningii	0	3.0	4.7	4.7	14.0 c	46.7
Control	0	5.3	7.0	9.3	28.0 a	93.3
LSD 5%	-	-	-	-	2.6	

The different letters for the column mean significant differences at 5% level

The obtained results on the potential effect of six *Trichoderma* extracts on the hatchability of *Eobania vermiculata* eggs 1, 2, 3, and 4 weeks of treatment are shown in Table (4).

Statistical analysis of the data indicated that there were significant variations among the six *Trichoderma* extracts in their toxic effect on the hatchability of *Eobania vermiculata* eggs.

The lowest hatchability percentages of *Eobania vermiculata* eggs were recorded (0.0 and 0.0 %) after four weeks of exposure to *Trichoderma harzianum* and *Trichoderma asperellum* extracts treatment, followed by *T. pseudokoningii* extract at 26.7 %.

Table 4: The potential effect of *Trichoderma* extracts on the hatchability of *Eobania vermiculata* eggs.

Trichoderma extracts	Number of hatched eggs/30eggs				Total	H%
	One week	Two weeks	Three weeks	Four weeks		
Trichoderma hamatum	0	3.0	4.0	4	12.0 b	40.0
Trichoderma viride	0	1.3	3.0	3	9.0 c	30.0
Trichoderma harzianum	0	0.0	0.0	0	0.0 d	0.0
Trichoderma koningii	0	3.3	4.3	5	11.0 b	36.7
Trichoderma asperellum	0	0.0	0.0	0	0.0 d	0.0
Trichoderma pseudokoningii	0	1.7	2.7	2.7	8.0 c	26.7
Control	0	7.0	9.3	9.3	28.0 a	93.3
LSD 5%					1.9	

The different letters for the column mean significant differences at 5% level

The obtained results in Tables (5&6), show the chemical analysis by GC-Mass spectrometry analyses of *Trichoderma harzianum* isolate number 3, and *Trichoderma asperellum* isolate number 5.

GC-Mass spectrometry analyses of *Trichoderma harzianum* Isolate number 3 revealed the presence of Glacial acetic acid at 50.26 % as the highest compound, as well as 2,3-butanediol at 33.77 %, it could be concluded that these compounds are the responsible for the toxic effect to snail eggs.

Furthermore, GC-Mass spectrometry analyses of *Trichoderma asperellum* Isolate number 5 revealed the presence of Trichodermaerin at 32.2 % as the highest compound, as well as Aspereline A at 19.2 %, it could be concluded that these compounds are the responsible for the toxic effect to snail eggs.

Table 5: Chemical analysis by GC-Mass spectrometry analyses of *Trichoderma harzianum* Isolate no 3.

Chemical name	Chemical structure	Abundance %
1-methoy-2-propanone	$C_4H_8O_2$	2.23
Glacial acetic acid	$C_2H_4O_2$	50.26
1,3-butanediol	$C_4H_{10}O_2$	2.50
2,3-butanediol	$C_4H_{10}O_2$	33.77
Phenylethyl alcohol	$C_8H_{10}O$	2.20

Table 6: Chemical analysis by GC-Mass spectrometry analyses of *Trichoderma asperellum* Isolate no 5

Chemical name	Chemical structure	Abundance %
Trichodermaerin	$C_{20} H_{28} O_3$	32.2
Aspereline H	$C_{47} H_{82} N_{10} O_{12}$	15.7
Aspereline A	$C_{45} H_{80} N_{10} O_{11}$	19.2
Aspereline E	$C_{45} H_{80} N_{10} O_{12}$	16.7
Methylecordysinin A	$C_{12} H_{20} N_2 O_3$	5.5

The obtained results are in harmony with those of Hussein and Sabry (2019) who reported that indoxacarb and abamectin can be used as promising molluscicides against the adults of *E. vermiculata* especially in conventional crops such as wheat and added that imidacloprid and fipronil can be used as soil treatment against the eggs of *E. vermiculata*. Also, Ghareeb (2023) found that the fungal isolates of *Verticillium alboatrum* and *Trichoderma harzianum* caused significant molluscicidal potency against the different ages of *Eobania vermiculata* snails, in addition, the safety and efficacy of these isolates, make them excellent candidates for use as environmentally friendly molluscicidal agents for control land snails. Moreover, Ahmed *et al*. (2023) tested the molluscicidal activity of a fungal isolate, *Trichoderma harzianum* against the land snail, *M. cartusiana* as a natural and environmentally safe alternative to synthetic chemicals, where under laboratory and field conditions, and the obtained results reported that *T. harzianum* exhibited molluscicidal activity 7 and 21 days after exposure, respectively. Moreover as a result, *T. harzianum* may be used as an eco-friendly bioagent molluscicide in land snail control program instead of harmful synthetic molluscicides. Recently, Abo-Elwfa *et al*. (2024) assessed under laboratory experiments and field trials the effectiveness of bacterial, *Bacillus thuringiensis* and fungal, *Metarhizium anisopliae* and *Trichoderma harzianum* isolates, compared to methomyl as a carbamate compound against the terrestrial snails, and revealed that *Bt* and *M. anisopliae* effectively combatted the terrestrial snail, making them viable options in integrated pest control as substitutes for pesticides.

Declarations:

Ethical Approval: All animal procedures were accomplished in accordance with the guidelines for the care and use of experimental animals established by Menoufia University, Faculty of Agriculture, Science and Education Committee which approved the experimental animal methods.

Conflicts of Interest: There is no conflict of interest.

Informed consent: The author of this manuscript accepted that the article is submitted for publication in the Egyptian Academic Journal of Biological Sciences, B. Zoology, and this article has not been published or accepted for publication in another journal, and it is not under consideration at another journal.

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ARABIC SUMMARY

التأثير السام لمستحضرات مختلفة من عزلات فطريات الترايكوديرما علي فقس بيض قواقع *Monacha cartusiana* ، *Eobania vermiculata*

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تم تقييم التأثير السام لتركيبات مختلفة لستة عزلات من الترايكوديرما على نسبة فقس بيض *Monacha cartusiana* و *Eobania vermiculata* تحت الظروف المعملية (*T. hamatum* , *T. viride* , *T. harzianum* , T. *asperellum* , *T. koningii* , و *T. pseudokoningii*). أجريت التجارب في معمل البيولوجي التابع لقسم الحيوان الزراعى والحشرات الاقتصادية ، كلية. الزراعة، جامعة المنوفية. اثبت التحليل الإحصائي للنتائج وجود فروق معنوية بين التراكيزات الثلاثة لكل عزلة من عزلات جرثومة الترايكوديرما في نسبة فقس بيض *M. cartusiana*. زادت السمية بزيادة تركيز كل عزلة. علاوة على ذلك، كانت هناك اختلافات معنوية بين عزلات جرثومة الترايكوديرما الستة في تأثيرها السام على نسبة فقس بيض *M. cartusiana*. تم تسجيل أقل نسبة فقس في بيض *Monacha cartusiana* صفر٪ بعد أربعة أسابيع من التعرض لمعاملة *Trichoderma harzianum* و T. *asperellum* بتركيز 6CFU/g x10^{6}، تليها معاملة T. *asperellum* بتركيز 6CFU/g x10^{4} بنسبة 23.3٪. أما في تجربة بيض *E. vermiculata* فقد كانت هناك فروق معنوية بين عزلات جراثيم *Trichoderma* الستة في تأثيرها السام على نسبة فقس بيض *E. vermiculata*. تم تسجيل أقل نسب فقس لبيض *E. vermiculata* 3.3 ٪ و 6.7٪ بعد أربعة أسابيع من معاملة *T. harzianum* و *T. asperellum* بجرعة 6CFU/g x10^{6}، تليها معاملة *T. asperellum* بجرعة 6CFU/g x10^{4} بنسبة 16.7٪. تم تسجيل فروق معنوية بين مستخلصات الترايكوديرما الستة المختبرة في تأثيرها السام على نسبة فقس بيض *M. cartusiana*. تم تسجيل أقل نسبة فقس في بيض *M. cartusiana* صفر٪ و 3.3٪ بعد التعرض لمستخلصي *T. harzianum* و T. *asperellum* لمدة أربعة أسابيع. بالإضافة إلى ذلك، تم تسجيل أدنى نسبة فقس لبيض *E vermiculata* صفر٪ و صفر٪ بعد التعرض لمستخلصي *T. harzianum* و *T. asperellum* لمدة أربعة أسابيع، يليها مستخلص *T. pseudokoningii* بنسبة 26.7٪. كشفت تحليلات مطياف الكتلة GC لعزلات *Trichoderma harzianum* عن المحتوى العالي من حمض الأسيتيك الثلجي، 2،3-بوتانيديول، Trichodermaerin، Aspereline A، والتي قد تكون مسؤولة عن التأثير السام على بيض القواقع .