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Research Article

Effect of botanical extract of garlic (*Allium sativum* L.) on larvae of tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Abstract
Tuta absoluta is one of the most devastating invasive pests affecting tomato crops in Algeria. Synthetic chemical pesticides to control this leaf miner, produce harmful effects on all organisms, they increase the risk rate for public health and the environment for this the present study aims to propose alternative solutions based on the use natural products "bioinsecticide", in order to fight against this bioaggressor. This study concerns the *in vitro* evaluation of the insecticidal activity of *Allium sativum* extract on the larvae of *T. absoluta*. The results of this test showed that garlic extract has remarkable larvicidal properties. Indeed, the L_1 and L_2 larval stages are the most sensitive towards all the concentrations, especially the concentration 30 which was the most influential. On the other hand, the requested plant requires an LD_{50} which exceeds 21.91% and an LD_{90} which exceeds 37.34% to kill 50 and 90% of the insects detected. The results obtained suggest that these natural compounds of plant origin could be successfully used in integrated pest management programs for *T. absoluta.*

Keywords: *Tuta absoluta*, tomato, bioinsecticide, *in vitro*, *Allium sativum*.

Introduction

Tomato (*Solanum lycopersicum* Mill.) is one of the most important vegetables with a very high production potential. However, with its various vitamins, organic substances and minerals, it is very important in human health and nutrition (Waheed *et al.*, 2020). Significant pests have been recorded during production, which may affect production directly or indirectly. Among these pests, tomato leaf miner *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) has been identified as the main pest of tomato today. The damage is caused by larval feeding on aerial plant parts, which may lead to 100% of crop loss (Biondi *et al.*, 2018), thereby affecting food security and livelihood of millions of growers and consumers (Fiaboe *et al.*, 2020).

Farmers have responded to this pest pressure through the sole injudicious use of chemical pesticides. However, this method of control is expensive, not effective (owing to reported insecticide resistance) and has potential adverse effects on the environment (including humans). To mitigate this, more environmentally friendly, bio-based and sustainable alternatives need to be put in place. Natural substances, for example, the use of pesticidal plant extracts, naturally occurring antagonists and related substances, can be used in this regard (Tarusikirwa *et al.*, 2020).
Botanicals are natural resources used to control different agricultural pests for long period of time. Crude extracts from seeds, leaves, bark, bulbs, and fruits of the different plant species have been extensively tested on agricultural pests for bioactivity worldwide (Isman and Seffrin, 2014).

Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents involve chlorophyll, proteins sugar and amino acids whereas secondary constituents contain flavonoids, steroids, alkaloids, resins, fatty acids, tannins and phenol compounds, etc. Due to the presence of these secondary constituent's medicinal plants show anti-insecticide activities (Ahmad *et al.*, 2017).

Taking into account the fact that for more than 5000 years, garlic has been recognized as a medicinal plant (Wu *et al.*, 2015). Garlic bulbs, which come from the plant *A. sativum* (family Amaryllidaceae), are a common flavoring ingredient in a range of recipes. It's also well-known for its therapeutic benefits. The garlic bulb and its numerous preparations, such as garlic oil, garlic powder, and various garlic extracts, are referenced in various traditional medical systems for their therapeutic effects (Adaki *et al.*, 2014). Garlic's remarkable biological activities include oxidative radical scavenging potential, cardioprotective abilities (Boonpeng *et al.*, 2014 ; Lee *et al.*, 2016), anti-glycating, antioxidant, and protein structural stability (Khan *et al.*, 2022), antimicrobial (Woods-Panzaru *et al.*, 2009; Bourgoin *et al.*, 2017; Steglinska *et al.*, 2022), antifungal (Sharma *et al.*, 2017) and insecticidal (Shiberu and Getu, 2017).

This study aims to propose alternative solutions based on the use of natural products "bioinsecticide" against the tomato leaf miner, *T. absoluta* (Meyrick), which is considered a serious threat to tomato production, and to study the chemical composition of methanolic extract of *A. sativum*.

Material and methods

Methanolic extract preparation

60g of garlic bulb was subjected to extraction by using 600 ml methanol as solvent in a Soxhlet apparatus. Then, the heating was stopped after 5 cycles and the mixture from the distillation flask was collected and cooled. After that this mixture was filtered and concentrated by using evaporator at a temperature of 45°C. The extract was stored in amber colored glass jar in a freezer and was used for further experiments (Raaman, 2006; Bichra *et al.*, 2012).

Determination of total phenolic content

The total polyphenol contents were determined using the Folin-Ciocalteu method (Miliauskas *et al.*, 2004). Nearly 1mL of extract was mixed with 5 mL Folin-Ciocalteu reagent (diluted 10 times with distilled water). After 5 min, 4 mL of sodium carbonate solution (7.5%) was added. Gallic acid was used as standard for the calibration curve. After 60 min, the absorbance was measured at 765 nm. The total phenolic content in garlic extract was determined from the calibration plot and expressed in milligram gallic acid equivalent per 1 g fresh weight (mg GAE/g fw) of garlic.

Insecticidal activity

The bioassays were carried out in the laboratory in Petri dishes 90 mm in diameter at a temperature of 17.5 ± 5°C, 52 ± 10% RH and a 12:12 h L:D photoperiod.

A healthy tomato leaflet is placed in a Petri dish with a bottom covered with a layer of moistened absorbent paper to keep the leaflet fresh, allowing the proper development of the larvae during the observation period. Five larvae of *T. absoluta* of the same larval stage in good condition were gently deposited on the leaflet in several Petri dishes and this for each larval stage.

Six concentrations (5, 10, 15, 20, 25 and 30%) of methanolic extract were tested. In addition, control insects were maintained under the same conditions without any extract. For the positive control groups acetone (10%) was used, for the negative control distilled water. The number of dead insects in each Petri dish was counted after 24hours until the end of 10 days exposure period.

Statistical analysis

Statistical analysis was performed using ANOVA analysis (STATBOX 6.01) followed by the Newman Keuls test. The differences were considered significant at $P<0.05$. All the variables studied and the lethal concentration (LC_{50} and LC_{90}) values of the metanolic extract were determined by probit analysis (Finney 1971).

Results and discussion

Extraction yield and total polyphenols content

The yield obtained from the methanolic extract of *A. sativum* bulbs is around 11.7% and the phenolic compound content of our methanolic garlic extract is (1.20 mg GAE/G FW). The latter is weaker compared to those obtained by Ciric *et al.* (2019) who detected a TPC of the garlic bulbs collected from Spain (26.44 mg GAE/g), followed by those from China (25.57 mg GAE/g fw) and those from Slovenia (14.44 mg GAE/g fw). However, the TPC of the samples were higher than those reported by Nuutila *et al.* (2003), who detected a TPC of 0.075–0.080 mg GAE/g in different *Allium* species.

Plant extracts are composed of the plant cells' secondary metabolites. Although the quality and quantity of these compounds depend on plant species, environmental growth conditions, pathogen incidence, harvesting season (Šernaite, 2017; Jiménez-Reyes *et al.*, 2019).

Larvicidal activity of garlic extract on *T. absoluta*

The results indicated that tomato leaf miner populations showed variable responses to different insecticide concentrations (Fig.1). The toxicity results of the extract of *A. sativum* on the different larval stages show a significant effect ($P<0.05$) on treated individuals compared to untreated ones (Tab. 01). The populations in the negative control batches continuously increased to reach an average maximum of (36%) on the last day of the test; garlic at different concentrations tested significantly reduced *T. absoluta* populations compared to the control. An amplification of the mortality rate is noted after two days for all concentrations; 72 hours after the application of the extract, the mortality rate reached 40% for the 5% concentrations; 10% and 20%, while the result obtained by the 25% concentration was less important with a mortality rate of 28%.
Four days after the treatment, the mortality rates recorded showed quite interesting proportions for the 20% and 30% concentrations, with rates of 55% and 57% followed by the 5% and 10% concentrations also causing mortality rates above 50% with 53% and 51% respectively. An interesting mortality rate (44%) was recorded for the 25% concentration. Whereas for the controls (positive and negative), mortality rates of 39% and 31% were noted respectively, highlighting the action of natural regulation factors which remains a non-negligible means of control. From the fifth day, we were able to observe a remarkable increase in the mortality rate for all concentrations, reaching a maximum of 97% for the 30% concentration, proving the effectiveness of the latter compared to the others. Garlic extract contain insecticidal properties that are lethal to a wide range of insects (Oparaeke 2007). Our work related to

contact toxicity bioassay was showed that the methanolic extract of *A. sativum* has an interesting insecticidal potential.

The insecticidal activities garlic extract have previously been evaluated against different larval stages of the tomato leaf miner (Shiberu and Getu, 2017). Indeed, the results obtained in this work were in agreement with those reported by Ghanim and Abdel Ghani (2014), who showed that highest effects of garlic on *T. absoluta* second instar larvae under laboratory conditions. In another hand, Hussein *et al.* (2015) studied the insecticidal potential of some plant extracts against *T. absoluta*. The results show that the highest reduction in the population of this insect was obtained after tomato plants treated with garlic extract. Also, garlic leaf lectin (ASAL) has been found to have detrimental effect on growth and survival of two important homopteran insect pests, *Lypaphis erysimi*, commonly known as aphids and *Dysdercus cingulatus* (red cotton bug) (Bandyopadhyay *et al.*, 2001). They reported that garlic extract was characterized by more polar compounds of phenolic, steroidal origin (glycosylated and flavonoids) which showing interesting pharmacological properties (Hussein *et al.*, 2015).

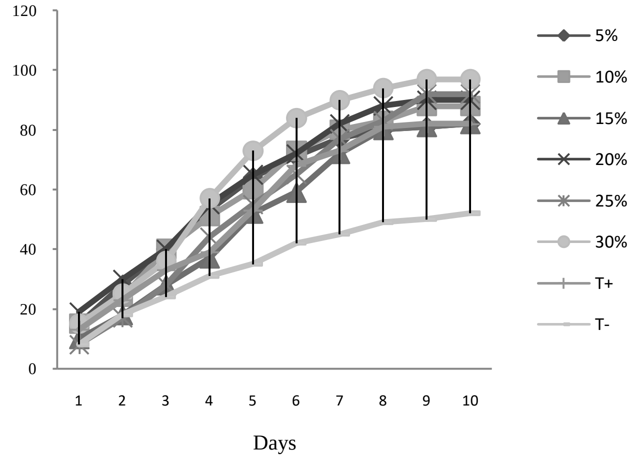

Figure 1: Cumulative mortality rate of *T. absoluta* larvae

Table 1. Abundance of dead *T. absoluta* individuals recorded in the *in vitro* test

	L1	L2	L3	L4
Positive control	3,37±0,32[b]	3,12±0,36[b]	2,74±0,42[c]	1,41±0,47[d]
Negative control	2,95±0,46[c]	2,21±0,3[c]	1,54±0,52[d]	0,83±0,84[e]
5%	4,14±0,31[a]	3,01±0,32[b]	2,06±0,49[c]	2,63±1,01[c]
10%	3,39±0,24[b]	2,96±0,68[c]	3,21±0,75[b]	2,52±0,96[c]
15%	3,45±0,4[b]	3,17±0,21[b]	2,37±0,30[c]	1,75±0,65[d]
20%	3,99±0,31[b]	2,94±0,49[c]	3,12±0,80[b]	2,48±1,46[c]
25%	3,92±0,42[b]	2,52±0,83[c]	2,34±0,36[c]	2,57±0,33[c]
30%	3,83±0,27[b]	3,79±0,44[b]	2,41±0,56[c]	3,16±0,12[b]

Means in columns followed by different letters are different ($P \leq 0.05$)

Determination of the DL_{50} and the DL_{90}

The analysis of the Probits (Finney, 1971) carried out on the corrected mortalities displays a lethal dose DL50 of the order of 21.91% and while the DL100 is around 37.34% (Fig. 2).

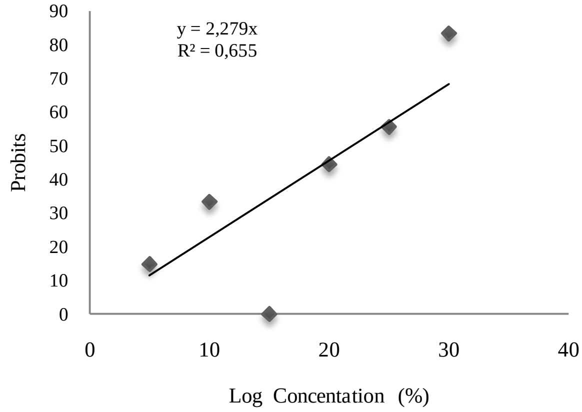

Figure 2. Effect of garlic metanolic extract against fourth instar larvae of *T. absoluta*: probits of corrected mortality as function the decimal logarithm of tested concentrations (R2: determination coefficient)

The rate of transformation of larvae into pupa

During the bioassays, the transition from the fourth larval stage to pupa was quite low. Indeed, a maximum rate of around 12% was recorded on the sixth and eighth day (Fig.3). The insecticidal effect of the extract of *A. sativum* with regard to larval individuals of *T. absoluta* is therefore confirmed following the results obtained.

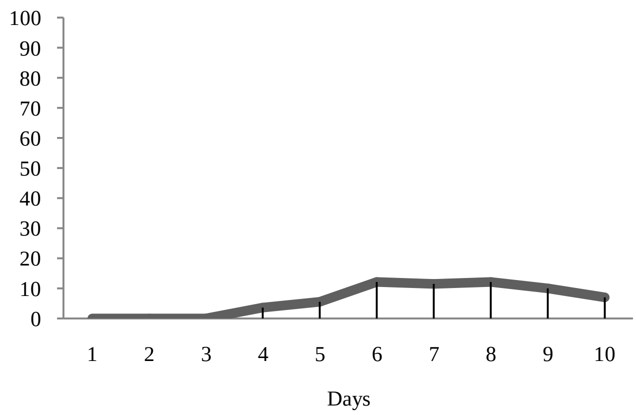

Figure 3: The larvae transformation rate into nymph during the test

Conclusion

The present study proved also the sensitivity of *T. absoluta* to *A. sativum* methanolic extract. This sensitivity varies according to the larval stage as well as the relative concentration and the exposure time. Lethal concentrations of 21.91% and 37.34% were estimated for 50 and 90% larval mortality, respectively. The results for this extract are more or less satisfactory for a concentration of 30%. The effectiveness of the latter on *T. absoluta* larvae was also observed during pupation, where only 12% of the larval population was able to reach the pupal phase. At the end of this study and depending on the results obtained, the methanolic extract of garlic showed a significant insecticidal effect, it can be used as a source of biological and natural treatment against the tomato leaf miner.

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