



**USE OF PLANT BIOCIDAL SUBSTANCES BASED ON CRATAEVA
RELIGIOSA AGAINST TUTA ABSOLUTA (LEPIDOPTERA:
GELICHIIDAE) DEVASTATOR OF TOMATO CROPS**



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Abstract:

*The tomato leaf miner, Tuta absoluta causes serious losses in production. It can feed on all the green parts of the tomato and can cause up to 100% damage if not controlled. Presently, the most effective method of pest control is the use of chemicals. The purpose of this study is to give alternative solutions based on natural products. The objective was to know the biocidal power of the aqueous extracts of Crataevareligiosa leaves on T. absoluta. The work was conducted on a field at the Niayes area. Three formulations were used: formulation with dry leaves (200g/l, 150g/l and 100g/l), formulation with fresh leaves (200g/l) and soapy formulation (100g Crataeva + 25g soapy) and two controls (C0, S). Each treatment was repeated twice as well as the controls. The results showed a significant decrease ($P < 0.0001$) of the larvae compared to the blank control. However, the fresh formulation (200g/l) had double effect by reducing the number of pest larvae and a positive effect on number of the predator *N. tenuis* in addition to a positive impact on the agronomic traits. The dry formulation, which certainly reduced the *T. absoluta* larvae, had no impact on *N. tenuis* and *H. armigera*.*

Keywords: *Tuta Absoluta; Biocides Extract; Crataevareligiosa.*

Cite This Article: Aissatou Raky BA, Toffène DIOME, Cheikh Abdou Khadre Mbacké DIA, Mbacké SEMBENE. (2019). "USE OF PLANT BIOCIDAL SUBSTANCES BASED ON CRATAEVARELIGIOSA AGAINST TUTAABSOLUTA (LEPIDOPTERA: GELICHIIDAE) DEVASTATOR OF TOMATO CROPS." *International Journal of Engineering Technologies and Management Research*, 6(8), 63-71. DOI: <https://doi.org/10.29121/ijetmr.v6.i8.2019.442>.

1. Introduction

Tomato speculation takes capital importance given its role in the agri-food industry and local consumption [1]. Which explains the rapid increase in world production over the last decade. However, this production is unevenly distributed: Asia ranks first, followed by Europe, then America and Africa [2]. In Senegal, the tomato is the second market gardening speculative, its production for the industry is estimated at 80 000 tonnes [3]. However, under greenhouse and in the field its production is slowed by the pressure of pests and pathogens that degrade the quality. Tomato moth *Helicoverpa armigera* and tomato leafminer *Tuta absoluta* are the main pests of tomato in Senegal. The latter, discovered for the first time in Senegal in 2012, is considered the pest that causes the most damage to tomato crops. The damage can lead to abandonment of plots

by farmers [4]. Therefore, different strategies of struggle have been developed to reduce the damage. The main current means of combating these pests is essentially chemical control. Moreover, in view of their harmful effects on humans and the environment, in addition to the selection of resistant populations, the search for alternative solutions is needed [5]. The purpose of this study is to quantify the biocidal power of aqueous extracts of *Crataevareligiosa* leaves on tomato leafminer *T. absoluta*.

2. Materials and Methods

Study Site

This study is conducted in the Niayes area, which is an agro-ecological zone of major importance in the economy of Senegal. It is characterized by these physical conditions: mild and humid climate, shallow water table depth, typology of soils, favorable to agro-pastoral activities. Administratively, it covers part of the regions of Saint-Louis and Louga, the departments of Tivaouane and Thiès and part of Dakar. It is formed by series of dunes that alternate with interdune basins [6]. The climate is tropical sub-Canarian dominated by the maritime trade wind, derived from Azores antantone. The water resources of the Niayes come mainly from groundwater and surface water [6; 7].

Biocidal Substance Extraction

After harvest, the leaves were crushed with mixer (fresh formulation) or dried for a dozen days in the shade and crushed and the powder stored in glass jars (dry formulation). To prepare the extracts of the dry formulation, 200g of leaves were macerated in 1 liter of water for the C1 concentration. The mixture was filtered using a sieve and a tissue. The same process was used to obtain C2 and C3 concentrations with respectively 150g and 100g of *C. religiosa* leaf powder. For the fresh formulation the only tested concentration was prepared with 200g of fresh crushed leaves and put in infusion in 1 liter of water. The soapy formulation was composed of 100g of dry leaves added to 25g of soap in 1 liter of water. The control was not treated while the soap control was treated with water and 25g of soap.

Cultural Techniques

To set up the culture, a plot of 28m² was delimited and divided into 2 blocks spaced 2,6m apart. The latter consisted of 14 elementary plots (7 plots per block) with an area of 1.69m² each. The elementary plot (1.30m × 1.10m) carried 4 tomato feet divided into two crop rows spaced 0.70 m apart. We obtained a total of 56 tomato plants. The distance between the elementary plots was 0.40 m. The name of the applied treatment is assigned to each parcel.

The nursery was set up on a floor laid out three days before. The area covered by the nursery was 3m² (2m * 1.5m). The soil has been cleared of old vegetation, stones and objects that may be an obstacle to the proper development of seedlings. Then it was plowed and then watered once a day. Streaks made on the ground allowed the tomato seeds to be placed at a depth of 2 cm and covered with soil. It was lifted 3 days after sowing. The transplanting soil, making 28m², was rooted, mixed with manure and then watered. Plants 15 to 25 cm high with 3 to 6 leaves were transplanted by hand. Each plot received 4 tomato plants spaced 0.70m apart.

Phytosanitary Treatment

The plots received aqueous treatments fifteen days after transplanting. The treatment was by spraying at different concentrations and formulations. The different doses of plant extract were sprayed one after another, the spraying of a dose was followed by a cleaning of the material used for handling. The jets were oriented so as to cover the lower and upper faces of the leaves of each plant to reach a large population of pests. The control plot received no treatment, it was simply watered.

Studied Settings

In order to evaluate the biocidal effect of leaf extracts, surveys were conducted throughout the crop. These were carried out before the application of the treatments and three days later on the whole of the feet of tomatoes. Surveys included the count of insects according to the three phenological stages, the number of tunnels dug and the number of plants attacked. The damaged fruits and the marketable fruits were weighed according to the treatment. These observations made it possible to determine several parameters: Abundance of the larvae according to the concentrations which corresponds to the number of individuals recorded for a taxon; frequency of occurrence: which is equal to the number of surveys where the insect is present in the total number of surveys; the weight of the fruits according to the concentrations (a summation of the weight of the fruits of each treatment on the different crops was carried out to have the total weight). The incidence of attacks: it was determined by the following formula: $I = PA / PT * 100$; I% = percentage incidence; PA = number of plants attacked; PT = total number of plants.

Statistical Analysis

Data processing was performed using Microsoft Excel 2013 spreadsheet to enter data and plot histograms and R version 3.4.4 software for statistical testing. To evaluate the effect of treatment, the Kruskalwallis test was used, the analysis focused on the average insect population observed on the plants after spraying. The Student-Newman-Keuls (S-N-K) test was used for a two-to-two comparison of the modalities. A correlation test was performed between the number of damaged fruits and the presence of pests. Before any analysis, the Shapiro-Wilk normality test was applied to our data. The difference between two values is considered significant when the p-value is less than 5% ($p < 0.05$).

3. Results and Discussions

Result

Inventory of Insects Present

The entomological fauna encountered (22 species) during the establishment of the tomato crop is divided essentially into 5 orders: *Lepidoptera*, *Hymenoptera*, *Diptera*, *Hemiptera* and *Orthoptera*. The majority of pests belong to the order *Lepidoptera*. These insects listed during our test were manifested according to the phenological stage of the plant. In the nursery, only *Liriomyza* was present and digging mines on some leaves and this all the period of the culture. The pests of the families of the noctuids and plutellids appeared in the fructification and maturation of the fruits. It should be noted that for the species *Plutellaxyllostella*, adults were the most frequent. The *T. absoluta* pest was encountered one month after transplanting from the sixth survey (31/12/2017) corresponding to the leafing-flowering period. The predator *Nesidiocoris tenuis* and spiders were

found in the right plots after transplanting. At the senescence of the tomato plants appeared lastly the red spiders.

Incidence of *Tutaabsoluta* Larvae on Tomato Crops

For our study, the incidence corresponds to the proportion of plants with at least one gallery. Thus, IT plots and C1 plots had the highest incidences of 100% and 87.5%, respectively. In contrast to S and SC where only 25% of the plants had at least one gallery of the tomato leafminer. For plots C2 and FF, the incidence was 37.5% while for C3 half of the plants had *T. absolutagalleries*. It should be noted that at the beginning of the test the leafminer was not present on the crop and over time it settled. Its damage multiplied and was mainly located on the plot TI.

Effects of Treatments n *Tutaabsoluta* Larvae

The number of larvae present on the plots differs very significantly according to the treatment assigned ($P < 0.0001$). There is a strong presence of tomato leafminer larvae on the TI and C0 plots compared to the C2, C3 and SC plots with relatively low values (see Figure 8). In addition, no larvae were recorded on the S plots during the entire period of the test, despite the presence of galleries. However, comparisons of averages have highlighted significant differences. Thus, the TI plots had a significantly higher number of larvae compared to the other plots. Plots C1, FF and SC showed no significant differences ($P > 0.05$) between them, as did plots C3, C2, and S. On the other hand, they had a significantly lower number of larvae ($P < 0.001$) to control plots C0. The significant difference is represented in the figure by letters (Figure 1).

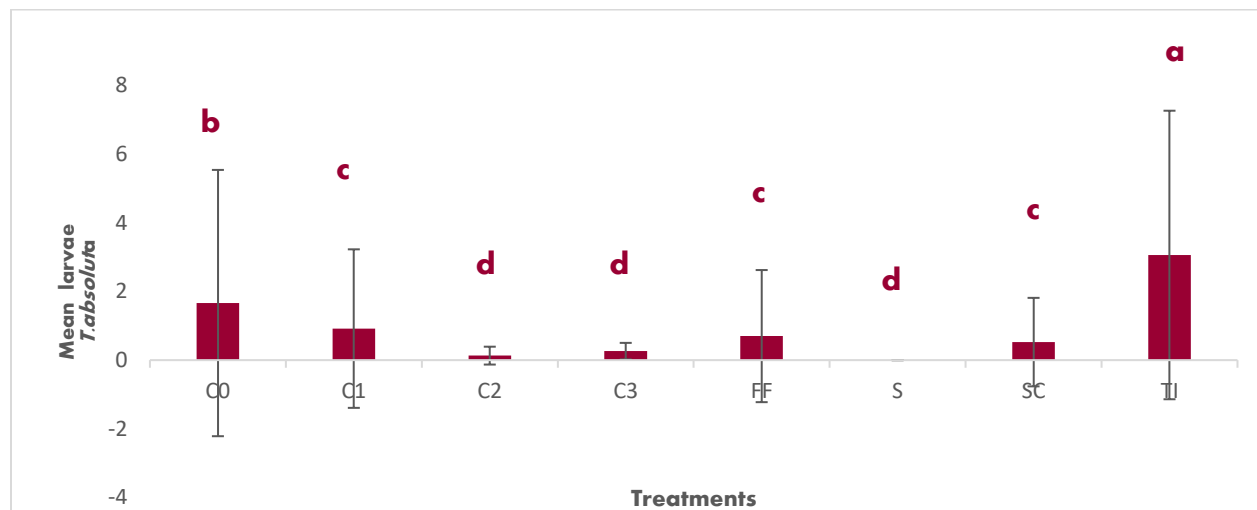


Figure 1: Mean of *Tutaabsoluta* larvae present on the plots according to the treatments attributed.

Distribution of the *Predatornesidiocoris Tenuis* According to the Treatments

The predator of *T. absoluta* was present on all plots. Nevertheless, the presence was rather heterogeneous with a larger number of *N. tenuis* (62) on the FF plots. The lowest value was recorded on IT plots with 2 individuals, followed by C2 and C1 with 8 and 10 individuals, respectively. Plots C0, C3 and S had a number of *N. tenuis* quite similar. The comparison test of the means showed a significant variation ($P < 0.0001$) in the number of *N. tenuis* according to the treatment attributed. The significant difference is represented in the figure by letters (Figure 2)

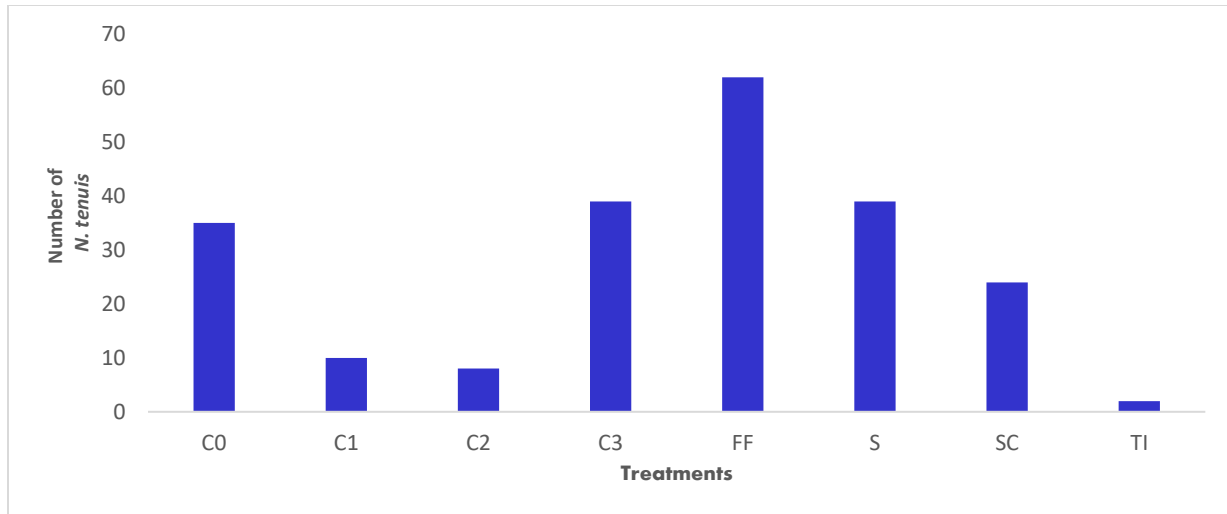


Figure 2: Number of individuals of the predator *N. tenuis* according to treatments attributed.

Comparison Between 200g / L Formulations

Plots C1 and FF were treated with 200g / l of aqueous extracts of *C. religiosa* leaves respectively dry in maceration and fresh in infusion. However, they do not present the same evolution. In fact, the incidence of the leafminer was higher in C1 (87.5%) than in FF (37.5%). The FF plots harbored a larger number ($P < 0.001$) of *N. tenuis*. In contrast, the variation of *T. absoluta* larvae was not significantly different ($P > 0.05$). In addition, the weight of the harvest for the plots treated with the aqueous extract of fresh leaves was estimated at 16.5 kg while the plots treated with aqueous extracts of dry leaves had a weight of 4.5 kg which is close to a quarter of the total harvest plots FF.

Comparison Between Formulations At 100g / L

Plots treated with 100g / l dry leaves of *C. religiosa* (C3) had an estimated leafminer incidence of 50%. On the other hand, it was 25% for plots treated with 100g / l of dry *C. religiosa* leaves supplemented with 25g of soap (SC). It is the same for the weight of the harvest which was 13,5kg for the parcels SC and of 5,5kg for C3. In addition, the number of *N. tenuis* was significantly higher on the C3 plots as opposed to the number of *T. absoluta* which was significantly lower ($P < 0.001$) on the C3 treated plots. The evolution of the pest and its predator were inversely proportional on these plots.

Impact of Aqueous Extracts on the Tomato Crop

The weighing carried out after each harvest made it possible to estimate the weight of the whole crop at 80,2kg. In addition, weighing was done by plots. Thus, the smallest crop was obtained on plots treated with 150g / l of dry leaves (C2) with a value of 3.5kg. For the control plots, a total weight of 6kg was recorded. It is superior in absolute value to the weight of plots C1, C2 and C3 respectively 4.5kg, 3.5kg and 5.5kg which are substantially equal to the weight of C3. Compared to the harvest from the control plots, FF, SC, S harvests are significantly higher at 16.5kg, 13.5kg and 15.7kg respectively (Figure 3). The correlation between the mean *T. absoluta* larvae and the damaged fruits was not significant $r = 0.014$; $P = 0.609$. Nevertheless, the test was significant and positive between the average larvae of *H. armigera* and the damaged fruits $r = 0.099$; $P = 0.0003$.

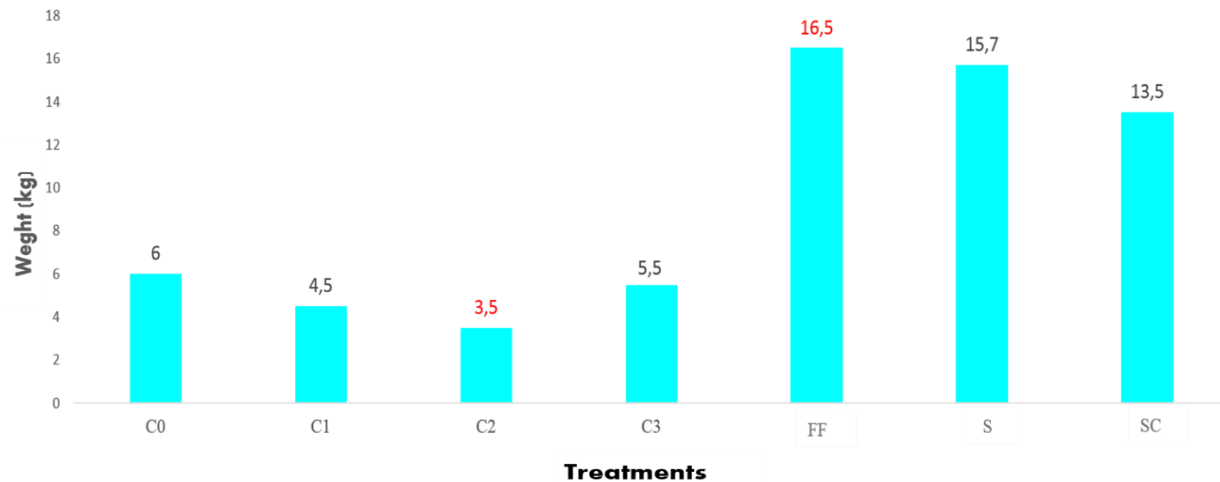


Figure 3: Total weight of fruits according to the treatments attributed.

4. Discussion

At the end of the inventory, insects were listed on the tomato crop: 22 species divided into 10 orders and 15 families. The analysis of these results reveals a very varied fauna in the culture site. A wide range of pests, mainly belonging to the order Lepidoptera and Hemiptera, can be distinguished. Added to this is a very marked presence of auxiliaries, especially predators such as *N. tenuis* and spiders. These results on entomological biodiversity are in line with the observations of Mochiah *et al.* [8] which stipulates that the application of plant extracts helps maintain an ecological balance between pests and auxiliaries. In addition, the population of the latter was greater on the tomato plots treated with the biocidal substance compared to the insecticide control plots. Similar results were obtained by Amoabeng *et al.* [9] which, according to them, populations of *Coccinellamagnifica*, *Episyrphusbalteatus* and predatory spiders were greater in plots treated with plant extracts than in those treated with synthetic insecticides. The pest *T. absoluta* appeared on the plots one month after transplanting corresponding to the end of December. Nevertheless, the number of larvae and the incidence were rather low until early February. Similarly, the work of Syllaet *al.* [10] showed that the incidence of the leafminer was very low at the beginning of the dry season (October to January). This same conclusion is obtained with the study of Allacheet *al.* [11] in Egypt, which according to them populations of *T. absoluta* had a small population during this period. The rarity of the preferred host plant of the pest during the rainy period remains until then the cause advanced by previous research. Indeed during the rainy season the weather conditions do not allow the establishment of the tomato crop; which leads to a drastic decrease in the population of *T. absoluta*.

In spite of the period the damage of the leafminer was very important on the tomato plots. In fact, the incidence was maximal (100%) for the untreated control plots and those treated with synthetic insecticides. This value of the incidence noted on the latter can be justified by the endophytic behavior of the larvae making the use of synthetic products difficult [1]. For plots treated with aqueous extract from dry leaves the highest incidence (87.5%) was noted on C1 and the lowest (37.5%) on C2. To this is added a mean larvae of C2 plots significantly smaller ($p < 0.001$) than those of C1. It follows that the C2 concentration of the dry leaf formulation appears to be effective for the reduction of *T. absoluta* larvae. In addition, the results on the number of *N. tenuis* predators

did not show any significance between C1 and C2 but the C3 plots had a significantly higher number of *N. tenuis* compared to the first two. At equal concentration, the fresh leaf formulation and the dry leaf formulation did not differ significantly in the larval average of the *T. absoluta* pest. However, the proportion of plants with at least one gallery (incidence) was much greater on the C1 plots (87.5%) than on the FF plots (37.5%). This difference noted on the damage caused by the leafminer could be explained by a strong presence of the predator in the FF plots (see Figure 1). Indeed, the predatory bug *N. tenuis* is likely to reduce the populations of the pest, this with 97% of infestation on leaves and 100% on the fruits [12]. According to Arno et al. [13], adults of *N. tenuis* are able to consume more than 100 eggs of *T. absoluta* per day. In addition, the study by Sylla et al. [14] in Senegal reveals that *N. tenuis* can consume different stages of the *T. absoluta* pest with a preference for eggs.

In addition, the plots treated with the aqueous extract of dry leaves at 100 g / l supplemented with 25 g of soap (SC) had an incidence of 25%, equal to that of the soap control and lower than C3 (37.5%). However, a significant difference was noted in the mean larvae of *T. absoluta* and *N. tenuis* between the soap control S and the treated plots SC respectively 0.00 ± 0.00 and 0.52 ± 1.27 for *T. absoluta*. It emerges that the soap, initially used as wetting by market gardeners in the Niayesarea, seems to have a biocidal effect on the larvae of the leafminer. Indeed, the treatment was sprayed on the tomato leaves while the leafminer feeds them by digging galleries. The number of larvae and the rather low incidence would probably result from the death of the larvae following the consumption of the treated leaves. These results are in line with the work of Taboneet al. [15] who recommends the use of soap as a means of physical control against *T. absoluta*.

Mean pest larvae on the plots treated with the aqueous *C. religiosa* extract were significantly ($P < 0.0001$) lower than the C0 control plots. This could indicate a probable reduction in the number of larvae by treatment with *C. religiosa* leaf extract. Previous studies have shown the effectiveness of these extracts on insect pests of stored commodities [16; 17] despite the fact that no studies have been done on the insects of vegetable crops. Nevertheless, in Niger the neem extract used on tomato plants was more effective than the synthetic insecticides on the management of the *T. absoluta* pest [18]. Similarly, Kona et al. [19], Coelho and Deschamps [20] revealed a sensitivity of *T. absoluta* larvae to neem extracts. In addition, these averages were also lower than those of the insecticide treated plots (3.06 ± 0.13). This could be attributed to the irregularity of treatments on these IT parcels. In fact, during the growing season, the market gardener who had set up the crop had travelled by delegating the interview to another, and unfortunately the latter only performed treatments once a month. Insecticide resistance and the endophytic nature of the larvae may also be an explanatory factor [1; 19; 21]; since these products were very harmful for the other insects encountered, especially for *N. tenuis*.

The analysis of the results on the agronomic data reveals that the total weight obtained differs according to the treatment applied. Thus, the FF plots had the greatest weight value, followed by the S and SC plots, while all the plots treated with the dried leaf extracts had a very low weight and less than the C0 plots. This observation could be explained by the damage that tomatoes undergo on plots C1, C2 and C3. A correlation test made between the number of fruits attacked and the presence of pest larvae ($r = 0.099$, $P = 0.0003$ for *H. armigera* and $r = 0.014$, $P = 0.609$ for *T. absoluta*) revealed that the damage were largely due to *H. armigera* pest. This could also be explained by a possible effect of the fresh formulation on *H. armigera* larvae. In addition, the soap,

which seems to have a biocidal effect, could have reduced the damage due to its larvae. Especially since the work of Traoré et al. [22], showed the effectiveness of plant extracts on the tomato moth.

5. Conclusions and Recommendations

The resistance to insecticides observed on the *T. absoluta* pest, combined with the harmful effects of these synthetic products on man and the environment, imply the adoption of new methods. The study generally quantified the biocidal effect of *C. religiosa* leaf extracts on *T. absoluta* tomato leafminer. Thus the tests revealed an effectiveness of the aqueous extracts of *C. religiosa* on *T. absoluta* larvae. Several formulations have been used but the most promising is the formulation fresh leaves that not only reduces the larvae of the pest, but has a negligible effect on the predator *N. tenuis* and contributes to the increase in the quantity of production. In addition, our work shed light on the real utility of soap that market gardeners used as wetting for plant extracts they use in the protection of their crops. In fact, soap is harmful to pests. Nevertheless, it should be noted that the cultivation period had a positive effect on the results obtained since it did not correspond to a period of pest outbreak, hence the interest of integrated pest management. This is where our study proposes alternative solutions based on the use of natural products "biopesticide", to fight against the tomato leafminer, *T. absoluta* is considered a serious threat to tomato production. It is also necessary to make market gardeners aware of the importance of the tomato growing season and the real usefulness of the soap. In view of these results it would be relevant to increase the surface and spread the tests in open fields and laboratories over time with different doses of the fresh formulation to confirm our results and determine the optimal dose for a better efficiency of aqueous extracts.

Acknowledgements

I thank the Genetics Team for Population Management for monitoring and supervising the work.

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