**Full Length Research Paper**

**Efficiency of the entomopathogenic fungus Verticillium lecanii in the biological control of Trialeurodes vaporariorum, (Homoptera: Aleyrodidae), a greenhouse culture pest**

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Our investigation in the region of Jijel revealed that whiteflies are the predominant greenhouses pests; they are polyphagous, moreover, some species can transmit many plant viruses. The treatment method is based on the systematic use of insecticides that have side effects on both the consumer and the farmer. The objective of this study was to evaluate the use of biological control in situ and in vitro as an alternative method by using an entomopathogenic fungus *Verticillium lecanii*. In vitro experiments showed that the fungus was active during all stages of development of the insect, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae): Eggs ($LD_{50} = 0.59 \times 10^7$ spores / ml) larvae ($LD_{50} = 0.5 \times 10^8$ spores / ml) and adults. Our results showed the influence of spore concentration, contact time and relative humidity on the development of the parasite to reach an efficient anti-larval effect of 100%.

**Key words:** Verticillium lecanii, Trialeurodes vaporariorum, entomopathogenic, alleyrodidae, whitefly, biological control.

**INTRODUCTION**

The green house aleurode *Trialeurodes vaporariorum* is a current polyphagous pest of leguminous cultures, for example, tomatoes, pepper, courgette, bean etc. (Vet et al., 1980; Van lenteren and Noldus, 1990). It was detected in 250 plants all around the world (Landa, 1994); it can multiply quickly and without interruption to many annual generations under greenhouse conditions. In parallel to its multiplication, sap puncture by different stages of development, virus transmission and secretion of honeydew on the lower face of leaves promote sooty mold development (Coffin and Coutts, 1995; Guzman, 1997; Mckee, 2007; Guevara-Coto et al., 2011). Thus, a depreciation of the product is seen, and a considerable decrease in photosynthesis making the leaves yellowing and falling (Dipietro, 1977).

In the region of Jijel, the control method used today against the green house aleurode is chemical; however, spraying chemical pesticides causes an increase of insecticide resistance, environmental pollution and can be toxic to natural enemies (Faria and Wraight, 2001; Liu et al., 2011), in addition to phytotoxicity problems and residual pesticides on legumes (Quinlan, 1988). Other control methods were proposed, including biological control by using biopesticides based on ascomycetes and hyphomycetes.

*Verticillium lecanii* which was reclassified by Zare and Gams (2001) as *Lecanicillium lecanii* is an important pathogen of insects, isolate from coccids, aphids and whiteflies (Cortez-Madrigal et al., 2003; Liu et al., 2011).

In the present work, we attempted to measure the efficiency of the microscopic fungus *V. lecanii* against the multiplication of *Trialeurodes vaporariorum*.

**MATERIALS AND METHODS**

**Sampling**

Samples of infected leaves with different larval stages of the aleurode (Figure 1) were collected from two agricultural regions: El Kennar (36° 49' 35" N/ 5° 57' 17" E, latitude 12 meter) and El Aouana (36° 46' 26" N/ 5° 37' 09" E, latitude 20 m) (Figure 2). They were put in plastic bags and conserved in the refrigerator. Larvae were used for species identification (Colles, 1958; Martin, 1987).
Figure 1. The whole white insect *T. vaporariorum* on the lower face of bean leaves in a greenhouse.

Figure 2. Regions where samples were collected.

**Spore germination and preparation of *V. lecanii* doses**

*V. lecanii* strain provided by the University of Kiev (Ukraine) was plated on Czapek–dox (CD) medium pooled in Petri dishes. After incubation at 25°C for 7 days, cultures were conserved in a freezer (-10°C). Frozen fungus spores were then collected and used to inoculate a CD agar plate and incubated at 23°C for 7 days. A volume of 10 ml distilled water supplemented with Tween 80 at 0.5% was pooled on the surface of the mycelium. The plate was slowly agitated and spore suspension was recovered, vigorously agitated by vortex and filtrated, spore count was carried out using a Malassez hematimetric cell. For every test, different concentrations were studied: 10⁵, 10⁶ and 10⁷ spores/ml. 0.1 ml of spore suspension (10⁴ spore /ml) was plated on agar (2%). The tests were realised in triplicate and the whole material was maintained at 23°C at a relative humidity (RH) of 100%. Spores of the germing fungus were counted by optical microscope every hour.
Insect culture

Two young bean plantations in the stage of the apparition of the two first leaves were infected by several aleurodes during 24 h in an insect cage (60x60x60 cm). Aleurodes were eliminated after setting their eggs on the lower face of the leaves, so that the obtained population of eggs, larvae and adults will be homogenous.

Eggs and stage II larvae treatment

Leaves were observed under a magnifying glass to localize the infected zones. 7 mm diameter discs of leaves were cut, each disc contains at least 2 to 20 eggs or stage II larvae. Discs were immersed during 10 s in a freshly prepared fungus spore suspension, and then 10 of these discs were placed on the surface of the agar 1.5%. The Experiment was carried out in triplicate. Controls were provided for each experiment. Incubations were done in desiccators at 20°C under a relative humidity of 100% with a photoperiod of 16 h per 24 h. Mortality percentage was determined from the 5th day of contact. LD50 were calculated according to the method of Probit (Bliss, 1935; Drummond et al., 1987).

Adults treatment

Adults of the same age were captured after cooling during 5 min in the refrigerator. They were then recovered and distributed in tubes to get 30 insects per tube. They were cooled again and put quickly in contact with a 7 days fungus culture. After 30 min of contact, spore infected adults were released in the desiccators each containing a host plant. Desiccators were adjusted to a relative humidity of 100% at 20°C and exposed to light during 16 h per 24 h in a phytotron incubator (Drummond et al., 1987; Ekborn, 1979).

Effect of relative humidity (RH)

Eggs and Stage II larvae were treated with a dose of about 1.5x10⁷ spores/ml. The whole (eggs/ larvae/ spores) were incubated at 20°C, first at 100% RH during 16 h and then at 70% RH during 4, 8, 12, 20 and 96 h. It was finally put at a RH of 100% during 7 days compared to control. For each case, the mortality rate was estimated after 7 days.

RESULTS

Species identification

Samples of infected leaves with different larval stages of the aleurode were collected from two agricultural regions (El Kennar and El Aouana) (Figure 2). Slides were prepared and examined by the optical microscope (Colles, 1958). Identification was done according to Martin (1987). Leaves samples analysis allowed noting the total predominance of *T. vaporariorum* species. The species exists on greenhouse cultures and even on other outside adventitious plants.

Spore germination

The spore germination starts since the first hour and reaches a rate of 65% after 9 h and 92% after 12 h of incubation (Figures 3 and 4).

Effect of fungal spore concentration on different development stages of *T. vaporariorum*

Effect on eggs

The percentages of mortality after treatment showed an increase of about 6.9, 14 and 61.8% for the three doses and contact times used. LD₅₀ were: 4.2x10⁷ spores/ml in day 5; 2.1x10⁷ spores/ml in day 6 and 0.59x10⁷ spores /ml in day 7 (Table 1). Thus, LD₅₀ in
Figure 4. Germination of *V. lecanii* spores after 24 h of incubation at 24°C and at a RH of 100%. G 40X0.75.

Table 1. Probit analysis of egg treatment.

<table>
<thead>
<tr>
<th>No. of insects (n)</th>
<th>Slope ± SE</th>
<th>LC (95%)</th>
<th>Chi-square</th>
<th>LD50</th>
</tr>
</thead>
<tbody>
<tr>
<td>788</td>
<td>0.69±0.10</td>
<td>(13.060-86.134)10^6</td>
<td>12.709</td>
<td>4.2x10^7</td>
</tr>
<tr>
<td>788</td>
<td>0.73±0.09</td>
<td>(93.573-37.621)10^6</td>
<td>15.929</td>
<td>2.1x10^7</td>
</tr>
<tr>
<td>788</td>
<td>1.02±0.10</td>
<td>(41.553-79.95)10^6</td>
<td>23.169</td>
<td>0.59x10^7</td>
</tr>
</tbody>
</table>

Figure 5. Effect of *V. lecanii* on *T. vaporariorum* eggs. Day 5 (a), day 6 (b) and day 7 (c) after treatment.

Day 5 was 7 folds higher than that found in day 7 (Figure 5a, b and c). Microscopic examination showed that the infected *T. vaporariorum* egg was surrounded with *V. lecanii* mycelium (Figure 6a and b).

**Effect on larvae**

As seen in Figure 7, the percentage of mortality increased according to contact time, whatever was the dose of spores used. LD50 were: 11x10^3, 3.4x10^3 and 0.5x10^3 spores/ml for the three incubation times (Table 2). Figure 8 showed *T. vaporariorum* larvae with an abnormal shape surrounded with *V. lecanii* mycelium.

**Effect on adults**

After 7 days of incubation of 120 adult insects, only 81
Figure 6. A *T. vaporariorum* egg control (a) A *T. vaporariorum* egg surrounded with *V. lecanii* mycelium, (b) G 40X0.75.

Figure 7. Effect of *V. lecanii* on *T. vaporariorum* larvae after 5, 6, and 7 days of treatment.

Table 2. Probit analysis of larvae treatment.

<table>
<thead>
<tr>
<th>No. of insects (n)</th>
<th>Slope ± SE</th>
<th>LC (95%)</th>
<th>Chi-square</th>
<th>LD50</th>
</tr>
</thead>
<tbody>
<tr>
<td>604</td>
<td>0.26±0.08</td>
<td>17.357 10³-94.40 10⁵</td>
<td>72.25</td>
<td>11X10³</td>
</tr>
<tr>
<td>604</td>
<td>0.30±0.08</td>
<td>42.156 10⁵-33.495 10⁵</td>
<td>61.43</td>
<td>3.4X10³</td>
</tr>
<tr>
<td>604</td>
<td>0.28±0.09</td>
<td>25.64 -13.045 10⁵</td>
<td>44.32</td>
<td>0.5X10³</td>
</tr>
</tbody>
</table>

were found to be completely invaded by means of the fungus mycelium (Figure 9).

Influence of humidity

The incubation at 70% relative humidity did not show any inhibitory effect on the pathogenesis of the fungus, since the mortality rate of the larvae was 100% and that of eggs was relatively high (Figure 10).

Information about probit analysis (No. of insects (n=885), Slope ± S: 0.88± 0.10, LC (95%): 3.74-7.23, Chi-square: 1.44).

DISCUSSION

Experiments on the efficiency of the fungus against *T. vaporariorum* investigated the influence of various environmental factors. Experimental infections of
larvae, adults and eggs showed that the observed mortality rates on the different stages treated by fungal spores are important and in particular in the case of larvae (LD$_{50}$: 0.5x10$^3$ spores/ml).

Drummond et al. (1987) found an LD$_{50}$ of 1.5x10$^5$ spores/ml in the same conditions of temperature and humidity which confirmed the efficiency of the used strain against the development of *T. vaporariorum*. In contrast to the results obtained with larvae, the efficiency of eggs treatment by fungal spores is lower with an LD$_{50}$ of 5.9x10$^6$ spores/ml. These results are conforming to those obtained by Hall (1982), which observed that, for adult insects, fungal spore treatment was very efficient in small experimental greenhouses. In our experiments, the substitution of experimental greenhouses with desiccators confirmed this efficiency. Therefore, 81 adults over 120 used were completely invaded by the fungal mycelium. Others died by drowning due to water condensation on desiccator’s walls.

Eggs, larvae as well as adults treatment showed that the used *V. lecanii* strain is very pathogenic against *T. vaporariorum* particularly in larval stage. The lower pathogenesis on eggs could be explained by the presence of the rigid cuticle which hinders the penetration of the mycelium. Given that insect
development inevitably passes by larval stages, which let us suppose that eggs that have escaped the treatment will be attacked in the larval stage. The percentage of mortality of spore-treated eggs and larvae increased throughout the incubation period in experimental conditions, thus, fungus efficiency requires some contact time.

Treatment efficiency depends closely on a high relative humidity rate (95 to 100%) necessary to the fungal spore germination. A decrease in humidity attenuates the fungal infectious capacity (Milner and Luton, 1986). It is then recommended to maintain a very high relative humidity after treating the aleurode by the fungus (Drummond et al., 1987). It is clear that humidity in greenhouses is not constant, its recording performed in Jijel during May and June 2008, showed a decrease in the weekly mean from 57.34% in the morning to 96.22% in the night. The other important factor is the capacity of fungal spore germination which should be higher than 90%, for the studied strain, spore germination reached 92% after 12 h of incubation in the experimental conditions, which is considered as a good result.

Germination capacity could be influenced by several factors such as spore age, temperature as well as relative humidity; consequently, evaluation of germination capacity of the studied strain should be carried out permanently before each biological control treatment. It was reported that one of the properties of highly pathogenic and virulent strains is their rapid germination. According to Hall (1984), 50% of the spores of virulent strains germinate after 9 h of incubation at 100% RH.

Spore germination rate of the studied strain reached 65% after 9 h of incubation at 100% RH. This fungal property is very important because a relative humidity higher than 95% rarely persists in greenhouses during more than 12 h, as showed by the recordings carried out in a greenhouse of Jijel. Because of the rapid germination of the fungal strain, it depends a little on the RH after an incubation period of 16 h at 100% RH. Development stages transfer of the insects maintained at an RH of 70% for 96 h did not influenced the mortality rates as compared with the control maintained at 100% during the complete experimentation period.

Drummond et al. (1987) observed that more the fungus is pathogenic more it is independent on low humidity. However, Fargues et al. (2005) showed that the entomopathogenic Hyphomycetes have strong potential for microbial control of whitely larvae infesting tomato crops at moderate ambient humidity in Mediterranean greenhouses. The characteristic of V. lecanii regarding the relative humidity could be of great importance for its application in greenhouses where important humidity fluctuations are noted.

Conclusion

Based on the realised tests, the entomopathogenic fungus V. lecanii is particularly infectious for larval and adult stages, and slightly infectious for eggs. In larvae, LD50 is relatively low (0.5x10^6 spores/ml) in the experimentation conditions. The action time of the fungus reached its maximum in day 7. Concerning the effect of humidity on the development of the fungus and on its virulence, results presented here allowed us to consider a future practical application of V. lecanii in the biological control of T. vaporariorum. Further applied research is required to determine the greenhouse application conditions.

REFERENCES


