The Study Of The Insecticidal Effect Of Nigella Sativa Essential Oil Against Tuta Absoluta Larvae

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Abstract: To contribute to the development of an integrated strategy of pests associated with the tomato crop, the insecticidal potential of the essential oil of *Nigella sativa* was evaluated on larvae of *Tuta absoluta*, which is considered the most destructive insect in the production of tomato (*Solanum lycopersicum*) and which causes serious damages in invaded areas. Rearing larvae of *Tuta absoluta* and the testing of toxicity were conducted in laboratory conditions in petri dishes at a temperature of 26±2°C, with a relative humidity ranging from 60 to 70% and a photoperiod of 16 / 8. The method of Finney based on probits regression of mortalities according to the logarithms of essential oil doses allowed us to determine the LD_{50}. The results of these tests showed that the essential oil of *Nigella sativa* has remarkable larvicidal properties. After four hours of exposure, they induced 100% of mortality of larvae in the concentration of 0,203µl/cm². The measured LD_{50} indicated the value of 0,105µl/cm². Field trials are needed to confirm the practical relevance of these results in the development of a natural pesticide against the larvae of *Tuta absoluta*.

Index Terms: Tuta absoluta, Nigella sativa, insecticidal effect, repellent activity.

1 INTRODUCTION

Tomato (Lycopersicum esculentum Mill) of the Solanaceae family, is an annual herbaceous plant which originates from the Andes and America. It is widely grown for its fruit consumed fresh or otherwise [1]. In Morocco, industrial tomatoes occupies 75-80% of the areas of vegetable crops for agri-business, which is the equivalent of 6000 to 7000 ha / year, with an average production during the last five years of about 200 000 to 300 000 tonnes per year [2]. Since 2008, this crop has been attacked by the leaf miner Tuta absoluta (a microlepidoptera of 6 to 7mm) whose larvae cause considerable damage on leaves, stems and fruits of tomato; such damage can reach 100% losses in some cases [3]. It also grows on other species such as eggplant, potatoes, pepper and other solanaceous products [4]. Tuta absoluta is native to South America. The first appearance was in 1964 in Argentina and subsequently it was spread to other countries in Latin America. It recently appeared in the Mediterranean, Spain (2006), Algeria and Tunisia (2008) and brought to Morocco for the first time in April 2008 in the region of Nador (Bouaareg). It is currently widespread in the major horticultural areas [5]. The main method of struggling against Tuta absoluta is spraying insecticides, which is harmful to both humans and the environment [6]. Because of the feeding behavior of larvae that protects them from the application of phytosanitary and important cases of resistance to certain insecticides, the fight against this pest is particularly difficult [7]. This study aims to propose alternative solutions based on the use of natural products "biopesticides" to fight the tomato leaf miner caused by Tuta absoluta which is considered a serious threat to tomato production and other Solanaceae. To meet this goal, our work is based on the evaluation of the insecticidal effect of the essential oil of Nigella sativa vis-à-vis the Tuta absoluta larvae.

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2 MATERIALS AND METHODS

2.1 Rearing caterpillars

In order to obtain a homogeneous and sufficient population of *Tuta absoluta* larvae for various biological tests, mass breeding was done with larvae. The latter was collected from the plots affected by the pest *Tuta absoluta* in the area of M'nasra in the region of "Gharb-Chrarda-Beni hsen". The breeding was carried out in Petri dishes of 90mm diameter, with fresh tomato leaves, a temperature of 26 ± 2 ° C, a relative humidity ranging from 60 to 70%, and a photoperiod of 16/8.

2.2 Bioassays

Bioassays were performed in the laboratory in Petri dishes of 90 mm of diameter, an average temperature of 26 ± 2 ° C, and a relative humidity of 60 to 70%.

2.2.1 Toxicity by contact of the essential oil on filter paper

Several preliminary tests were carried out to select the dose to be used. Thus, the different doses of each essential oils were prepared by diluting 2 ml of acetone in the volume of 40 ul of essential oil to be tested. Then, a cascade dilution based on ½ was performed in little bottles each containing 1 ml of acetone to obtain a range of doses of between 20 .mu.l and 2.5 .mu.l. Each of the solutions thus prepared was uniformly spread on a filter paper disk (Whatman No. 1) of 9 cm in diameter (or 63.62 cm² area) placed in a petri dish [8];[9] of glass of the same diameter. This washer has been left at room temperature for 15 minutes to allow the complete evaporation of the dilution solvent. Thus, if one wants to express different amounts of essential oil (2.5, 5, 10 and 15µl) with filter paper unit area (63.62 cm), this respectively corresponds to the doses 0,039, 0.079, 0.157 and 0,236µl/cm². For the fifth dose or witness, the washer was treated only with acetone. A batch of 20 larvae of *Tuta absoluta* Freshly drawn their rearing environment and aged more than three days were introduced into each petri dish containing a treated washer; then the dishes were immediately closed. Four replications were conducted for each dose and the dead larvae were counted (and kept in boxes) every hour for four hours.The mortality in the treated dishes (Mo) were expressed in the words of Abbott’s formula in corrected mortalities (Mc), taking into account natural mortality observed in the control dishes (Mt) using the following formula:

$$Mc = \frac{Mo - Mt}{100 - Mt} \times 100 \quad [10]$$

The method of Finney based on probit regression mortalities in view of the logarithms of essential oil of doses allowed us to determine the LD_{50} [11]. This was done by using BioStat Pro 5.9.8 software.

2.2.2 Repellent essential oil on filter paper

The repellent effect of the essential oil against the larvae of *Tuta absoluta* was evaluated using the method of the preferred area on filter paper described by McDonald et al. in 1970 [12]. Thus, the filter paper disks of 9 cm in diameter used for this purpose were cut into two equal portions each having a surface of 31.80 cm². Four doses of Oil were prepared (2.5, 5, 10 and 15µl) by diluting in 1 ml of acetone. Then, 0.5 ml of each of the prepared solutions was uniformly spread on half of the disc corresponding respectively to the doses 0.039, 0.079; 0.157 and 0,236µl/cm² while the other half received only 0.5 ml of acetone. After fifteen minutes, which is the time required for complete evaporation of the dilution solvent, the two halves of discs were reconstituted by means of an adhesive strip. The filter paper disc thus reconstituted was placed in a Petri dish and a set of 20 larvae aged more than three days was placed at the center of each disc. Four replications were conducted for each dose. After two hours, the number of larvae present on the portion of filter paper treated with essential oil (s), and the number of those present on the treated with acetone only (Nc) were identified. The percentage of repulsion (PR) was calculated using the following formula:

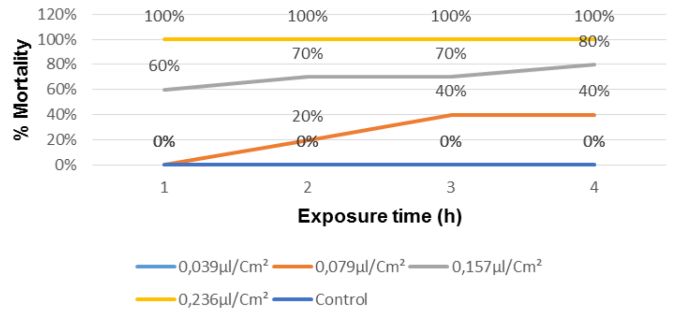
$$PR = \frac{Nc - Nt}{Nc + Nt} \times 100$$

The average percentage of essential oil repellency was calculated and assigned as ranked by McDonald et al. in 1970 to one of the different classes repulsive ranging from 0 to V: Class 0 (PR <0.1%), class I (PR = 0.1 to 20%), class II (PR = 20.1 to 40 %), class III (PR = 40.1 to 60%), class IV (PR = 60.1 to 80%) and Class V (PR = 80.1 to 100%).

3 Results and Discussion

Figure 1 illustrates the variation of the percentages of mortalities cumulated and corrected with respect to the control of larvae of *Tuta absoluta* according to time and dose of the essential oil of the used *Nigella sativa* seeds. There is a variation in the mortality rate with the dose of the tested essential oil and time. The dose of 0,203µl/cm² causes a total mortality (100%) of larvae at the fourth hour of exposure. The LD_{50} value is 0,105µl/cm² calculated after four hours of exposure which confirms the high degree of toxicity of this essential oil on filter paper vis-à-vis these insects.

Fig.1: Percentage of mortalities of larvae of *Tuta absoluta* according to the exposure time and the dose of the essential oil of *Nigella sativa* on filter paper



The percentages of the repulsion of the various doses of the essential oil of Nigella sativa seeds are summarized in Table 1. It shows that after two hours of exposure, the different levels of the essential oil of Nigella sativa seeds (0.039; 0.079; 0.157 and 0.236µl/cm²) have respectively caused 71.25, 82.5, 91.25 and 100% of repulsion vis-à-vis the larvae of Tuta absoluta. This clearly shows that the percentage of repulsion increases with the dose. In the light of these results, it can be noticed that the essential oil of the seeds of Nigella sativa also has a strong repellent activity against the larvae of Tuta absoluta and belongs, according to McDonald et al, to the repulsive class V with an average of repellency of 86.25%. In this study, the essential oil of Nigella sativa seeds was found to be highly repellent and toxic on filter papers against Tuta absoluta larvae. The volatile nature of the constituents of the essential oils leads us to believe that the active ingredient would probably be one or various volatile components contained in the essential oil. The toxic and repellents effects of this essential oil may depend on its chemical composition and the level of insect sensitivity [13]. Indeed, studies have highlighted the main constituents of the essential oil of Nigella sativa seeds [14]. By using GC-MS (Gas Chromatography-Mass Spectroscopy), Burits and Bucar in 2000 identified 32 constituents in which the majority are monoterpenes [15] such as α-pinene (5-14 %), p-cymene (38%), carvacrol (5-11%), in which the insecticidal properties have been shown vis-a-vis Tribolium confusum, Ceratitis capitata and Rhopalosiphum padi (phytophagous insects) [16];[17].

***Table 1:** filter paper repulsion percentages of the essential oil of Nigella sativa seeds vis-a-vis the larvae Tuta absoluta.*

Doses (µl/cm2)	repulsion rate in %
0,039	71,25 ± 2,5
0,079	82,5 ± 2,89
0,157	91,25 ± 4,79
0,236	100 ± 0
Average	86,25 ± 2,55
Repulsive Class	V

However, it would be difficult to think that the insecticidal activity of this oil is limited only to some of its major constituents since it could also be due to certain minority constituents or to a synergistic effect of several components [18].

4 Conclusion

The essential of oil Nigella sativa shows a strong repellent activity as well as an insecticidal activity in vitro against the larvae of Tuta absoluta. The use of this essential oil to protect tomato crops against this pest could thus be considered. This is why the in-vivo study of the insecticidal effect of the essential oil of Nigella sativa is necessary.

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