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Preliminary Assessment of the Insecticidal Activity of an Extract from an Algerian Field Ranunculaceae Species against *Phthorimaea absoluta* (Lepidoptera: Gelechiidae)

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ABSTRACT

Tomato crops are threatened by the destructive invasive pest *Phthorimaea (=Tuta) absoluta* (Lepidoptera: Gelechiidae), for which control usually entails the use of synthetic pesticides as an important part of management strategies. Nonetheless, environmentally friendly methods and resources are being used successfully; one such strategy is the use of plant extracts. Thus, we screened extracts from various plants to control *P. absoluta* larvae. From those we selected the *Clematis flammula* extract to further investigate its lethal effect and to characterize its contents using LC-ESI-MS/MS. Finally, we aimed to validate the extracts efficacy under open field conditions by studying the proportion of leaf area damaged, larval mortality and damage of harvested tomatoes. Findings demonstrate that *C. flammula* has a significant effect on *P. absoluta* with a lethal concentration (LC_{50}) of 0.07mg/ml. Fifteen compounds, comprising phenolic acids and flavonoids were identified from the ethanolic extract. In the open field different concentrations of the aqueous *C. flammula* plant extract did not significantly affect the damaged leaf area, nor did they have a significant effect on *P. absoluta* larvae inside their mines. Overall, 98% of the tomato yield was consumable and only 4% was destroyed, with no significant difference between the extract-treated plants, the Emma Gold-positive control and the untreated negative control. Thus, the wild Ranunculaceae plant species from Algeria *C. flammula* could be considered as a potential sustainable source for a control program against *P. absoluta* but further validation of its efficacy under field conditions is necessary.

keywords: Tomato, Screening plants, Lethal concentration, LC-ESI-MS/MS, Damaged leaf area

Introduction

Nowadays, millions of hectares are devoted to the cultivation of tomato (*Solanum lycopersicum* L.), the most widely cultivated vegetable crop in the Solanaceae family and the second most important crop after potato. For instance, African countries such as Egypt, Nigeria, Morocco, Tunisia, Cameroon, and Algeria produce millions of tons of tomatoes annually (**Gebermariam, 2025; Melomey et al. 2029**).

Tomato production in Algeria is thriving due to national programs, which support its cultivation in greenhouses and plastic tunnels, enhancing its role in the agricultural economy (**Rekibi, 2015**). Invasive pests attacking agricultural crops reduce the quantity and quality of production (**Aynalem, 2022**). In this context, the problems with *Phthorimaea absoluta* (Meyrick,1917), formerly known as *Tuta absoluta* (Lepidoptera: Gelechiidae) exacerbate (**Chang and Metz, 2021**). This South American pest has spread outside its endemic region through Europe, Africa, and Asia (**EPPO, 2025**). *P. absoluta* was initially discovered in Algerian greenhouse tomato crops in the spring of 2008 in Mostaganem province (**Guenaoui, 2008**). Until today, farmers struggle to effectively control the pest due to a lack of knowledge about pest management techniques and rely solely on the use of chemical insecticides (**Guenaoui et al. 2015**). In Europe, management studies with an increasing number of natural enemies of *P. absoluta* provide a clearer picture of their potential use against the pest, even though the effectiveness of control techniques varies (**Desneux et al. 2022**). Combining biocontrol agents with other methods for pest control, such as host plant resistance (**Zannou et al. 2025a, b**) and entomopathogens (**Koller et al. 2023**), can enhance pest management of *P. absoluta*. Nevertheless, the detection of *P. absoluta* in many countries is followed by a surge in usage of synthetic insecticides as a quick and effective method of pest control despite their negative effects on the environment and humans (**Rwomushana et al. 2019**).

For many years, studies on plant-derived pesticides have been conducted with the primary objective of reducing the negative effects of synthetic insecticides (**Adeyemi, 2010**). Many natural insecticides or biopesticides are safer for consumers and non-target beneficial insects like entomophages and pollinators (**Walia et al. 2017**). According to **Shivakumar et al. (2013**), more than two thousand plant species have insecticidal properties, and studies have shown that extracts from specific plants including Lamiaceae, Asteraceae, Liliaceae, and Euphorbiaceae, have high bioactivity and are effective against a variety of insects (**Moreno et al. 2012**). The usefulness of natural insecticides made from native mediterranean plants in managing significant insect pests in vegetable crops has been examined in recent years (**Karkanis and**

Athanassiou 2021). Algeria possesses a diverse flora, influenced by climate and soil types, which includes numerous aromatic plants, which are distributed across the country and known for medicinal and biological properties (**Sahi 2016**). Screening those plants for activity against insect pests supports the development of affordable, safe, and ecologically sustainable alternatives to conventional pesticides (**Tarusikirwa et al. 2020**).

Thus, the objectives of the present study are: (1) screening several plants named by farmers as being effective against pests for their activity against *P. absoluta;* (2) further investigating the efficacy of an extract from *Clematis flammula* as a promising plant with a mortality effect on *P. absoluta*; (3) characterizing the plant extract using LC–ESI-MS/MS technique, and (4), validating the efficacy of *C. flammula* plant extract under open field conditions.

2. Materials and methods

2.1. Bioassays

2.1.1. Insects and tomato plants for laboratory bioassays

Tomato plants were grown in seedling trays and transplanted into pots, they were then grown in laboratory conditions at 23.4±2°C, 35±4% RH, and 12:12 L:D; with the windows opened for proper ventilation and natural lighting. Consequently, plants were exposed to natural infestation by *P. absoluta*. Studies were performed in 2024 with the tomato variety " Hayat F1". The larvae (L3 and L4) found in tomato leaves were exploited for bioassay 1, for which experimental runs 1 and 2 were conducted with those larvae. Another colony of *P. absoluta* larvae was provided from open-field tomato crops at Aine Djaser, Batna province (35°82'28.40" N, 5°95'15.02" E), from which larvae were used for the remaining bioassays, i.e. runs 3-5 of bioassay 1 (3^{rd},4^{th} instar), and all runs of bioassay 2 (3^{rd} instar).

2.1.2. Collection of plants and extraction method

The choice of plants was based on a survey, which we conducted with farmers in the Algerian province Batna in 2023 (**Bentrcia et al. 2023**). The collection of plant material for bioassay 1-2 was done in 2023 and 2024 in Foum Toub district (35°.38'46.80" N, 6°.46' 36.49" E) Batna province, (**Fig. 1**). After collecting the plants, we directly washed the aerial parts of the plants with tap water and cleaned them of all impurities and dust. Then, we dried them for a week at ambient temperature in a well-ventilated room. After finishing the drying process, we cut the plants into small pieces, then grinded them with an electric grinder until obtaining a

homogeneous powder. Extraction was conducted by maceration technique: 100 ml of ethanol/distilled water (70/30) to 10 g of powder, with moderate stirring at a speed of 100 Hz continuously for 24 hours. Afterwards, we filtrated the extract using filter paper (Fisherbrand/size: 210 mm). The same operation was repeated three times (three cycles). The filtrate from the three cycles was evaporated by using a rotary evaporator at 43°C and a speed of 145 rpm; the filtrate was put in a glass petri dish in an oven at 37°C to dry (**Abaza et al. 2015**).

2.1.3. Bioassay 1: Screening bioassay

Tomato leaf disks of 4 cm diameter were dipped in the crude extract for 20 s with gentle agitation. The treated leaf disks were removed and placed on a mesh surface to dry for 30 min. Once dried, they were transferred into petri dishes and placed on an absorbent paper disk soaked in mineral water. By using a fine soft brush, one *P. absoluta* larva was transferred into each petri dish covered with a transparent perforated cover to ensure ventilation. The screening experiment was performed in five replicates; each replicate consisted of 6 larvae tested at the concentration of 1mg/ml resulting in a total sample size of 30 larvae per treatment. The control leaves were soaked only in distilled water containing a drop of Tween 80 (0.1%). The experiment took place at room conditions (24±2°C, 46% RH and 16:8h L:D). The status of the larvae was checked at 24 h intervals for 5 days. Insects were observed under a stereomicroscope (OPTIKA: 4×10×20) and considered dead if they did not respond when probed with a fine paintbrush. Corrected mortalities were calculated by using the formula of **Abbott (1925**) given as:

Corrected mortality = (Observed mortality in treatment - Observed mortality in control/100-Control mortality) x 100

2.1.4. Bioassay 2: Evaluation of *C. flammula* extract against *P. absoluta* larvae

Based on the screening experiment, *C. flammula* was chosen for more detailed investigations. The same method of extraction as described above (2.2.) was applied in this experiment, except that the extract was prepared by adding 400 ml (280 ml of ethanol/120 ml of distilled water) to 40 g of powder. Four concentrations were obtained by diluting the crude extract: 0.5 mg/ml; 0.25 mg/ml; 0.1 mg/ml; 0.05 mg/ml. The bioassays were carried out as described above, using the four concentrations of the *C. flammula* extract and control with distilled water. Overall, 320 larvae in 3rd instar, split among seven replicates, each containing ten larvae were tested in this

bioassay. Assessment of mortality happened after 24 h, 48 h, and 72 h and was calculated by using the formula of **Abbott (1925**).

2.2. Characterization of *C. flammula* crude extract by LC–ESI-MS/MS

The ethanolic *C. flammula* extract was characterized by using the Agilent 6460 Triple Quad LCMS System to quantify bioactive compounds. All were controlled by LC-solution software (Agilent MassHunter Workstation). The chromatographic separation was performed on a Poroshell 120 EC-C18 (50 mm 4.6 mm I.D., 2.7 µm) column. A sample of 50 mg of crude extract was taken into a 2 ml Eppendorf tube and dissolved by adding 1 ml of acetonitrile-methanol-water solution (1-1-1). The sample was vortexed and further dissolved in an ultrasonic bath. Then it was centrifuged at 7000 rpm for 5 minutes. The pellet was taken and diluted at 1:4 (extract: methanol). In the last step, after filtering with a 0.25 µm filter, 4.00 µl were injected for LC MS/MS analysis. Chromatographic separation was performed at a flow rate of 0.400 mL/min., over a runtime of 40.00 min. The column temperature was 30 °C. The mobile phases consisted of two solvents: (A) water containing 0.1% formic acid and 5 mM ammonium formate and (B) Acetonitrile containing 0.1% formic acid (**Erenler et al. 2023**). The peaks in the resulting chromatograms were compared to the reference library for compound identification.

2.3. Open field study

2.3.1. Experimental setup

An experimental plot with a total surface area of 52.80 m^2 was chosen in the district of Arris, Batna province, Algeria (35°15' 15.2" N, 6° 20' 47.9" E). Plants of the variety "Ibiza" were grown in the nursery before being transplanted into the experimental field. The study was carried out in 2024 from Sep 1 to Dec 30. Temperature and humidity were recorded during the cultivation period (15±6°C; 58±6 RH and 12:12 L:D), no fertilizers and no pesticides were used during the study period. The experiment was laid out in a randomized complete block design with five treatments and ten replicates, each replicate of treatment corresponding to one plant across the ten blocks (**Fig. 2**). Water and delta traps, together with careful visual examination of leaflets, were used to check the arrival of *P. absoluta* at the experimental site, and once arrival was observed, application of treatments was started. Afterwards, application of treatments was done at weekly intervals. As a first step, we prepared an aqueous extract by using 1 kg of powder of *C. flammula* plants in 10 L distilled water. Then dilutions were prepared to achieve extracts

at 100% (0.1 g/ml), 50% (0.05 g/ml), and 25% (0.025 g/ml). In addition, a positive control "Emma Gold", with Emamectin benzoate 5% +Lufenuron 5% active ingredients (LEEDS), a licensed insecticide against *P. absoluta* was used and a negative control with water. Sprays were conducted on tomato plants, using a hand-held sprayer and a steady, sweeping motion to ensure even coverage. Safety and post-application care were taken, and regular pruning and physical barriers were used to control growth.

2.3.2. Proportion of leaf area affected by *P. absoluta* larvae

The presence of larvae was assessed by picking leaves showing visible signs of infestation during the weekly visit to the crop. Infested leaves were picked by the zigzag method (**Mahdjouba, 2018**), sampling a small proportion of the total infested leaves within the field; they were then photographed with the phone before entering pictures into the software Image J "(http://imagej.nih.gov/ij/). Following the measurements taken by image J, which allowed us to measure total leaf area and *P. absoluta*-damaged leaf area, we used the following calculation to calculate leaf damage percentage area: Damaged leaf area/Total leaf area ×100

2.3.3. Effect of treatments on *P. absoluta* larvae inside the mines

Monitoring the population of *P. absoluta* larvae was conducted weekly to assess the treatment effect. All plants were systematically checked, and all infested leaves were picked. The samples were placed in plastic boxes (40 cm x 30 cm x 20 cm) and brought back to the laboratory to be examined under a stereomicroscope for presence and viability of larvae inside the mines. The total number of infested leaves, the number of mines with alive larvae and mines without alive larvae were assessed.

2.3.4. Damage of harvested tomatoes

We assessed the damage rate of the tomato harvest from the experimental plots. Four harvests were carried out between December 2nd and December 4th, 2024. For each harvest, we recorded the number and weight of tomatoes from i) the positive control (T+), ii) the water control (T-) and iii) the plants treated with extract (T1-T3). We further assessed the number of holes (caused by *P. absoluta* or other factors). From those data, we calculated the damage rate as follows:

Damage rate = number of damaged fruits /total number of examined fruits ×100

2.4. Data analysis

All data were checked for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests, respectively. Because the mortality data from different plant extracts did not follow a normal distribution these data were analyzed using the Kruskal-Wallis test. To compare all possible pairs of means, we used the Newman-Keuls post hoc test. The mortality data for *P. absoluta* larvae were subjected to log probit analysis (log base 10), to determine concentrations leading to 25, 50, and 90% mortality with 95% confidence intervals. The data on the proportion of leaf area affected by *P. absoluta* larvae and the data on the effect of treatments on *P. absoluta* larvae inside the mines of infested leaves did not follow a normal distribution, thus, the Friedman test was used. All statistical analyses were performed using IBM SPSS Statistics, version 27.0.0.

3. Results

3.1. Bioassays

3.1.1. Bioassay 1: Screening bioassay

A significant effect of plant extracts on *P. absoluta* corrected mortality rate was found for all times (24, 48, 96, and 120 hours) except 72h after application of the extracts (**Table 1**). *Clematis flammula* evoked the highest mortality rate at 24 hours (58.33%±0.16; mean±SD) and together with *Euphorbia sp.* at 120 hours (74.99%±0.26/±0.14 resp.). High mortality after 24h was also observed with *Marrubium vulgare* (69.99%±0.18), and *Teucrium polium* (56.66%±0.09), followed by *Lonicera implexa* (48.32%±0.23) and *Hertia cheirifolia* (48.32%± 0.22).

3.1.2. Bioassay 2: Evaluation of *C. flammula* extract against *P. absoluta* larvae

Mortality of *P. absoluta* larvae increased with increasing concentration of the *C. flammula* extract (Table 2). The probit model was used as a suitable model for the concentration-dependent mortality based on those data after 72 hours, in which LC_{10}:0.001mg/ml, LC_{25}:0.006 £££mg/ml, and LC_{50}: 0.077mg/ml (P =0.98; and X^2=13.36).

3.2. Characterization of *C. flammula* crude extract by LC–ESI-MS/M

Characterization of the *C. flammula* crude extract by LC–ESI-MS/MS revealed several phenolic acids and flavonoids, as shown in the chromatogram in Fig. 3. The identified compounds, their retention times, and concentrations are presented in Table 3. The detected compounds showed concentrations ranging from 0.63 to 56.43 µg/g. Isoquercitrin was the most abundant compound (56.43 µg/g), followed by chlorogenic acid (40.71 µg/g) and trans-

cinnamic acid (35.41 µg/g). Other compounds were detected at moderate concentrations, including trans-ferulic acid (21.58 µg/g), kaempferol (15.39 µg/g), and caffeic acid (13.65 µg/g). In contrast, naringenin (0.63 µg/g), morin (1.54 µg/g), and p-coumaric acid (1.98 µg/g) were detected at the lowest concentrations. Overall, isoquercitrin and chlorogenic acid were the predominant compounds identified in the *C. flammula* crude extract.

3.3. Open field study

3.3.1. Proportion of leaf area affected by *P. absoluta* larvae

Between September and December, a total of 66 infested leaves were collected. Infested leaves were picked on each date and across the various treatments applied with T1=12, T2=8, T3=14, T+=2, T-=30. There was no significant effect of the different treatments on the proportion of leaf area damaged P=0.736, (Fig. 4).

3.3.2. Effect of treatments on *P. absoluta* larvae inside the mines

There was no significant effect of the different treatments on the number of leaves infested (P=0.14, X^2 =6.85), the number of mines with alive larvae (P=0.79, X^2=1.69), or the number of mines with dead larvae (P=0.31, X^2=4.76) (Table 4).

3.3.3. Damage of tomatoes harvested

Across the whole period of harvest, 452 ripe tomatoes in total were collected, weighing (34.01 kg). Some additional unripe fruits fell to the ground or did not fully ripen until the end of the experiment. There were 283 tomatoes in the three treatments (T1. T2. T3) weighing 20.07 kg, 101 tomatoes weighing 11.59 kg in the T+ and 68 tomatoes weighing 6.51 kg in the T-. From treatment plants 2.82% of tomatoes had holes induced by *P. absoluta*, no holes were observed in the T+ control, and 5.88 % in the T- control (Table 5).

4. Discussion

Laboratory bioassays demonstrated that some native Algerian plant species, which are *Clematis flammula*, *Marrubium vulgare, Euphorbia sp., and Teucrium polium*, have a significant effect on mortality of *P. absoluta*. Particularly, *C. flammula* seems the most promising plant as it had an important toxicity effect on *P. absoluta* larvae in laboratory assays. It should be noted that this is the first study to report the bioactivity of *C. flammula* on *P. absoluta*. Nevertheless, numerous works have examined the effectiveness of different plant extracts in controlling or deterring insect pests. The biological activity associated with these plant extracts can be produced by soaking roots, flowers, seeds, stems, leaves, fruits, and bark in water or organic solvents (**Civelek and Weintraub 2004**). Other studies have also shown the potential of several plants as a source of botanical insecticides with bioactivity on *P. absoluta*, such as piper species (**Brito et al. 2015**), *Tephrosia vogelii* (**Ndereyimana et al. 2019**), and *Haplophyllum perforatum* (**Turaeva et al. 2022**).

Quantitative evaluation of plant bioactive substances is an important study for many purposes such as medicine or managing pest insects; the effects of plants may be due to high concentrations of the compounds or to the synergistic action of matching compounds together (**Karan et al. 2018**). In this study quantitative analysis of compounds was performed and fifteen compounds were identified. Isoquercitrin was calculated as a major product in the ethanolic extract of *C. flammula* then Chlorogenic acid, Trans-cinnamic acid, trans-ferulic acid, Kaempferol, and Caffeic acid. The compounds identified in the ethanolic extract include two major chemical substance groups, flavonoids (Isoquercitrin, Vanillin, Naringenin, Morin, Diosgenin) and phenolic acids (Chlorogenic acid, Gentisic acid, Caffeic acid, Syringic acid, P-coumaric acid, Salicylic acid, Protocatechuic acid, Trans-cinnamic acid).

Flavonoids show great potential as toxicants against insect pests (**Pereira et al. 2024**). According to the literature, main classes of secondary compounds frequently detected in *C. flammula* are substances classified as flavonoids glucosides 1 and 2 (**Saidi et al. 2019**) and HPLC-MS analysis demonstrated the presence of flavonols (**Belkhir et al. 2024**). The differences in the compounds identified could be due to the type of solvents employed, extract concentrations, the method of analysis or the device used.

Under open field conditions, no significant difference in percentage of leaf area damaged in the treated group (T1.T2.T3), compared to the controls was found. This indicates the applied treatment was not effective in mitigating *P. absoluta* under the given conditions. However, the

absence of significant differences might also be linked to the relatively small size of the field experiment and the relatively low *P. absoluta* infestation observed during the experiment, which took place at the onset of September – December. While there are no other studies specifically investigating *Clematis* extracts against *P. absoluta*, several field studies have demonstrated the potential of botanical-based treatments for reducing *P. absoluta* damage. For example, *Tephrosia vogelii* extract and Nimbecidine reduced leaf damage by approximately 48% and 38%, respectively, compared with untreated controls under field conditions in the Democratic Republic of the Congo **(Mbuya et al. 2026).** Similarly, neem-based formulations have consistently reduced *T. absoluta* leaf infestation under field conditions in India, with previous studies reporting reductions of 70–83% following azadirachtin application **(Abd El-Ghany et al. 2018; Shashank et al. 2021).** Other field studies have also reported significant reductions in leaf damage following the application of neem, garlic, onion, eucalyptus, and tobacco extracts, although their efficacy varied among treatments and experimental conditions **(Khan et al. 2022).** These findings indicate that plant-derived products may represent promising alternatives for the management of *P. absoluta*; however, their efficacy can vary according to plant species, formulation, environmental conditions, pest pressure, and application regime **(Braham et al. 2012; Machekano et al. 2020; Mbuya et al. 2026).**

The present study showed that only 2.82% of the tomato yield from treated plants was damaged by *P. absoluta*, whereas 12.36% was affected by holes attributed to other causes. Although *P. absoluta* mainly damages leaves and stems, larval feeding can also extend to fruits, where galleries, exit holes, and frass are typical symptoms **(Ndereyimana et al. 2020).** The relatively low level of *P. absoluta*-related damage may explain why no apparent increase in yield was observed in the extract-treated plants. Similarly, **Rostami et al. (2021)** reported that the effect of *P. absoluta* infestation on tomato yield depends on the level and timing of infestation. In contrast, **Sarr et al. (2021)** found that effective treatments with azadirachtin reduced leaf and fruit damage and increased tomato yield. These differences suggest that the yield response to botanical treatments may depend on pest pressure, treatment efficacy, and the contribution of other sources of fruit damage.

8. Conclusion

Globally, aromatic plants exhibit high diversity, including the genus *Clematis* (Ranunculaceae), which is known for its potent odors and toxic compounds. These plants have been reported to

possess insecticidal properties and biologically active constituents. In our study, *C. flammula* showed promise in managing *T. absoluta* due to its abundance and demonstrated efficacy in laboratory bioassays, supporting its potential for practical applications. This activity may be related to the different compounds identified by LC-MS/MS, including phenolic acids and flavonoids, which are known for their toxic effects against insect pests and pathogens.

Although the extract exhibited promising insecticidal activity under laboratory conditions, its efficacy was not statistically significant under field conditions during the single growing season investigated. This discrepancy may reflect environmental degradation of active compounds, variability in pest pressure, limited residual activity, or application timing. Additionally, multi-season trials are necessary to further investigate the effects of *C. flammula* on the life cycle of *T. absoluta* and to assess the interactions of this plant extract with other biocontrol agents, such as natural enemies and microorganisms.

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Figure captions

Figure. 1. Locations and geographical coordinates of plant sampling sites at Foum Toub district; Batna province (Northern Algeria) with photographs of the plants and the location of their collection. The top-right plot represents the climatic diagram of the region (exploiting Google Earth Pro).

Figure. 2. Location and geographical coordinates of the open field tomato cultivation site at Arris -Batna province with diagram of the experimental design (exploiting Google Earth Pro).

Figure. 3. LC–ESI-MS/MS chromatogram of *C.flammula*.The peaks correspond to : (1) Chlorogenic acid, (2) Gentisic acid, (3) Caffeic Acid, (4) Syringic acid, (5) Isoquercitrin, (6) P-coumaric acid, (7) Vanillin, (8) Trans-ferulic acid, (9) Salicylic Acid, (10) Protocatechuic acid, (11), Kaempferol, (12) Trans-cinnamic acid, (13) Naringenin, (14) Morin, (15) Diosgenin

Figure. 4. Proportion of leaf area affected by *P. absoluta* larvae, when exposed to the different treatments according to picking date (T1: 0.1 g/ml; T2: 0.05 g/ml; T3: 0.025 g/ml of aqueous solution of *C. flammula*; T+: Emma Gold; T−: Water).

Table1. Means (±SD) of *P. absoluta* larval corrected mortality rate after five days of exposure to tomato leaf lets treated with ethanolic extract from different plant species at a concentration of 1mg/ml.

Plant species	Corrected mortality rate **(%) ±SD**				
	24h	**48h**	**72h(ns)**	**96h**	**120h**
Marrubiumvulgare	53.33±0.18 ^{bc}	66.66 ± 0.20 ^{b}	69.97 ± 0.25	69.99±0.18 ^{b}	69.99±0.18 ^{ab}
Loniceraimplexa	21.66± 0.13 ^{ab}	41.66 ± 0.2 ^{2a}	43.32± 0.19	43.32± 0.19 ^{a}	48.32± 0.23 ^{a}
Clematis flammula	58.33 ± 0.16 ^{c}	64.99 ± 0.19 ^{b}	64.99 ± 0.23	68.33 ± 0.25 ^{b}	74.99 ±0.26 ^{ab}
Teucriumpolium	29.99 ± 0.18 ^{abc}	49.99 ±0.11 ^{ab}	44.99 ± 0.16	56.66± 0.09 ^{a}	56.66 ± 0.09 ^{a}
***Euphorbia*sp.**	36.66± 0.21 ^{abc}	59.99 ± 0.22 ^{b}	61.66 ± 0.26	68.32±0.20 ^{ab}	74,99± 0.14 ^{b}
Hertiacheirifolia	21.66 ± 0.13 ^{a}	34.99 ± 0.19 ^{a}	38.32 ± 0.26	44.99± 0.16 ^{a}	48.32± 0.22 ^{a}
Control	6.66 ± 0.14 ^{a}	6.66± 0.14 ^{a}	16.66 ± 0.23	16.66± 0.23 ^{a}	16.66± 0.23 ^{a}

The different letters in the columns differ significantly among the plants (Kruskall-Wallis's test followed by N.K post hoc test), at 5% probability. ns: Nonsignificant

Table 2. Observed mortality of *P. absoluta* larvae caused by different concentrations of the ethanolic extract from *C. flammula* and estimated lethal concentrations necessary to cause 25%, 50% and 90% mortality after 72h.

N° of insects Tested	Conc. (mg/ml)	Mortality (%) ±SD	LC 10 (mg/ml) (LCL-UCL)95%	LC $_{25.}$ (mg/ml) (LCL-UCL)95%	LC $_{50(}$mg/ml) (LCL-UCL)95%	Slope± SD
320	0.5	57.1±1.41	0.001	0.006	0.077	1.25±0.187
	0.25	53.3±1.52	(0.000-0.006)	(0.000-0.023)	(0.017-0.135)	
	0.1	43.3±1.98				
	0.05	33. 3±1.31				

LC: Lower Confidence Limit. UCL=Upper Confidence Limit. SD: Standard Deviation, X^2: Chi-square value

Table 3. Major compounds identified in *C. flammula* crude extract by LC–ESI-MS/MS.

N°	Compound name	RT (min)	Conc. µg/g
1	Chlorogenic acid	2,67	40,71
2	Gentisic acid	2,86	9,45
3	Caffeic acid	3,49	13,65
4	Syringic acid	3,61	3,45
5	Isoquercitrin,	3,93	56,43
6	P-coumaric acid	4,84	1,98
7	Vanillin	5,29	7,74
8	Trans-ferulic acid	5,30	21,58
9	Salicylic acid	7,53	3,86
10	Protocatechuic acid	8.29	2,38
11	Kaempferol	9.91	15,39
12	Trans-cinnamic acid	10.89	35,41
13	Naringenin	11.52	0,63
14	Morin	11,52	1,54
15	Diosgenin	20.19	3,13

695 **Table 4.** Total and mean ±SD of leaves infected by *P. absoluta* larvae, mines with alive larvae,
696 and mines with dead larvae after exposure to the different treatments (T1: 0.1 g/ml; T2: 0.05 g/ml;
697 T3: 0.025 g/ml of aqueous solution of *C. flammula*; T+: Emma Gold; T−: Water).
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Model of study / Treatments	T1: 0.1 g/ml	T2 : 0.05 g/ml	T3 : 0.025 g/ml	T+ : EmmaGold;	T− : Water
Leaves infected	1.69±1.65 (22)	2.23±2.61 (29)	2.07±1.93 (27)	1.07±1.11 (14)	1.92±1.70 (25)
Mines with alive larvae	0.23±0.43 (3)	0.16±1.12 (8)	0.30±0.48 (4)	0.23±0.43 (3)	0.53±0.96 (7)
Mines with dead larvae	0.07±0.27 (1)	0.61±1.12 (8)	0.30±0.48 (4)	0.23±0.43 (3)	0.23±0.76 (5)

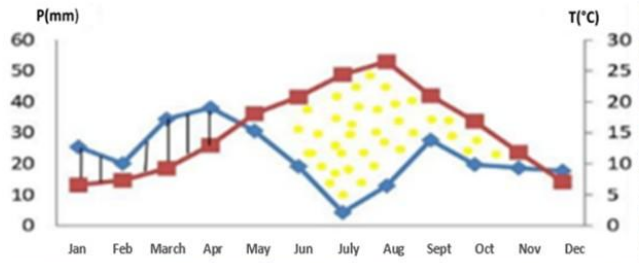
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719 **Table 5.** Damage rate of tomatoes harvested by *P. absoluta* holes and with other holes under
720 exposition to different treatments (T1, T2, T3) and T+ and T- and then tomato yield:
721 nonconsumable and consumable.
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Date of harvest	Total number of tomatoes	Damage rate by *P. absoluta holes*			Damage rate with other holes			Tomato yield:non consumable	Tomato yield:consumable
		Treatment	T+	T-	Treatment	T+	T-		
02/12/2024	50	7.69 % (2 : 26)	0% (0 : 1)	0% (0 : 9)	15.38 % (4 : 26)	0% (0 :15)	0% (0 : 9)	4% (2 : 50)	96% (48 : 50)
12/12/2024	45	0% (0 : 22)	0% (0 : 11)	8.33 % (1 : 12)	31,81% (7 : 22)	0% (0 : 11)	0% (0 : 12)	0% (0 : 45)	100% (45 : 45)
22/12/2024	175	0% (0 : 110)	0% (0 :35)	10% (3 : 30)	13.63 % (15 : 110)	0% (0 : 35)	6.66 % 2 : (30)	4% (7 : 175)	96% (168 : 175)
30/12/2024	182	4.8 % (6 : 125)	0% (0 : 40)	0% (0 : 17)	7.2% (9 : 125)	0% (0 : 40)	0% (0 : 17)	0% (0 : 182)	100% (182 : 182)

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Plants sampling sites	Pick up	Plants taxonomy
Site P1:(35°38'38.28"N,6°46'25.65"E) **Located:**9FM7+G2PFOUM TOUB(Tibikawine)	→	**Order:** Lamiales **Family:**Lamiaceae **Scientific name:***Marrubium vulgare*
Site P2:(35°38'46,58"N,6°46'34,76"E) **Located:**9FM7+V99FOUMTOUB(Tibikawine)	→	**Order:** Lamiales **Family:**Lamiaceae **Scientific name:***Teucrium polium*
Site P3 :(35°38'46.13"N,6°46'34.22"E) **Located:**9FM7+R9V FOUM TOUB (Tibikawine)	→	**Order:**Malpighiales **Family:**Euphorbiaceae **Scientific name:***Euphorbia Sp.*
Site P4:(35°36'74.46"N,6°46'33.41"E) **Located:**9F87+X8GFOUMTOUB(Tibikawine)	→	**Order:**Ranunculales **Family:**Ranunculaceae **Scientific name:***Clematis flammula*
Site P5:(35°36'76.00"N,6°46'34.73"E) **Located :**9F97+29QFOUM TOUB(Tibikawine)	→	**Order:**Dipsacales **Family:**Caprifoliaceae **Scientific name:***Lonicera implexa*
Site P6 :(35°38'47.82"N,6°46'37.32"E) **Located:**9FM7+WF9 FOUM TOUB (Tibikawine)	→	**Order:**Asterales **Family:**Asteraceae **Scientific name:***Hertia cheirifolia*

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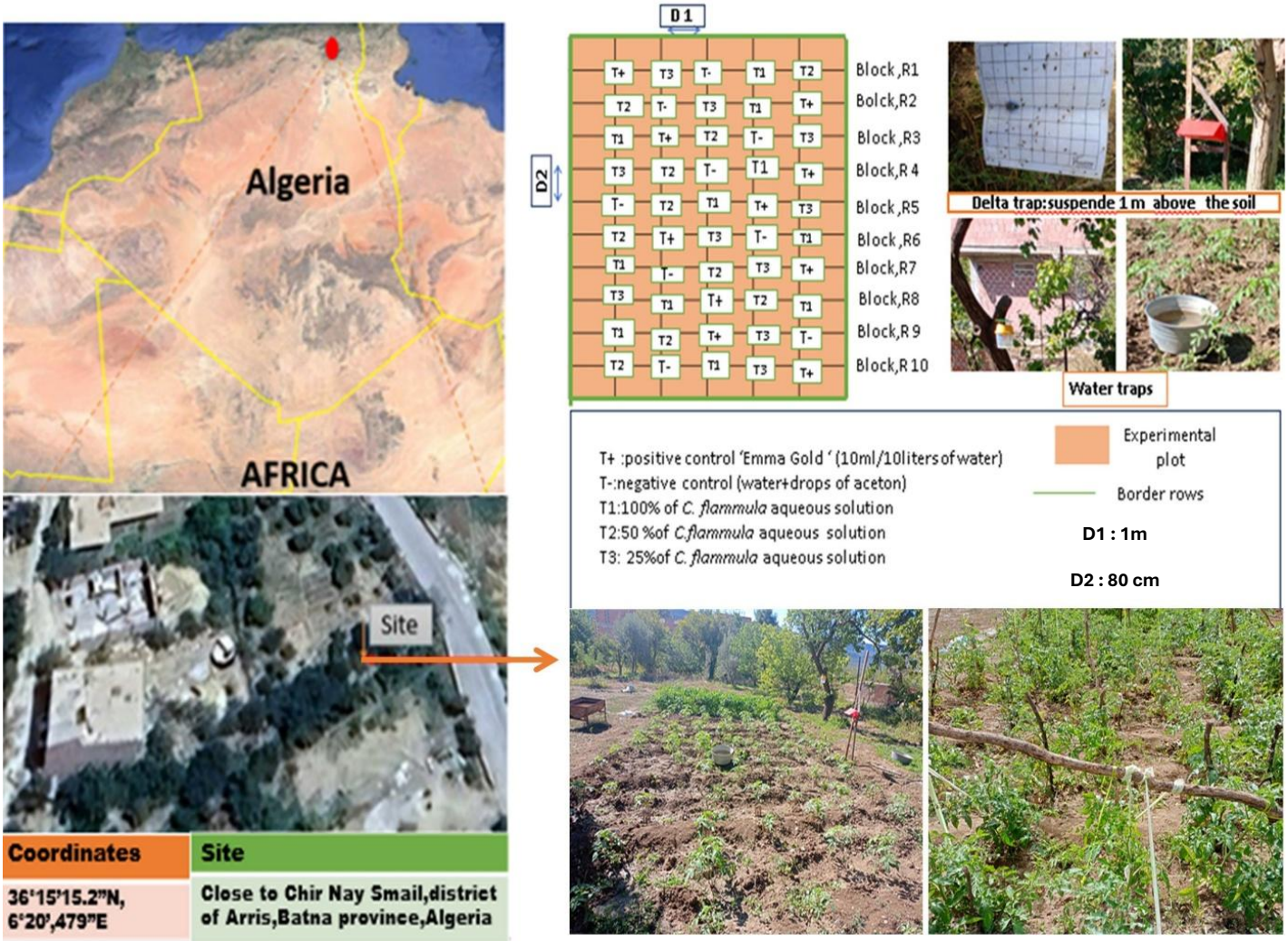
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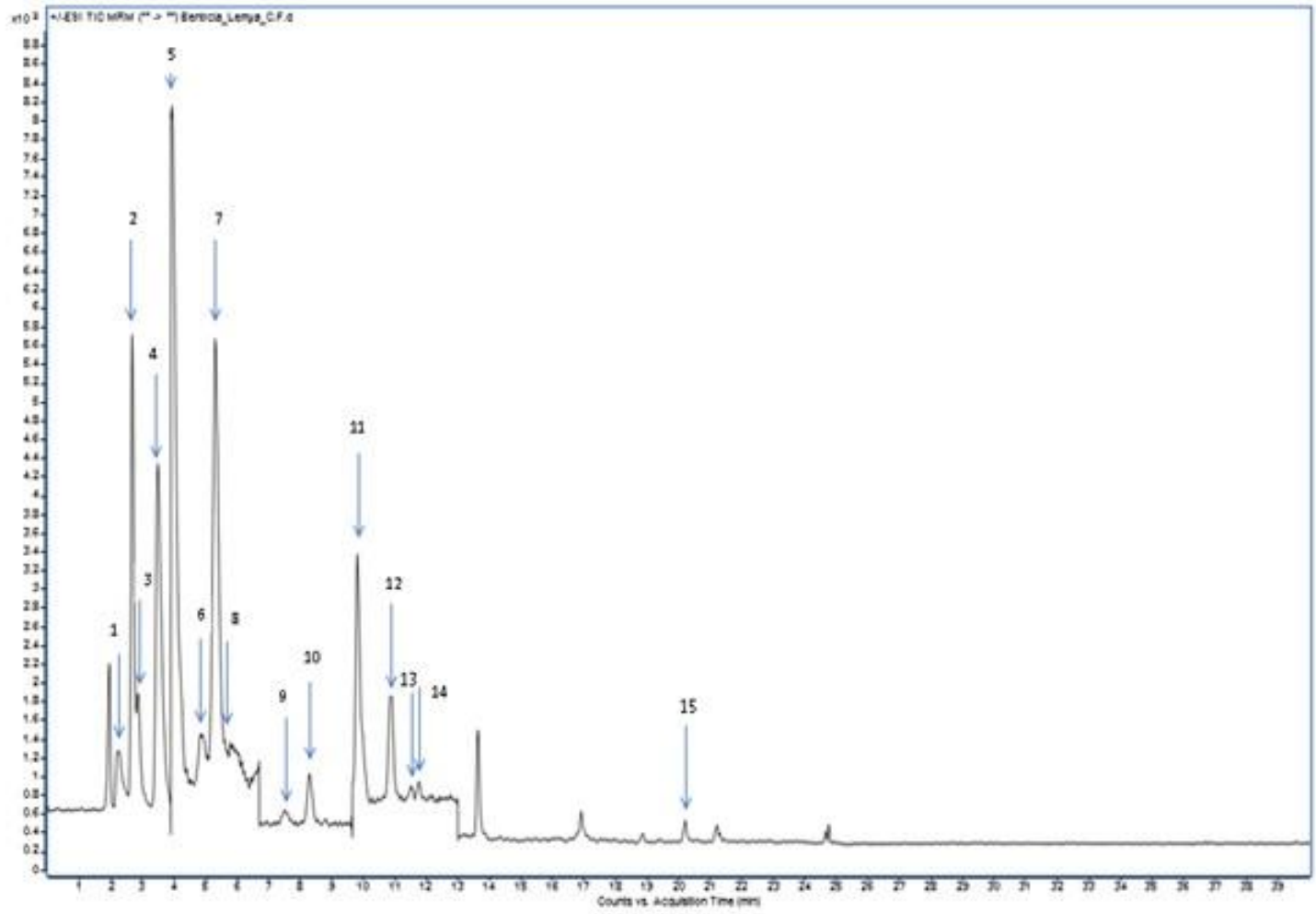
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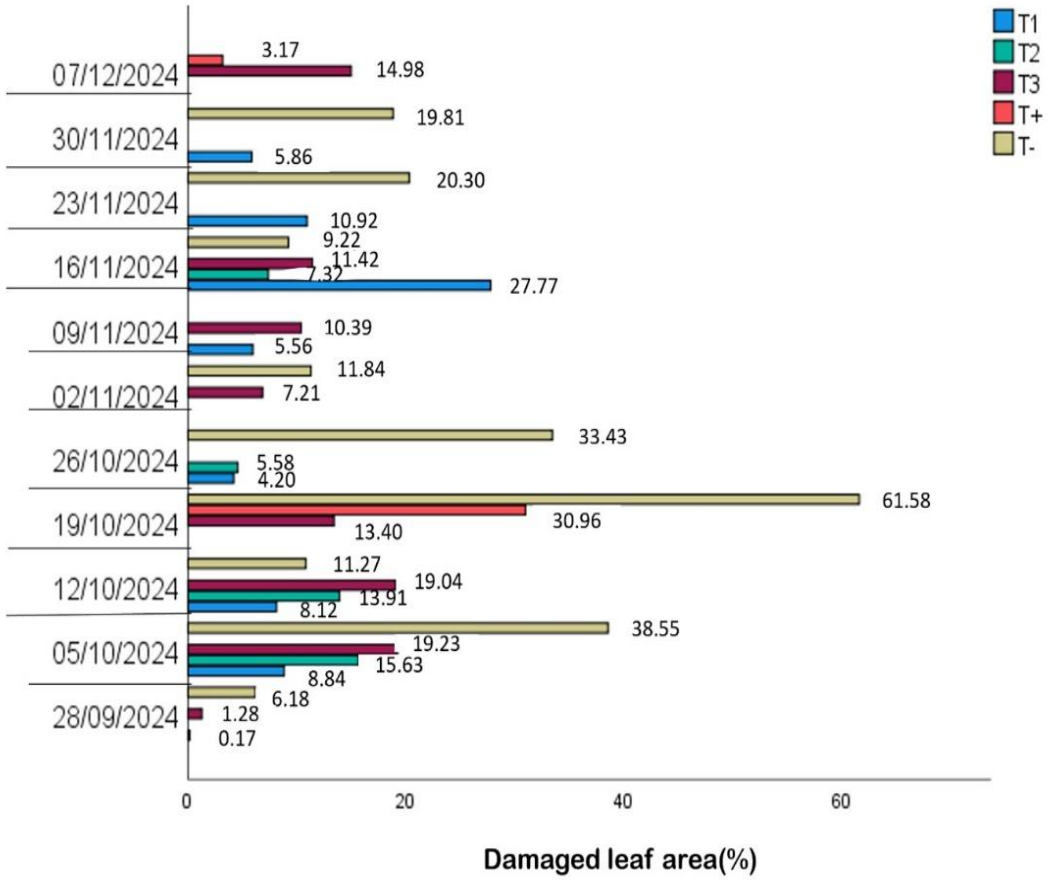
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Counts vs. Acquisition Time (min)


T1
T2
T3
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07/12/2024
30/11/2024
23/11/2024
16/11/2024
09/11/2024
02/11/2024
26/10/2024
19/10/2024
12/10/2024
05/10/2024
28/09/2024
3.17
14.98
19.81
5.86
20.30
10.92
9.22
11.42
7.32
27.77
10.39
5.56
11.84
7.21
33.43
5.58
4.20
61.58
30.96
13.40
11.27
19.04
13.91
8.12
38.55
19.23
15.63
8.84
6.18
1.28
0.17
0
20
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Damaged leaf area(%)