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STUDY OF THELARVICIDAL ACTIVITYOFHYDRO-ALCOHOLICEXTRACTS*NERIUMOLEANDER* L.AND*RICINUSCOMMUNIS* L. ON*TUTA ABSOLUTA*M.

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Abstract

The use of synthetic pesticides is now in frequent use in crop protection. But they are considered as a major problem on the environment, the flora and fauna or human health. Biopesticides of botanical origin are a good alternative to remedy this evil. Our study aimed at assessing the toxicity of hydro-methanoic, hydro-ethanoic and synergistic extracts of two toxic plants, including *Nerium oleander* and *Ricinuscommunis* growing spontaneously in Mostaganem area, on larvae of the tomato leafminer*T. absoluta* (Meyrick), which is considered as a serious pest on tomato crop in Algeria. The extraction yields (Method of Soxhlet) obtained by the twohydro-methanoicandhydro-ethanolicextracts are (26%, 18%) for *N. oleander* and (35%, 29%) for *R. communis* respectively. However, the hydro-methanoic extract remains the most effective. These extracts were tested adopting the method of toxicitybydirectcontact orspray". The biological tests on the larvae of *T. absoluta*have shown that there is a difference between the toxicity of hydro-méthanoic and hydro-ethanolic extracts within the same plant. The synergistic extract caused a remarkable mortality rate that exceeded 90%. *N. oleander*et*R. communis*presented a low toxicity not exceeding 30% for the hydro-ethanolic extracts and 40% for the hydro- methanoic extracts.After 24hours of exposure to the test,theLD50 values werevery high (above 50%) for hydro-ethanolicandhydro-méthanoicextracts for the two plants used. However, theLD50 values wererelatively low forthesynergisticextract(19%).The results showedthatL2andL1larvaeare most sensitiveto allextracts used. TheL3andL4are the most resistant, exceptforthesynergistic extract.

Key words: *Bioinsecticides-Tutaabsoluta- Nerium oleander- Ricinuscommunis-Toxicity-Hydro-alcoholic*.

Introduction

The tomato borer *Tutaabsoluta* (Meyrick) (Lepidoptera: Gelichiidae), a key pest of tomato invaded Algeria in March 2008, It is now devastating pest in tomato crops in Mediterranean basin and Maghreb. According to Gilardon*et al*. (2001) and Tropea*et al*. (2012), in most countries where *T. absoluta* occurs, the main control strategy consists of frequent sprays with chemical insecticides, because without control *T. absoluta* causes yield losses up to 100% and dramatically decreases fruit quality in both field and in greenhouse tomato crops.Both control *T. absoluta* population and care ecosystem, natural products are increasingly required for sustainable agriculture, the indiscriminate use of conventional synthetic pesticides have had a negative ecological and health impact (pest resistance, contamination of environment and ecosystems, loss of biodiversity, etc.).

The use of vegetable kingdom and molecule that allowed the plants to protect against natural enemies becomes indispensable (Regnault-Roger, *et al*., 2008; Lacava and Azevedo, 2014). Plant derived insecticides and insect hormones are receiving significant interest as alternatives

to chemical pesticides and key components of integrated pest managements systems, which are cheaper, safer, and ecofriendly (Attathom, 2002; Adedire and Ajayi, 1996 in Adedire and Akinkurolere, 2005). We can cite as active elements of plants: phenols, flavonoids, tannins, anthocyanins, saponins, vitamins, and minerals glucosides (Chevallier, 2001).

Material and Methods

To improve the bio-insecticide effect of the natural plants substances, we have chosen two plants known for their toxicity and high content of active ingredients: oleander (*Nerium oleander* L.) and Ricin (*Ricinuscommunis* L.) in order to use it to control larval population of *T. absoluta* on tomato crop and to secure the production by reducing the damage caused by this pest; also for limiting the harmful effects of these on the environment and human Health. It is also important to encourage and promote the production of organic crops in Algeria. Other studies have highlighted the effectiveness of plant extracts on *T. absoluta*larvae (Da Cunha *et al*., 2008; Nilahyane*et al*., 2012; Moreno *et al*., 2011; De Brito *et al*., 2015).The larvae sensitivity of extracts varies from one plant to another.

a) Extraction Method (The Soxhlet Extractor)

We treated 20g of Ricin fresh leaves by ether petroleum, and then allowed to dry for 10 minutes at room temperature. After, the solute is introduced in a cartridge placed in Soxhlet. Extraction solvents used are: a hydro-methanoic mixture and hydro-ethanoic (Jordan *et al*., 2009).The flask contents (more solvent materials dissolve) are concentrated by rotary evaporator.

b) Larvicidal Activity (Test Contact)

The use of plant extracts to control *T. absoluta* larvae has been proposed by many authors. Moreira (2007) tested extracts of such toxic plants, *Datura stramonium* and *R. communis*.

In Algeria, the studies on the larvicidal activity of toxic plant extracts to the lepidopteran larvae are so limited. In this context we have been able to evaluate the larvicidal activity of the hydro-methanoic extracts and hydro-ethanoic of toxic plants (*N. oleander*, *R. communis*), and a synergistic extract.

The tests were carried out as of T°= 25±3°C, H°= 48±8% and natural photoperiod. *T. absoluta* larvae were levied by a brush and placed in airy Petri dishes containing agar;each box carrying a healthytomato's leaflets that serve as food support for the larvae. The concentrations of each extract (hydro-methanoic, hydro-ethanoic, and synergistic of two plants used) were tested: at 10, 20, and 30%, then the control treated with distilled water. The mortality rate (%) is determined for each treatment after 24h, 72h, 48h, 7 days, and 15 days after spraying. The larval mortality rate depending on time contact is estimated with Abbott's formula (Busvine, 1981).

Results and Discussion

Humidity Rate of Plant Material

Plants are rich in water, the analysis of the samples showed a high humidity, between 39 and 50%, which means approximately half weight of fresh plant is constituted by water.*N. oleander*leaves gave us an approximate weight to equivalent proportions of dry matter and water (50%). whereas*R. communis* is very rich in dry matter (61%) and only 39% water.The results show a variability of the water content between different species (Fig. 1).

Extraction Efficiency

Hydro-methanoic extract noted a higher yield compared to hydro-ethanoic. Then it was recorded higher yield for *R. communis* compared with *N. oleander* (Fig. 2). Mohadjerani

(2012) showed that aqueous methanol and pure methanol were the most effective solvents for the extraction of phenolic compounds from the leaves and flowers of *N. oleander* in Iran.
The difference between yields' extraction for the same plant is rated on the solvent used. The highest yield was observed by hydro-methanoic extracted with 26% and 35% for respectively *N. oleander* and *R. communis*, followed by hydro-ethanoic extract with 18% and 29% respectively for both plants tested (Fig. 2).

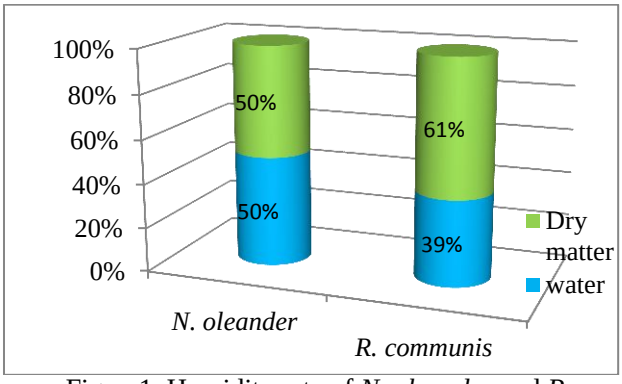

Figure1. Humidity rate of *N. oleander* and *R. communis*on *T.absoluta*

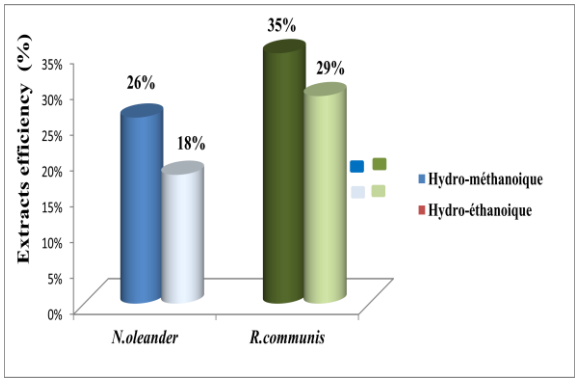

Figure2.Comparison of *N. oleander* and *R. communis*hydro-alcoholic extracts fresh leaves

Larvicidal Activity

T. absoluta larvae were exposed to different treatments of hydro-methanoic and hydro-ethanoic extractsfrom fresh leaves of *N. oleander* and *R. communis* at different periods: 24, 48, 72 hours, 7,and 15 days. The results showed that the same mortality was obtained after 24 hours which is explained by the larvicidal activity that is not related to the duration of exposure to treatment but by the action of extracts that have had an immediate effect. Moreover, the live larvae were able to continue their development after 24 hours to reach pupation. No mortality was recorded in all control groups we tried even after 15 days (the development of larval instars was not unaffected).

Larvicidal Activity of *N. oleander* Extracts of *T. absoluta*

The results presented in Figure 3, indicate that the two hydro-alcoholic extracts of *N. oleander* caused more or less significant larvicide efficiency compared to the control. This activity increases proportionally with the concentrations tested and it varies from one sample to another for the same plant.For both *N. oleander* extracts, larval mortality lower rate reached 50% even for the highest concentration (30%).
The LD50 (dose causing the death of 50% of *T. absoluta* population) have been very high for both extracts of *N. oleander*, which values are 54% and 75% for hydro-methanoic and hydro-ethanoic extracts respectively. And for even extracts the LD90 obtained are exceeded 100%. All these lethal doses explain the low toxicity of the extract of this plant. A positive correlation was obtained between the dosages of the extracts and mortality with a correlation coefficient of 0.933 and 0.9 respectively for the two extracts.
Furthermore, the Sankarasubramanian *et al*. (2007)tests carried out onto *Bipolarisoryzae* have revealed that, in vitro studies of Nerium oleander leaf extract, wasexerted a higher percent inhibition to mycelial growth (77.4%) and spore germination (80.3%).

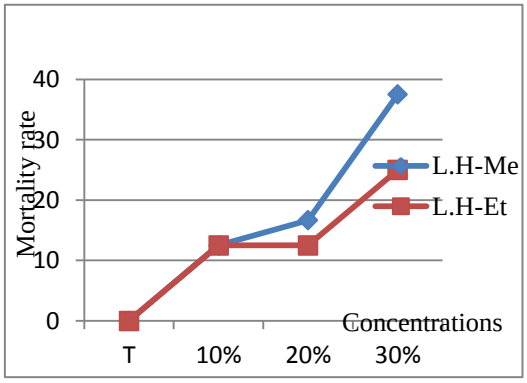

Figure3.Larvicidal activity of hydro-methanoic and hydro-ethanoic extracts of *N. oleander*

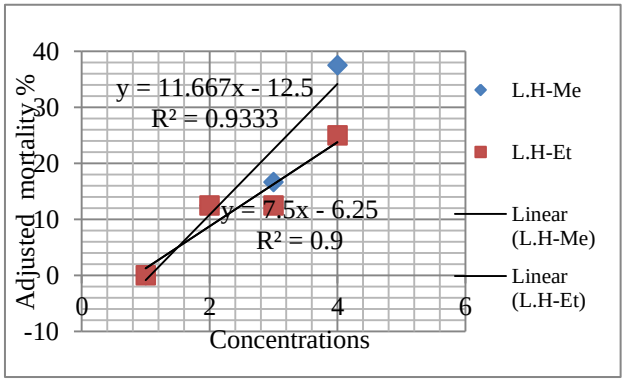

Figure 4. Mortality adjusted of hydro-methanoic and hydro-ethanoic extracts of *N. oleander*

Larvicidal Activity of *R. communis* Extracts on *T. absoluta*

The results shown in Figure 5, indicate that the two hydro-alcoholic extracts of *R. communis*illustrate a significant larvicidal efficacy compared to control. For both extracts of *R. communis* larval mortality reached rate below 50% even for the highest concentration (30%).The observations indicate that the hydro-methanoic extract has a higher mortality rate thanhydro-ethanolic extract.

LD50 values were very high for both hydro-alcoholic extracts of *R. communis*. LD50 values obtained are 51% and 63% respectively for hydro-methanoic and hydro-ethanoic extracts. While the LD90 obtained were very important. We recorded 86% and 100% rates respectively for the two extracts raised; this explains the low toxicity of these extracts. A positive correlation obtained between doses extracts and mortality with respective correlation coefficients of 0.956 and 0.968 for both extracts.

The synergistic extract caused remarkable mortality rate exceeded 90% mortality. While *N. oleander* and *R. communis* showed only low toxicity not exceeding 30% for hydro-ethanoic extracts and 40% for hydro-methanoic extracts (Fig. 7).

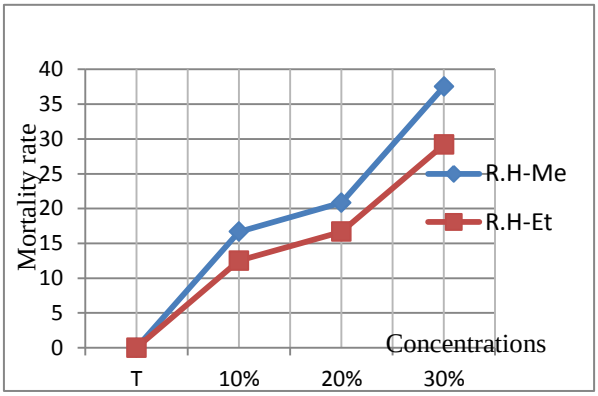

Figure5.Larvicidal activity of hydro-methanoic and hydro-ethanoic extract of *R. communis*on *T. absoluta*

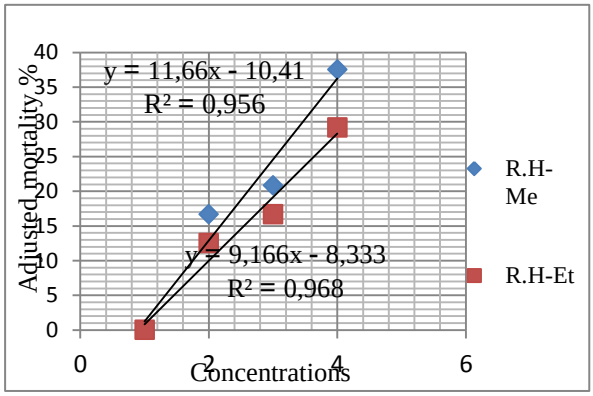

Figure6. Mortality adjusted of hydro-metanoic extracts of *R. communis*on *T. absoluta*

The results of comparison between all the extracts used report that the best rate of mortality is caused by the synergistic extract; in fact, by a concentration of 10% it's caused the death of 87% to *T. absoluta* larvae. While at the same concentration, the other extracts showed only a low efficiency about 12 and 17% for *N. oleander* and *R. comminus*.

The larvicidal activity of the extracts of *N. oleander* and *R. communis* remains low for both extracts even at 30% compared to the synergistic extract which presented a very high rate around 92% (Fig.7).

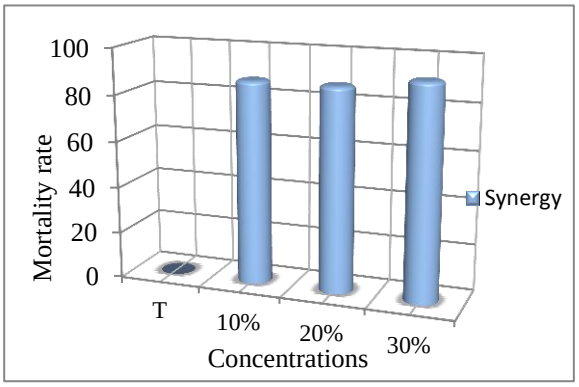

Figure 7. Evolution of the larvicidal synergistic extract activity on *T. absoluta*

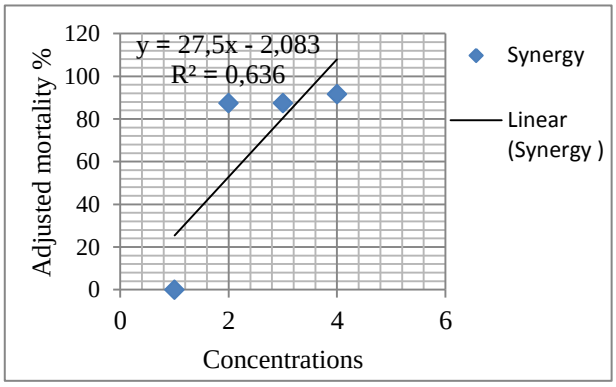

Figure 8. Mortality adjusted of synergetic extracts on *T. absoluta*

Table 1. Result of Lethal dose (50 and 90) for the different hydro-alcoholic extracts

Extracts / Dose	N.H-Me	N.H-Et	R.H-Me	R.H-Et	Synergy
LD50	54%	75%	51%	63%	19%
LD90	88%	128%	86%	107%	33%

According to the results shown in Table 1, we observed that the hydro-methanoic extracts and hydro-ethanoic of *R. comminus* and *N. oleander*, require doses greater than 50 and 86% to cause a death 50 and 90% respectively. While the synergistic extract requires a low dose of 19 and 33% for the respective mortality rates of 50 and 90%. These indicate the high toxicity of the product after mixing the two plants' methanolic extracts.

Larvicidal Activity of Hydro-Alcoholic Extracts on *T. absoluta* Larval Instars

During *in vivo* assay should be taken into account mortality in the general population. The best way for using our extracts in biological control, is to define in the first the sensitivity *in vitro* of each instars.

The effect of different hydro-alcoholic extracts of the two plants tested on *T. absoluta* biological instars showed a high sensitivity of the first two instars (L1 and L2). While L3, L4 were more resistant. The synergistic extract showed a very interesting effectiveness on the four larval instars, it has been enabled us to record a 100% mortality for L1 and L2 and a respective rate of 83 and 67% for L3and L4, for 10 and 20% of extract doses (Fig. 9-10).

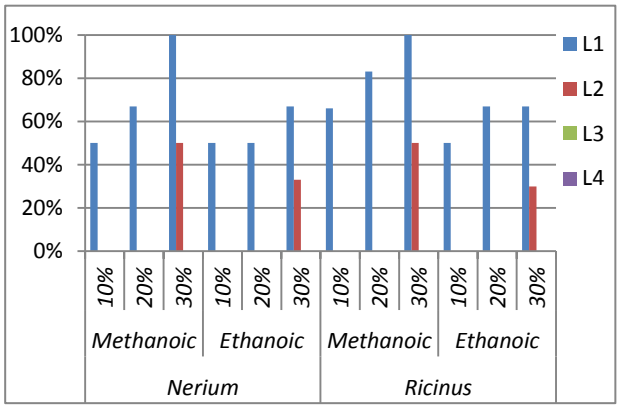

Figure 9.Larvicidal activity of hydro-alcoholic extracts on *T. absoluta* biological instars

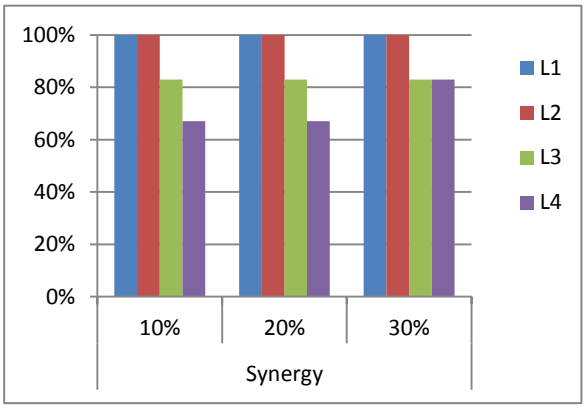

Figure 10.Larvicidal activity of synergistic extract on *T. absoluta* biological instars

Conclusion

The main objective of this work was to find an alternative to synthetic insecticides via insecticides solely of natural and organic compounds by testing the hydro-alcoholic extracts of medicinal plants. The tests were performed on tomato leafminer*Tutaabsoluta* in order to control its population, by extracts of medicinal plants: *Nerium oleander* and *Ricinuscommunis* and their synergistic extract. The results obtained during this research note that hydro-alcoholic extracts of the two plants show insecticidal activity of *T. absoluta*. Hydro-methanoic extract was more efficiencies than a hydro-ethanoic extract. Further, the synergistic extract showed greater results compared to Ricin and oleander; indeed, very significant mortality rates were recorded for the synergistic extract by the different doses tested, 10, 20, and 30%, when a respective percent mortality 87, 87, and 92% was registered. The *T. absoluta* larval instars have showed the highest sensitivity to different extracts on the two first larval instars namely the L1 and L2 with greater sensitivity to the effect of the synergistic extract, where a mortality rate of 100% was recorded. These results need to be deepened by the various polyphenolic's study compounds of different substrates used for a better assessment of the quality of the solute.

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