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Research Article
Euphorbia guyoniana Ethanolic Extract Efficiency Against Tomato Leaf Miner in Southeastern Algeria

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Abstract

Background and Objectives: The abusive use of insecticide on tomato crop may cause several impacts to environment and human as well as they can provoke resistance to plants. For that reason, a biological alternative was tested by using the ethanolic extract of *Euphorbia guyoniana*, spontaneous plant collected from arid regions in Southeastern Algeria, against eggs and larvae of tomato leaf miner *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). **Materials and Methods:** The 120 tomato leaf miner larvae *T. absoluta* of the L2-L3 stages and eggs were tested into contact with *E. guyoniana* ethanolic extract within 3 doses chosen after several preliminary tests: 20, 30 and 40 µg. **Results:** Experimentation showed the larvicidal effect of this plant varied with doses and time. The greatest mortality was obtained by the D3 (40 µg) with 50.83±19.54 and 95.42±03.15% noted after 2 and 96 h, respectively. The DL_{50} recorded was between 22.69 and 19.49 µg/larva, involving 50% of mortality. The treatment of eggs showed mortality rates of 15±05, 35±05 (D 30 µg) and 95±05% (D 40 µg) at the end of the test. **Conclusion:** The ethanolic extract of *E. guyoniana* revealed that it could constitute a good means of managing *T. absoluta* that might be introduced in sustainable organic agriculture.

Key words: Bio-insecticide, *Euphorbia guyoniana*, *Tuta absoluta*, tomato, larvicidal, ovicidal

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Algerian agriculture is constituted with cereals and vegetables. The later sector is encouraged by the presence of fertile area[1] of about 238,174.1 ha. Tomato (*Lycopersicon esculentum* Mill.) occupies a very important place among agricultural crops and presents a national annual production exceeded[1] 975 thousand tons in 2013. However, several pests confront this vegetable such as whiteflies and aphids, but also tomato leaf miner *Tuta absoluta* Meyrick, 1817 (Lepidoptera: Gelechiidae). This lepidopteron, which was observed for the first time in Algeria[2] in 2008, is originated from Latin America. Each year, this pest produces considerable yield losses of the crop that can be destroyed[3] at 100%. Also as known in the worldwide, the abusive use of insecticide may cause several impacts to environment and human as well as they can provoke resistance to plants. Recently, biological alternatives were found to control crop pests like plant bio-insecticides. Different types of plant preparations such as powders, solvent extracts, essential oils and whole plants are being investigated for their insecticidal activities including their action as fumigants, repellents, anti-feedants, anti-ovipositions and insect growth regulators[4-7]. Plant extracts can take an important place in the control of the tomato leaf miner. Indeed, Algeria has several plant species known for their biocidal effects[8]. A large number of them do exist in Saharan regions[8,9] and tested on several crop pests in Algeria[10-14] and Africa[15-18]. Using of secondary compounds properties of these plants can help to develop organic agriculture and reduce the chemical insecticide applications which are a great danger for the environment and the consumer. In this direction, the main objective of this work was to test *Euphorbia guyoniana*'s ethanolic extracts against *T. absoluta*.

MATERIALS AND METHODS

Study site: The present study was carried out at the entomology laboratory in the National Institute of Agronomic Research of Algeria (station of Touggourt) from September, 2017-June 2018. It is located at the valley of Wadi Righ in Southeastern Algeria. This region extends from 2 provinces, Ouargla from the South and El Oued to the North. It is crossed by the Righ canal which serves to evacuate sewage of agglomerations and drainage of palm groves towards the big chott of Merrouane. Indeed, a slope is recorded, from an altitude of 70 m at Tamacine for the highest point -39 m at El Meghaïr for the lowest point[19], which make the flow of water easier towards this point. Climatic data of study area show that the valley of Wadi Righ enjoys a Saharan climate that is characterized by a warm summer and a temperate winter. The maximum temperature was noted in August with a monthly average of 33°C and the minimum was in January with 9°C. The humidity of the air is low, registering only an annual average of about 48%. Precipitations are rare and random not exceeding 17 mm in the rainiest month[13]. Consequently, agriculture in this region is based exclusively on irrigation, which is favored by a high potential for underground water resources, since this part of Algeria extends over 3 reservoirs of water: The intercalary continental, the terminal complex and the groundwater table[19].

Plant material: *Euphorbia guyoniana* belongs to the family of Euphorbiaceae. Samples were collected from El Hedjira region (Southeastern Algeria). This species, locally known as Lebbina, occurs in all the desert and pre-desert regions of Algeria[9,20,21]. Its high is about 30-100 cm with erected and branched stems that contain very toxic white latex[22]. The genus *Euphorbia* contains more than 2000 species that are known in traditional medicine for their ability to treat skin diseases such as eczema[23], gastrointestinal disorders[24], bacterial and fungal infections[25] or even some types of cancer[26]. In addition, Saharan nomads of Algeria frequently use this plant against snakebites[8]. Moreover, a study has noted that it also has important antioxidant properties[27]. The beneficial effects of *E. guyoniana* are due to its high content of secondary compounds, such as terpenoids[28], alkaloids and flavonoids[29].

Plant Preparation of ethanolic extract of *Euphorbia guyoniana*: Soxhlet extractor was used to prepare the crude ethanolic extract of *E. guyoniana.* Aerial parts of this plant were collected at the flowering stage, dried in the shade in open air and crushed to obtain homogeneous powder. A quantity of 25 g of this later was added to 700 mL of ethanol. After 4 reflections, the extract was ready for use. The solution was then evaporated by using rotary vacuum evaporator at 40-50°C and kept in glass vials in freezer for further use. Three dilutions had been prepared to obtain different doses (20, 30 and 40 µg).

Breeding of *Tuta absoluta*: *T. absoluta* larvae used in this study were obtained from infested tomato leaves. They were collected in a greenhouse of untreated tomato installed in the station of INRAA of Touggourt. Pairs of *T. absoluta* were placed in plastic breeding cages (43 cm Length×30 cm width×36 cm height) at a temperature between 20 and 25°C and moisture of 60±10%. Insects were fed with a mixture of honey and water that were presented to them on a piece of cotton wool suspended inside the breeding enclosures[30]. Eggs, larvae and adults of tomato leaf miner recovered were used in the various tests carried out in this study.

Toxicity tests

Larvicidal effect: The 120 tomato leaf miner larvae of the L2-L3 stages were taken from the breeding with a fine brush and brought into contact with *E. guyoniana* ethanolic extract. Three doses were chosen after several preliminary tests: 20, 30 and 40 µg. Treatment consisted in the application of solution on each larva using a micropipette. Treated larvae were placed on fresh tomato leaves in petri dishes to continue their development. The dishes were then covered with perforated covers. The control was treated in the same way but the larvae were exposed only to ethanol. Three repetitions were retained for each preparation with 15 larvae for each one. The development of the larvae was followed under a binocular lens. The number of dead individuals was recorded after 02, 04, 24, 48, 72 and 96 h of treatment. This method was conducted, in consideration of real field situation, because of the exit of larvae to get food from other leaves.

Ovicidal effect: This test was done by recovering the eggs from leaves inside the breeding cages. Ovicidal effect was carried out in Petri dishes by covering the eggs with a quantity of *E. guyoniana* ethanolic extract by using a micropipette. The effect of this solution was revealed by counting the eggs that did not be hatched. Control was obtained by covering the eggs with ethanol only. Batch of 90 eggs divided into 03 replications were used in this test. For that, two doses chosen after several preliminary tests were used: 30 and 40 µg.

Parameters used: The effect of crude extract of *E. guyoniana* on larvae and eggs of *T. absoluta* was evaluated by using larval mortality rate, lethal dose (DL_{50}) and eggs non-hatched rate.

Mortality rate was calculated by using the equation of Abbott[31]:

$$\text{Mortality rate (M\%)} = \frac{\text{Number of dead individuals}}{\text{Number of introduced individual}} \times 100$$

The corrected mortality was calculated by using the equation of Schneider-Orelli[32]:

$$\text{Corrected mortality (MC)} = \frac{\text{Responded (\%)} - \text{Responded in control (\%)}}{100 - \text{Responded in control (\%)}} \times 100$$

The DL_{50} was calculated by transforming used doses into decimal logarithms as well as values of percentage of corrected mortality to probits using the table of Cavelier[33]. The DL_{50} is given starting from the straight regression lines probits = f (log doses).

Statistical analysis: Data was subjected to statistical analysis of ANOVA in order to evaluate larvicidal and ovicidal effect against tomato leaf miner; the data were collected as mortality rates in each replication. The statistical processing of the experiment data was carried out by using SPSS software (version 20.0).

RESULTS

Larvicidal effect of *Euphorbia guyoniana*: Ethanolic extract of *E. guyoniana* showed a very marked larvicidal effect on larvae of *T. absoluta* (Fig. 1). The first dose (20 µg) noted mortality rates of 45.42±15.36 and 74.58±07.12% at the beginning (after 2 h of treatment application) and the end (after 96 h) of the test. Also, the 2nd dose (30 µg) recorded higher values with a mortality of 50.00±12.77% noted after 2 h, only by contact of larvae with extract, compared to that noted at the end of test after 96 h (90.42±04.38%). The greatest mortality was obtained by D3 (40 µg) noted after 2 and 96 h of treatment application, respectively. Control mortality rate was between 33.18±07.98 and 48.34±12.17% at the beginning and the end of experiment. Statistical analysis revealed a highly significant difference ($p<0.001$) between these values and those of control. *Euphorbia guyoniana* toxicity increased with time for all doses and the statistical analysis showed a significant difference ($p<0.001$) between these values in time. Ethanol only showed a remarkably toxicity because of larval mortality rate equal to 48.34±12.17% that obtained at the end of the test (after 96 h of the treatment).

The lethal dose (DL_{50}) for the crude ethanolic extract of *E. guyoniana* was calculated for each observation in time by using the regression equation (Fig. 2).

Obtained results (Fig. 2) showed that values of DL_{50} decreased in time after 24 h from the treatment application, which explains the good efficiency of the extract. Indeed, obtained DL_{50} after 24 h was about 22.72 µg/larvae. Data obtained after 48, 72 and 96 h recorded 22.69, 21.37 and 19.49 µg/larva, respectively, involved 50% of mortality. All these lethal doses lie between doses of 20 and 30 µg.

Ovicidal effect of *Euphorbia guyoniana*: Treatment of *T. absoluta* eggs by crude ethanolic extract of *E. guyoniana* produced a very important effect of this preparation on emergence of larvae. In present experiment, it should be note that eggs not hatched are taken as dead. Control mortality rates were of 15±05, 35±05 and 95±05% at the end of test for D 30 µg and D 40 µg, respectively. Statistical analysis revealed a highly significant difference ($p<0.001$) between the 3 treatments (Table 1).

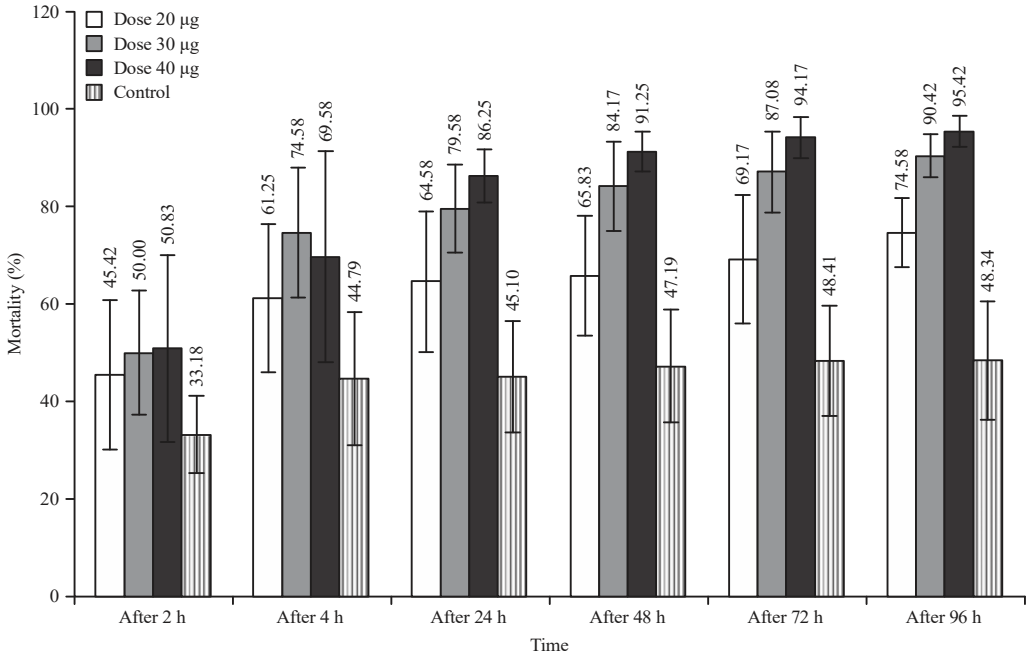

Fig. 1: Mortality of *Tuta absoluta* larvae treated with ethanolic extract of *Euphorbia guyoniana*

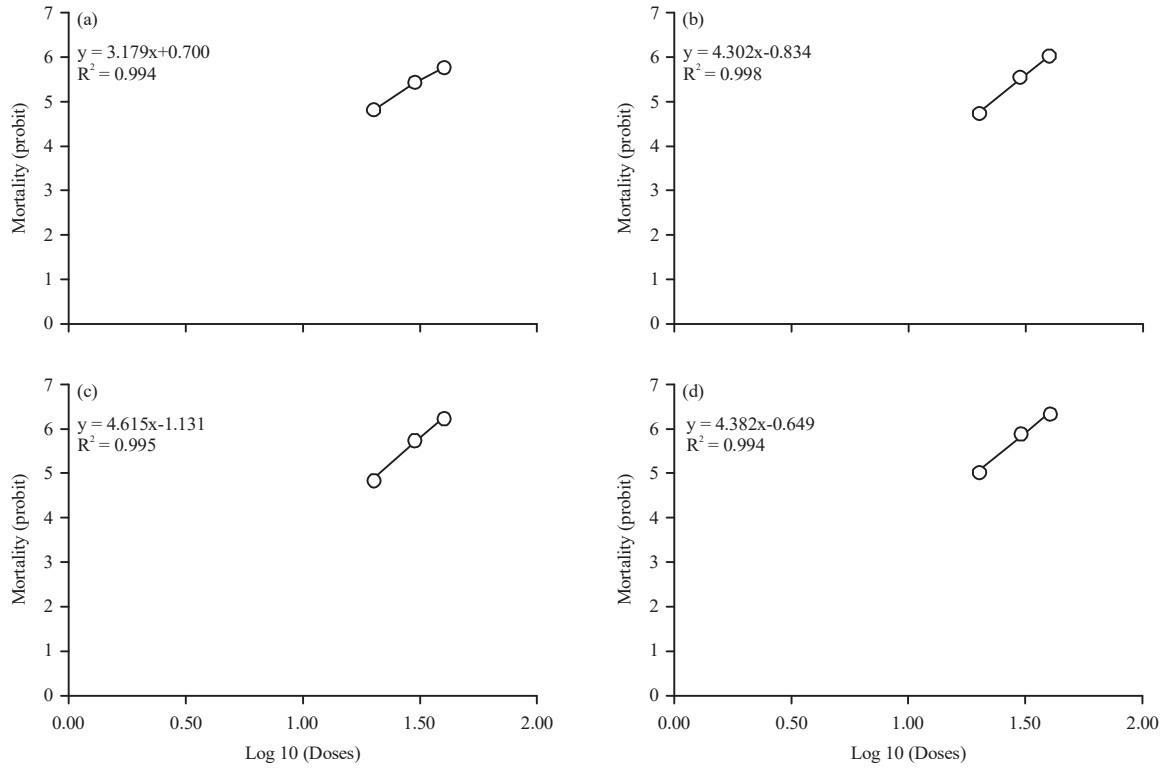

Fig. 2(a-d): Regression lines Probit = f (log dose) of *T. absoluta* larvae treated with *Euphorbia guyoniana* crude ethanolic extract, (a) DL_{50} after 24 h = 22.72 µg/larva, (b) DL_{50} after 48 h = 22.69 µg/larva (c) DL_{50} after 72 h = 21.37 µg/larva and (d) DL_{50} after 96 h = 19.49 µg/larva

Table 1: Effect of *Euphorbia guyoniana* crude ethanolic extract on *Tuta absoluta* eggs hatching

Parameters	N	Control (Mean±SD)	Dose 30 µg (Mean±SD)	Dose 40 µg (Mean±SD)
Mortality (%)	90	15±05	35±05	95±05
Significance (Control×Dose)			p = 0.003**	p = 0.000**
Significance (Dose×Dose)			p = 0.000**	p = 0.000**

**Significant difference at level $p \leq 0.01$

DISCUSSION

Some plants synthesize secondary metabolites with pesticidal and repellent effects that allow them to defend themselves against different bio-aggressors. Using extracts of these plants as insecticide is known for a long time. Indeed, pyrethrin, nicotine and rotenone were already used to control several pest insects[34]. Euphorbiaceae family species are known to be among the important poisonous plants due to presence of great diversity of secondary metabolites that allow them to have a worldwide distribution[35]. *Euphorbia* species growing in Algeria contain several chemical families such as tannins, flavonoids, lipids, sterols, saponins, phenolic compounds and terpenes which have bio-insecticide, antimicrobial and antioxidant properties[36]. The last property is also detected in other *Euphorbia* species in the world[37]. Due to crude ethanolic extract of *E. guyoniana* tested against *T. absoluta*, the results showed larval mortality rates of about 45.42±15.36 and 95.42±03.15%. Value of 95±05% was also recorded in the treatment of eggs of this insect. In previous study, Dehliz *et al*.[13] noted that the aqueous extract of *E. guyoniana* did not exert any toxicity on *T. absoluta* eggs hatching whereas, an important mortality about 77% was observed on larvae. This result was perhaps due to the solvent used which made extract of secondary compounds that have insecticidal effects possible. Same authors also revealed that aqueous extract of this plant disposed a repulsive effect on females of *T. absoluta* because the number of eggs laid on the treated leaves (06±1.41 eggs/leaflet) was less important than that obtained with control (37.25±13.15 eggs/leaflet).

A treatment application by acetonic extract of *E. guyoniana* recorded, after 11 days, a mortality rate of 100% against larvae[38] and adults[39] of *Schistocerca gregaria* (Orthoptera: Cyrtacanthacridinae). Moreover, the later at a low dose (1%) recorded mortality rates of about 57% of larvae of *Culex pipiens* L. (Diptera: Culicidae)[40]. Furthermore, a work led on the Algerian endemic species *E. guyonina* showed an important antibacterial activity against six bacteria strains[41]. Several species such as *Euphorbia caracasana* Boiss and *Euphorbia cotinifolia* L. from Venezuela showed also antibacterial activity[42]. Also, another work on *Euphorbia kopetdaghi* showed that extract of this species has inhibitory activities on cancer cells[43]. A study on Algerian *E. guyoniana* showed that their flavonoids extracts are important inhibitors of the steel corrosion[44].

Several vegetable substances were tested on tomato leaf miner *T. absoluta*. Taadaouit *et al*.[16] studied the effect of ethanolic extracts of *Argania spinosa* and *Thymus vulgaris* on the larvae of this micro-lepidopteron insect where a very high mortality rate (90%) was recorded with the first plant. Allal-Benfekih *et al*.[10] obtained a toxicity rate of more than 75% by using the aqueous extracts of *Inula viscosa*, *Salvia officinalis* and *Urtica urens* to control this pest. Berima and Osman[45] obtained a toxicity rate of 63% against adults of *T. absoluta* treated with the ethanolic extract of *Jatropha* curcus. Similarly, Konan *et al*.[46] obtained a mortality rate of 100% against the larval populations of *T. absoluta* after only 4 days of the application of ethanolic extracts of *Azadirachta indica* and *Jatropha curcus*, whereas, Hussein *et al*.[47] obtained a mortality of more than 63% by using aqueous extracts of *Cymbopogon citratus* and *Allium sativum*. At the same time, Ghanim and Abdel Ghani[48] reported 73, 80, 84, 86 and 91% of toxicity rates with *Oscimum basilicum* L., *Allium cepa* L., *Allum sativum*, *Pelargonium zonale* and *Melia azedarach*, respectively. In a study using the extract of *Z. album,* Lakhdari *et al*.[12] argued that its acaricidal effect against the yellow mite of the date palm *Oligonychus afrasiaticus* Meg. (Acari: Tetranychidae), could be an important means of controlling this pest because a mortality of about 76%.

The present study confirmed and supported that this plant contributes to organic agriculture as an eco-friendly product as well as an alternative to avoid the wrong use of chemical products and their negative effects on human and environment.

CONCLUSION

The present study showed significant data on this kind of biological treatment against the tomato leaf miner. According to the obtained results, crude ethanolic extract of *E. guyoniana* revealed that this substance could constitute a good means of managing *T. absoluta* that might be introduced as an eco-friendly way to control this in sustainable organic agriculture.

SIGNIFICANCE STATEMENT

This study discovers the insecticidal effect of ethanolic extract of *Euphorbia guyoniana*, spontaneous plant collected from arid regions in Southeastern Algeria, against eggs and larvae of tomato leaf miner *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) as novel and original study in this region. This study will help the researcher to uncover the critical area of using the bio-insecticidal against this pest that many researchers were not able to explore. Thus, a new biological alternative against *T. absoluta* conducted as an eco-friendly product in this experimentation may be arrived at.

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