RESEARCH PAPER

Biocontrol test against the leaf minerof tomato *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) by using entomopathogenic fungi in the Algerian Sahara

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Received 02 January 2016; Revised 13 March 2016; Accepted 18 March 2016

Abstract

Devastating insects constitute one of strains for cultivate tomato. Among these insects, tomato leafminer (*T. absoluta*) has been recently introduced in Algeria (2008), it constitute a challenge for both agricultures and scientists. Firstly, this insect is introduced without their natural enemies which may reduce their damage. Secondly, this species has developed insecticide resistance to many active matters. To contribute to establish a control strategy for *T. absoluta* we have made an inventory of their enthomopathogenic fungi. Two fungi were identified among others taken from adults and pupae. These fungi are *Aspergillus flavus* and *Fusarium sp.* A study was conducted in laboratory of plant pathology to recognize the efficiency of these antagonists. These species had unregistered a mortality mounts of 100 % in 6 days.

Keywords: *Tuta absoluta*; Tomato; Enthomopathogenic fungi; *Aspergillus flavus*; *Fusarium sp.*; control strategy.

Essai de lutte biologique contre la mineuse des feuilles de la tomateTuta absoluta (Meyrick) (Lep: Gelechiidae) par l'utilisation des champignons entomopathogènes dans le Sahara algérien

Résumé

Les insectes ravageurs constituent l'une des contraintes de la culture de la tomate. Parmi ces derniers, la mineuse de la tomate (T. absoluta) qui a été signalée récemment en Algérie (2008), constitue un défi pour les agriculteurs et les chercheurs ; parce que cet insecte a été introduit sans son cortège d'ennemis naturels. En plus, cette espèce a développé une forme de résistance contre certaines molécules insecticides. Dans le but de contribuer à l'établissement d'une stratégie de lutte contre T. absoluta, un inventaire des champignons entomopathogènes de celle-ci a été réalisé. Deux souches fongiques ont été isolées et identifiées à partir des adultes et des nymphes de ce déprédateur. Il s'agit d'Aspergillus flavus et Fusarium sp. L'essai a été conduit au laboratoire de la phytopathologie de l'INRAA(Station Sidi Mehdi, Touggourt). Les résultats obtenus montrent l'importance de ces micro-organismes car un taux de mortalité de 100 % a été enregistré par ceux-ci.

Mots-clés: *Tuta absoluta; Culture de la tomate ; Champignonentomopathogène; Aspergillus flavus; Fusarium sp.; Stratégie de lutte.*

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1. INTRODUCTION

Among all agricultural productions in Algeria, tomato takes up a great place and constitutes an important economic interest. However, this production is confronted, like all agricultural productions, with certain constraints in particular biotic ones. Tomato leafminer (*Tuta absoluta*) is the second devastating insect introduced in Algeria in the last twenty years. The first insect was Phyllocnistis citrella(Guénaoui &Dahliz 1996). T. absoluta was observed in Algeria firstly in the west regions, in 2008 (Guénaoui 2008). Controlling this insect makes a new challenge to rise. Biological control may be used with predators, parasitoids and microbiologic agents (fungi and bacteria).

A good result was obtained with entomopathogenic fungi in laboratory conditions against a Lepidoptera P. citrella (Lakhdari, 2009) and an Orthoptera Brachytrupes megacephalus(Lakhdari et al., 2015). The aim of this work is to conduct an experiment control T. absoluta with entomopathogenic fungi.

2. MATERIALS AND METHODS

2.1. Study Area

This study was conducted in the region of Oued Righ. This area is located in the Southeastern part of Algeria (Fig. 1). It is a saharian region with a temperate winter and a hot summer. It covers a South-North axis whose latitude is 32°54' to 39°9' north and longitude 05°50' to 05°75' east.

Figure 1: Study site

2.2. Isolation of fungi

The incubationperiod of infected larvaeis between 4 and 7 days at 27 °C. Fungi were isolated from nymphs and adults of T. absoluta. Leaves with tomato leafminer larvae were sampled at greenhouses located in the region of Touggourt (Fig. 2), in the province of Ouargla. Leaves were placed in plastic containers until emergence of adults. Dead individuals (nymphs and adults) were collected and placed in Petri dish with agar-agar. Healthy nymphs were disinfected with bleach (2 %) for two minutes, cleaned with distilled water and dried in blotting paper. Then nymphs were placed in Petri dish with agar-agar to insure the development of fungi. Four nymphs were placed in every Petri dish. Petri dishes were placed from20 to 25°C. Different breeding grounds were used.

2.3. Purification of fungi

Fungi Colonies developing around nymphs and adults of T. absoluta in Petri dish were usually made with many types of fungi. Purification was necessary. For that, pieces of colonies were taken and sub-cultivated in a new Petri dish. This operation was repeated until obtaining just one fungusspeciesper Petri dish.

2.4. Identification of fungi

The identification of purified fungi was based especially on morphological aspect. For that reason, macroscopic and microscopic aspects of fungi were examined. Two fungi were identified. It's about*Aspergillus flavus and Fusarium sp.* Other fungi are waiting for identification.In our identification of fungi, we have based on the determination key (Barnett & Hunter, 1977; George et al., 1984; Wraight & Roberts, 1987).

2.5. Effect of fungi on larvae of tomato leafminer

The aim of this test is to recognize the effect of isolated fungi on the tomato leafminer larvae.

2.5.1. Breading of *Tuta absoluta* larvae in the laboratory

Leaves containingtomato leafminer larvae were sampled at a tomato greenhouse in Sidi Mahdi INRAA station. Treated Leaves were placed in Petri dishes with cotton soaked in water in order to keep leaves cool. Larvae of L2 and L3 stages were used. Twenty Petri dishes were used. Every Petri dish contains only one larva.

Figure 2: Sampling of leaves in the greenhouse of tomato (Lakhdari et al., 2015)

2.5.2. Pathogenicity of fungi on tomato leafminer larvae

Leaves were treated by methods. The method was used in our experimentation; leaves were soaked in the spore solution. Witness test was used with twenty larvae treated by distilled water which contain a drop of Tween 80. Two concentrations (10^4 and 10^6) of *Aspergillus flavusand Fusarium sp*.were prepared and tested on T. absoluta larvae (2^{nd}& 3^{rd} instar) to study the effect of these agents on larval mortality. The test was repeated three times. Observations were recorded daily in order to recognize the effect of fungi on the behavior of tomato leafminer larvae.

Schneider and Oreilli method was used to calculate the mortality of larvae. This method allows eliminating the risk of natural mortality.

Corrected mortality (MC %) = (M2 - M1)x100/100 - M1
M1: Rate of mortality in the witness lot
M2: Rate of mortality in the treated lot

2.6. Statistical analyses

STATBOX was used in the statistical analyses. The effect of fungi on the mortality of larvae was very significant (1%) whereas, the effect of treatment method was non- significant. Mortality in the treated lots was observed since the third day of the experiment.

3. RESULTS AND DISCUSSION

3.1. Isolation and identification of entomopathogenic fungi from adults of T. absoluta

3.1.1. Macroscopic and microscopic appearance of strains

The identification of isolated fungi was conducted by examining the color and shape of the biological material.

Aspergillus flavus: The fungus grows rapidly on the PDA environment. It forms colonies fluffy dusters, first white, and then yellow. The conidial head is first removed and then is divided into several yellowish colonies. The conidiophores are hyaline and subglobose vesicles. Phialides are inserted directly into the bladder. Conidia are globose and subglobose (Fig. 3).

Fusarium sp.: This species forms fluffy or cottony white colonies and become light pink. The main morphological character is the presence of *Fusarium macroconidia* fusiform and compartmentalized. Phialides are short and wide, formed on the aerial mycelium. Micro-conidia are absent (Fig. 4).

3.2. Mortality of tomato leafminer treated by entomopathogenic fungi

The table below shows that the mortality started in

Table 01: Mortality percentage of leafminer treated by entomopathogenic fungi

Days	Fusarium sp.		Aspergillus flavus		Witness
	106	104	106	104	
1st day	0%	0%	0%	0%	0%
2nd day	0%	0%	0%	10%	0%
3rd day	10%	20%	30%	40%	0%
4th day	20%	30%	40%	30%	0%
5th day	30%	30%	20%	20%	0%
6th day	20%	10%	10%	0%	0%
7th day	20%	10%	0%	0%	5%
Total	100%	100%	100%	100%	5%

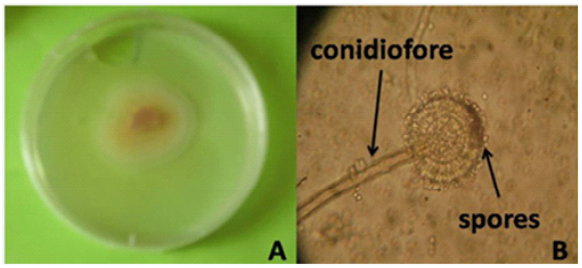

Figure 3: Macroscopic aspect (A) and microscopic (B) of *Aspergillus flavus sp*. (G X 40)(Lakhdari et al., 2015)

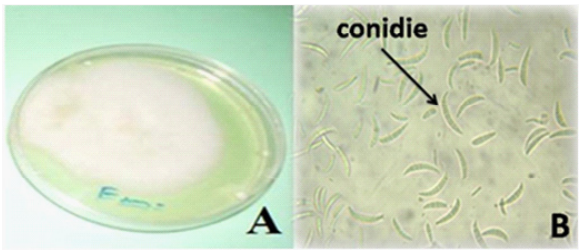

Figure 4: Macroscopic (A) and microscopic aspect (B) of *Fusarium sp*. (G X 40) (Lakhdari et al., 2015)

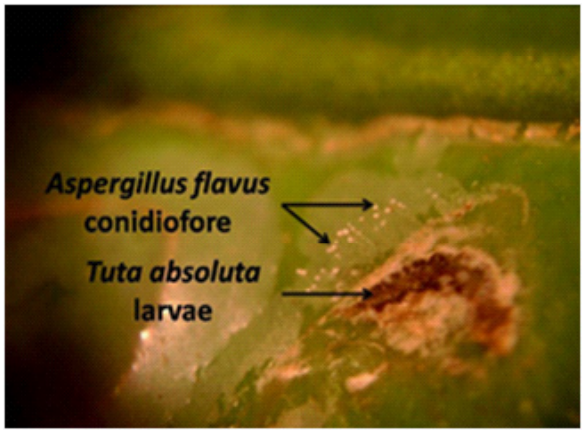

Figure 5: Action of *Aspergillus flavus* on the larvae of Tuta absoluta after 6 days of treatmen (Lakhdari et al., 2015)

the 2nd day to reach 100% in the 5th day observed in the leafminers of *Tuta absoluta* treated by *Fusarium sp*. with a concentration of 10^6 and the larvae treated by the 2nd concentration (10^4) reached 100 % in the 6th day. Unlike the other fungus which is the *Aspergillus flavus* have attained 100 % of mortality in the 7th day with two concentrations.

Thus, it was evident that the highest effective concentration of *Fusarium sp.*on the second instar larvae of T. absoluta was 10^4 & 10^6 conidia/ml where these concentrations gave 100% reduction rapidly in the 5th day of the evaluation.

According to the statistical analysis, we note that both fungi (*Aspergillus flavus and Fusarium sp*.) have a highly significant effect for larvae mortality of T. absoluta in comparison with the witness (F= 18% and F= 19%) respectively. As against there is no significant difference between the two doses used.

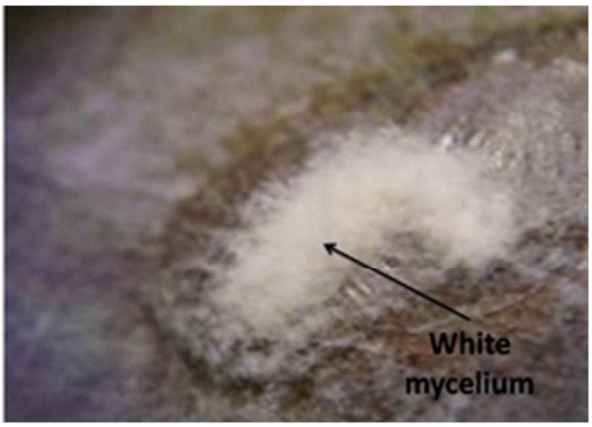

Figure 6: White mycelium covers the larvae of *Tuta absoluta* after 5 days of treatment by *Fusarium sp*. (Lakhdari et al., 2015)

According to recent searches, in a biological control test against larvae Phyllocnistis citrella by several fungi, the most striking effect was that of *Fusarium sp.* which caused the highest mortality rate with 75% (Lakhdari et al., 2015).

Several studies of biological control against Orthoptera by the use of insect pathogenic fungi were made (Doumandji-Mitiche et al., 1999; Kaidi, 2006; Chaouch, 2009; Saiah et al., 2010; Outtar et al., 2014) interesting results were strong obtained.

Our results are similar to those obtained by (Lakhdari 2015), has tested *Fusarium sp.* against an orthoptera Brachytrupes megacephalus which has found a mortality rate 100% in the 11th day from the treatment.

Also Saiah (2010), found that the mortality rate in its biological control by *Fusarium sp.* against a lepidoptera (Phyllocnistis citrella) was superior to 55%.

Several studies of biological control against *Tuta absoluta* by the use of different insect pathogenic fungi like Metarhizium sp., Alternaria sp., Penicillium sp., *Aspergillus niger,* Verticillium lecaniiet Beauveria bassiana(Saiah 2010; Abdel-Raheem et al., 2015).

3.3. Treatment effect on *Tuta absoluta* larvae

A. flavus either by penetration of the hyphae in the body of the larvae giving the appearance of needle sticks, which causes destruction of the tissues of larvae (Fig. 5).

Fusarium sp. wraps with mycelium whitish over the entire surface of the larva, leaving only appear not even one millimeter. Shortly after, one notices the disintegration of the larva (Fig. 6).

CONCLUSION

At this stage, the tests demonstrated the possibility of biological control on the populations of *Tuta absoluta* with the use of the fungi (*Aspergillus flavus and Fusarium sp.*) but before considering a field use of this entomopathogenic, it would be desirable to extensive experiments in terms controlled.

ACKNOWLEDGMENT

This work was supported by the Algerian National Institute for Agricultural research (INRAA), Sidi Mehdi Station, for which we are grateful. Additional grateful to the farmers of Megarine region: Mr. Seddiki Halim,Ghougali Abdelwahab, Chafou Rettmi and Miss Soltani Souadfor their helpful in this experiment.

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