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Research Article
Effect of *Pseudomonas* as a Preventive and Curative Control of Tomato Leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae)

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Abstract

Background and Objective: *Tuta absoluta* is a devastating pest of tomato crops. Currently, *T. absoluta* control is mainly based on chemicals control. However, these products have a harmful impact on environment. This work aims to evaluate the preventive and curative effect of *P. fluorescens* Q036B, *P. fluorescens* Q110B and *P. putida* (Q172B and Q110B) against *T. absoluta*. **Materials and Methods:** The three strains were tested directly on mortality of *Tuta absoluta* adult. Indeed, the effect on larva was done on both sides as a preventive and curative trials. Each bacteria suspension was tested at 10^8 CFU mL^{-1}. The mortality rate was recorded 24-120 h after application. **Results:** The results indicated that all tested bacterial isolates has a toxic effect on *T. absoluta* larva and adults. Within the 24-120 h of exposition period, *T. absoluta* mortality rates up to 81% for adults and to 100% for larva for Q110B and Q036B isolates, respectively. The Q036B isolates induced higher mortality rates than Q172B and Q110B in both preventive and curative control. Regarding the mechanism of action, all isolates produced hydrogen cyanide, siderophores in addition to the exhibited protease and cellulose activities, while only Q036B and Q172B possess chitinase activity. **Conclusion:** Our results concluding that the *P. fluorescens* Q110B and *P. fluorescens* Q036B were a promising candidates bio-agent for biological control of *T. absoluta* and has potential to be an efficient component in an integrated pest management program. Then the fruits produced will be qualified as safe for consumers and the environment.

Key words: *Tuta absoluta*, *P. fluorescens*, *P. putida*, biocontrol, chitinase, tomato

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), was originated from south America where it is considered as one of the most devastating pests of tomato crops[1-3]. This pest infests tomato plants and fruits destined to fresh market. The larvae causes severe damage to different part of the plants: Leaves, flowers, stems and fruits[4,5]. In 2008, *T. absoluta* was detected in Morocco, especially in the tomato growing regions, where it causes considerable economic losses[6,7]. The most common control practice of *T. absoluta* is based on chemical insecticides[8]. However, these products have negative impact on environment and effects on natural enemies which are based on biological control[9,10]. In addition, chemicals can also lead to insect resistance[8,11,12], environmental pollution and risk for human health[13]. Therefore, integration of other control methods (cultural, biological and biotechnological methods) becomes more and more imperative. After the introduction of *T. absoluta* in the Mediterranean Basin, some indigenous parasitoids and predators have been reported to prey on this exotic pest[14-17]. Different botanicals with efficient insecticidal activities against *T. absoluta* has been known at length and more recent studies have also shown the efficacy of plant extracts against larva[18,19]. In the same context, the use of micro-organisms as insecticides has been widely known as well. The *B. thuringiensis* has been reported as efficient on *T. absoluta* larval stages in many countries[20-22]. Several reports about plant growth promoting rhizobacteria (PGPR) like *Pseudomonas*, *Bacillus* and *Serratia* indicated that these micro-organisms are efficient root colonisers and able to protect plants from different crop pests[23-26]. Most of the studies that focused on the effect of *B. thuringiensis* on *T. absoluta* have been performed and reported from originated region of *T. absoluta*[27-29]. Commercial formulates based on *B. thuringiensis* have been used for decades to control insect pests as an alternative to chemicals. Such formulates are ecofriendly, harmless to human and other vertebrates[30-32] have shown high compatibility with the use of natural enemies[33-35]. Furthermore, the biological products are also recommended when insect populations have developed resistance to other products or when treatment is required just before harvest[36]. Thomas and Ellar[37] reported that *B. thuringiensis* involve an insecticidal mechanism in which interaction of toxin with specific plasma membrane lipids causes a detergent-like rearrangement of the lipids, leading to disruption of membrane integrity and eventual cytolysis. *Pseudomonas fluorescens* isolates were found effective in killing or causing morphological defects in a widely used laboratory pests[26,38]. The ingestion of *P. fluorescens* bacteria by *D. melanogaster* larvae causes both lethal and non-lethal phenotypes, including delay in the onset of metamorphosis and morphological defects in surviving adult flies, which can be decoupled[39]. Accordingly, few studies have evaluated the efficacy of *Pseudomonas* on *T. absoluta*. The aim of this study is to evaluate the preventive and curative efficacy of three *Pseudomonas* isolates as bio-insecticides against *T. absoluta* along with the establishment of the mechanisms involved in the control of this pest.

MATERIALS AND METHODS

All experiments were done in the plant protection laboratory of INRA-Agadir Morocco from March and the end of July on 2018.

***Pseudomonas* isolates:** The three isolates of *Pseudomonas*, *P. putida* Q172B, *P. fluorescens* Q110B and *P. fluorescens* Q036B were isolated from the rhizosphere of tomato plants and stored in glycerol at -20°C in the plant protection laboratory, INRA-Agadir[26].

Insect rearing: A colony of *T. absoluta* was obtained from the *Tuta*-rearing glasshouse at the experimental farm-Belfaa (Agadir, Morocco). They were reared under glasshouse conditions, on tomato plants at 26±2°C, L: D 16:8 and 70±5% RH and no pesticides were applied during the process of mass rearing.

Effect of *P. putida* Q172B, *P. fluorescens* Q110B and *P. fluorescens* Q036B on *T. absoluta* adults: The effect of bacterial isolates on *T. absoluta* adults was studied under laboratory conditions using a leaf-dip bioassay[12]. A leaf cage was prepared using vials (5 cm in diam. and 8 cm in h) containing Whatman paper soaked in sterile distilled water. A 1.5 cm diam. hole was made in the lid of bottles and covered with muslin tissue. Tomato leaflets were dipped in each isolate concentration (10^8 CFU mL^{-1}) for 30 sec. The treated leaflets were dried under a laminar hood then transferred to leaf cages. The control leaflets were dipped only in sterile distilled water. Eleven *T. absoluta* adults (male to female sex ratio: 1-1.2) were then transferred to leaf cages. The cages were incubated in growth chamber (Ehret, Type KLT/04) at 25±2°C, 70% RH and 16 h: 8 h light-dark. Four replicates for each isolate were used and the bioassay was replicated thrice. The *T. absoluta* mortality rate was assessed 24, 48, 72 and 120 h after treatment and were corrected using Abbott's equation[40] (Eq.1):

$$CrrM = \frac{DIN - DINC}{ITN - DINC} \times 100 \quad (1)$$

Where:
CrrM = Corrected mortality
DIN = Dead insect number
DINC = Number of dead insect in control
ITN = Total insect number

Toxicological effect of *P. putida* Q172B, *P. fluorescens* Q110B and *P. fluorescens* Q036B on *T. absoluta* larva

Preventive control: Larva of *T. absoluta* were collected from the *Tuta*-rearing greenhouses. Infested tomato leaves were transferred to the INRA Plant Protection Laboratory in Agadir. Bioassays were carried out on the same day. A leaf cage was prepared using Petri dishes (9 cm) containing Whatman paper soaked in sterile distilled water. A 1.5 cm diam. hole was made in the lid of Petri dishes and covered with muslin tissue. Tomato leaflets were dipped in each isolate concentration (10^8 CFU mL^{-1}) for 30 sec. The treated leaflets were dried under a laminar hood then transferred to leaf cages. The control leaflets were dipped in sterile distilled water. Five *T. absoluta* larva (L2) were then transferred to cage leaflets. The cages were incubated at 25±2°C with a photoperiod of 16: 8 h (L: D) and *T. absoluta* mortality rate was assessed 24, 48, 72 and 120 h after treatment. Five replicates for each isolate were used and the bioassay was four times replicated.

Curative control: The *T. absoluta* infested tomato leaves were collected from tomato plants in an infested greenhouse. The leaves containing newly hatched eggs (5-7) were dipped in each bacterial isolate (10^8 CFU mL^{-1}) for 30 sec. The treated leaflets were dried under a laminar hood then transferred to leaf cages. The control leaflets were dipped only in sterile distilled water. The cages were incubated at 25±2°C with a photoperiod of 16: 8 h (L: D). The rate of damage of *T. absoluta* larva was estimated 24, 48, 72 and 120 h after treatment. Four replicates (2 leaves/replicate) for each isolate were used and the bioassay was replicated thrice. After incubation period, the mesophyll area consumed was estimated and the anti-feeding rate (AFR) was calculated using the equation[41] (Eq. 2):

$$AFR = 1 - \frac{Dt}{Dc} \times 100 \quad (2)$$

Where:
Dc = Area of mesophyll consumed in the control leaves
Dt = Area of mesophyll consumed in the treated leaves

Mechanisms of action of *P. putida* Q172B, *P. fluorescens* Q110B and *P. fluorescens* Q036B: The ability of an isolate to produce chitinase was determined as described by Cattelan *et al.*[42]. For the cellulase activity, M9 medium agar amended with cellulose and yeast extract was used to test the cellulase activity[43]. Protease activities of *Pseudomonas* were determined according to the method reported by Jha *et al.*[44]. The ability of volatile organic compounds production, hydrogen cyanide (HCN), was determined as described by Bakker and Schippers[45]. The phosphatase activity was determined by development of a clear zone in NBRIP medium[46] while, Siderophore production was determined using the FeCl3 test[47] and the chrome azurol S (CAS) assay[48].

Statistical analysis: The percentage of mortality was calculated for each *Pseudomonas* isolate. To determine the efficacy of *Pseudomonas* on *T. absoluta*, mortality and anti-feeding rates were subjected to the analysis of variance test (ANOVA- one-way) with Duncan's multiple range test at 1% level of significance using Statistica software (Ver 6).

RESULTS

Effect of *Pseudomonas* isolates (*P. putida* Q172B, *P. fluorescens* Q110B and *P. fluorescens* Q036B on *T. absoluta* adults: The results indicated that *P. fluorescens* Q110B was most effective and caused 82% of mortality compared to *P. putida* Q172B and *P. fluorescens* Q036B with 55 and 34%, respectively at 120 h after treatment (Table 1).

Toxicological effect of *P. putida* (Q172B and Q110B) and *P. fluorescens* Q036B on *T. absoluta* larva

Preventive control: The three tested bacteria showed strong larvicidal effects with mortality rates ranging from 80-100% at 120 h after treatment for *P. putida* Q172B and *P. fluorescens* Q036B, respectively (Table 2).

Curative control: The results of curative control show that all these three tested *Pseudomonas* are highly efficient in reducing the damage caused by *T. absoluta* larva.

Table 1: Mortality rate caused by three *Pseudomonas* isolates (*P. fluorescens* Q036B, *P. fluorescens* Q110B and *P. putida* Q172B on *T. absoluta* adults

	Hours after treatment			
Variables	24	48	72	120
Q172B	30.00±15.28[a]*	33.81±10.42[a]	50.05±20.81[a]	55.56±23.13[a]
Q110B	56.67±15.28[b]	81.48±12.83[b]	81.83±7.87[b]	81.95±0.00[b]
Q036B	13.33±0.00[a]	29.63±11.11[a]	22.80±15.73[a]	33.81±10.42[a]

*By column, the rates followed by the same letters are not statistically different at p<1%

Table 2: Larvicidal effect of three *Pseudomonas* isolates *(P. fluorescens* Q036B, *P. fluorescens* Q110B and *P. putida* Q172B: preventive control

	Hours after treatment			
Variables	24	48	72	120
Control	0.00±0.00[a]*	0.00±0.00[a]	33.33±0.00[a]	33.33±0.00[a]
Q172B	13.33±18.26[ab]	33.33±0.00[b]	60.00±27.89[b]	80.00±18.26[b]
Q110B	26.67±14.91[bc]	66.67±0.00[c]	66.67±14.91[b]	86.67±18.26[bc]
Q036B	40.00±14.91[c]	66.67±23.57[c]	73.33±23.57[b]	100.00±0.00[c]

*By column, the rates followed by the same letters are not statistically different at p<1%

Table 3: Larvicidal effect of three *Pseudomonas* isolates *(P. fluorescens* Q036B, *P. fluorescens* Q110B and *P. putida* Q172B: curative control

	Hours after treatment			
Variables	24	48	72	120
Q172B	28.89±25.58[a]*	44.62±18.37[a]	50.00±13.98[a]	55.86±12.29[a]
Q110B	26.67±18.59[a]	49.23±12.87[a]	53.75±8.39[a]	69.66±4.50[ab]
Q036B	28.89±19.88[a]	64.62±17.71[a]	85.00±3.42[b]	91.03±3.08[c]

*By column, the rates followed by the same letters are not statistically different at p<1%

Table 4: Mechanisms involved by three *Pseudomonas* isolates *(P. fluorescens* Q036B, *P. fluorescens* Q110B and *P. putida* Q172B

Variables	HCN*	Siderophores	Protease	Chitinase	Cellulase	Phosphatase (mm)
Q172B	+	+	+	+	+	10.75±1.71[a]*
Q110B	+	+	+	-	+	7.75±2.06[a]
Q036B	+	+	+	+	+	12.00±3.16[a]

Hydrogen cyanide detected by discoloration of filter paper when incubated with reagent and siderophores detected by orange halo of CAS medium. The chitinase, protease and cellulase and phosphatase activity were detected by surrounding the colony. *By column, the rates followed by the same letters are not statistically different at p<1%

P. fluorescens Q036B showed highest toxicity on *T. absoluta* larva compared to control with an AFR of 91%.

Mechanisms of action of *P. putida* (Q172B and Q110B) and *P. fluorescens* Q036B: The results showed that all three *Pseudomonas* have a positive activity for cellulase and protease activity and produce hydrogen cyanide (Table 4). They produce siderophores in solid medium, as indicated by orange halo surrounding the colony in CAS medium. Regarding phosphatase enzyme, all three *Pseudomonas* produce phosphatase as indicated by clear halo surrounded the colony. The chitinase plate tests of both *Pseudomonas* isolates Q036B and Q172B manifested a clear halo surrounding the colony which, confirmed their ability to induce chitin degradation, however, no chitinase activity was involved for Q110B (Table 4).

DISCUSSION

The genus *Pseudomonas* is known to have biocontrol propriety against some pest species such as *Cnaphalocrocis medinalis* (Lepidoptera: Crambidae)[49], two-spotted spider mites (*Tetranychus urticae*) and red spider mite (*Oligonychus coffeae*)[26,50-52]. The integration of micro-organisms as alternative to chemical pesticides shows an important interest[53]. Treatments based on *B. thuringiensis* provide a safe strategy to manage *T. absoluta* pest, as *B. thuringiensis* control larva[54]. To our knowledge, the present work is indeed the first report related to the high curative effect of *Pseudomonas* isolates against *T. absoluta* in the Mediterranean Basin. This study provides further evidence that *P. putida* Q172B, *P. fluorescens* Q110B and *P. fluorescens* Q036B cause increased mortality of the adult and larvae of *T. absoluta* and induce potential preventive and curative effect against this pest. The tested bacteria reduce damage caused by *T. absoluta* larva at 100% with Q036B in laboratory conditions. These differences explain the mode of action involved by each bacterium against this insect. The achieved results are consistent with those found by Qessaoui *et al.*[26], which showed that *Pseudomonas* isolates provided significant death and repellence to spider mite *T. urticae* adults.

The results obtained in this study have shown that it is possible to reduce the pest's impact to very low levels without chemical insecticides and by using only *Pseudomonas* isolates based-treatment. Fluorescent *Pseudomonas* bacteria act by several mechanisms for insect and mite pests although the ability to degrade chitin is often considered the primary factor involved[26]. Chitinolytic organisms such as *Pseudomonas* sp. isolated from the rhizosphere have shown that they have potential as biological control agents[49]. This study demonstrates that *P. putida* Q172B, *P. fluorescens* Q110B and *P. fluorescens* Q036B control effectively both adults and larva of *T. absoluta*. Accordingly, it is possible to design control programs based on this bacterium that will successfully manage this pest while having low impact on the auxiliary fauna and the environment[26,53,55]. Additionally, the integration of this technology in biological control methods focusing on management of *T. absoluta*, will reduce certainly the use of chemicals and, consequently residues on fruits. The technology related to biological control is thus improving food safety, quality and a sustainability of agroecosystems[56,57].

CONCLUSION

This study indicate that fluorescent *Pseudomonas*, especially *P. fluorescens* Q036B, *P. putida* Q172B and *P. fluorescens* Q110B have a high biological control potential on tomato leaf miner *T. absoluta*, which is promising in both preventive and curative management strategies. These bacteria occur through various mechanisms of action, such as the production of HCN gas, and enzymes like chitinase.

Future research has to be planned to evaluate the impact of these bacterial isolates under greenhouse conditions. The aim will be to confirm this curative effect and to assess toxic and repellent effects so that they can control other key pests of tomato. For the forthcoming works we suggest additionally molecular studies of the factors involved in these toxic effects.

SIGNIFICANCE STATEMENTS

This study discovered the *Pseudomonas* genus bacteria effect that can be beneficial to control the tomato leafminer *Tuta absoluta*. These 3 isolates of Pseudomonas identified as *P. putida* and *P. fluorescens* would effectively prevent the infestation and even avoid the crop damage after the infestation occurrence, which has been confirmed by the curative and preventive trials. This study will help the researchers to uncover the critical areas of *Pseudomonas* application in the biocontrol of *T. absoluta* that many researchers were not able to explore.

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