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Effect of antagonistic bacteria on *Dactylopius opuntiae* (Hemiptera: Dactylopiidae)

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Abstract

Cactus pear has important economic roles in Morocco as a source of food, animal feed, and income for rural populations. However, this crop is currently suffering from the attack of the wild cochineal *Dactylopius opuntiae* (Cockerell), which has caused huge production losses. A range of insecticides are widely used to control *D. opuntia* and other scale insects worldwide. However, the problems associated with the harmful effects of pesticides on humans, the environment and non-target organisms, require that alternative options should be developed for the eco-friendly control of *D. opuntiae*. This study aims to evaluate the potential of three rhizospheric bacteria in controlling *D. opuntiae*, from which two isolates have a notable impact on both nymphs and adult females. Particularly, isolate TRD16 caused 80% mortality against nymphs, while BRS35 and ZTRS2 show 97% and 84% pathogenicity, respectively, against adult females.

Keywords: *Opuntia ficus-indica*, cactus cochineal pest, rhizospheric bacteria

Introduction

Cactus pear is a plant species commonly found in semi-arid and arid regions worldwide. It is an important crop in many countries for mitigating water scarcity and soil erosion owing its resistance to drought and heat and alleviates desertification (LeHouérou, 1996; Yahia *et al.,* 2011). The Moroccan Green Plan (PMV 2008-2020) advocates for cactus pear cultivation in harsh areas, which extended from 45,000 hectares during the early 1990s to over 150,000 hectares by 2017 (MAPMDREF, 2017). Unfortunately, the crop is currently being attacked by a sap-sucking insect pest named *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae). In Morocco, this insect was initially reported in the Sidi Bennour region in September 2014 (Bouharroud *et al*., 2016) and since then, it has spread to various regions, posing a threat to the entire value chain. Due to its high propagation speed, this pest could potentially cause significant damage to cactus pear in a short time.

In response to *D. opuntiae*, the National Office of Alimentary Security in Morocco authorized several insecticides to control this pest (ONSSA, 2024). However, the use of synthetic residual chemicals not only contributes to pest resistance but also poses a threat to human health and the environment and negatively affects natural enemies (Ramírez-Bustos *et al.,* 2018; Isman, 2019). To minimize these problems and keep pest populations below economic damage levels, alternative control techniques are required for an integrated management. In this study, bacteria isolated from the cactus pear rhizosphere were evaluated for their insecticidal effects on *D. opuntiae* under laboratory conditions.

Materials and methods

Soil samples

Cactus pear rhizosphere soil samples were collected from cacti in Tioughza, located in the Sidi Ifni province, Morocco (29°26’13.97”N/10° 1’55.73”W), during March 2022, transported to the laboratory and stored at 4°C until further analysis.

Bacterial isolation

To isolate bacteria from the rhizosphere, 1 g of each soil sample was added to 9 mL of sterile distilled water

and mixed. Subsequently, decimal dilutions up to 10^{-6} were prepared. Next, 0.1 ml of each dilution was used to inoculate three Petri dishes containing nutrient agar medium, which were then incubated at 28 ± 2 °C for 48 h (Qessaoui *et al*., 2019). All colonies that appeared on the culture media were streaked on fresh nutrient agar media. After incubation for 48 h at 28 ± 2 °C, well-isolated colonies were considered pure and stored on nutrient agar at 4 °C for three months. Eppendorf tubes containing nutrient broth supplemented with 20% sterile glycerol were used for long-term storage at -20°C (Fonseca *et al*., 2006; Caplik *et al*., 2022).

Bactericidal activity of isolates against *D. opuntiae* females and nymphs

The activity of three selected isolates against *D. opuntiae* females and nymphs was evaluated under laboratory conditions (26 ± 2 °C, relative humidity of 75%, and photoperiod of 14 h light/10 h darkness). For this test, pieces of cladode of the same size containing ten females were used. Ten first-instar nymphs of *D. opuntiae* were placed separately on the cladodes. Afterward, the infected cladodes were treated with each bacterial isolate (10^6 CFU/ml) using a hand sprayer (**Fig. 1**). The infected cladodes were placed in a disinfected plastic box. For control, sterile distilled water was used to treat the cladodes. Three repetitions for each treatment were used.

Nymph mortality was recorded 48 and 72 h after applying the treatments. Mortality of adult females was examined through a binocular stereoscope and recorded 6 and 9 days after treatment. Dead nymphs displayed color changes and immobility, while the dead females exhibited desiccation and a dark brown hue (Ramdani *et al.*, 2021).

Statistical analysis

Data was subjected to ANOVA, and presented as means ± standard deviation. Any difference mentioned is significant at $p< 0.05$ using Newman–Keuls test through the SPSS software.

Results and discussion

The nymph mortality rates increased by all three bacteria at 48 and 72 h of treatment (**Table 1**). After 48 h of treatment, TRD16 isolate shows the highest percentage of nymph mortality (53.33 ± 20.82%) compared to the control. At 72 h after the treatment, although the means were clearly different and TRD16 still causing the highest percentage of nymph mortality (80 ± 26.46%), no statistically significant differences were observed among the treatments.

Regarding the effect of bacteria on female mortality, the results indicated a significant difference between the tested bacteria ($P < 0.05$) at six and nine days after treatment. By six days, BRS35 isolate exhibited the highest percentage of female mortality (70.16 ± 13.10) compared to the control. After nine days of treatment the same isolate had 97.62 ± 4.12% of mortality followed by ZTRS2 (83.64 ± 12.50), and consistently, the control obtained the lowest percentage of mortality (44.79 ± 5.5).

Exploring the bacterial diversity of soil for biological control of pests remains an attractive alternative (Achouak *et al.*, 2000). The current study was carried out in laboratory conditions to assess the potential effect of three isolates alone with a black soap for controlling nymphs and adult females of *D. opuntiae*. TRD16 and BRS35 provided the highest toxicity on *D. opuntiae* nymphs and adult females, respectively. The potential of bacteria as biopesticides has been shown against

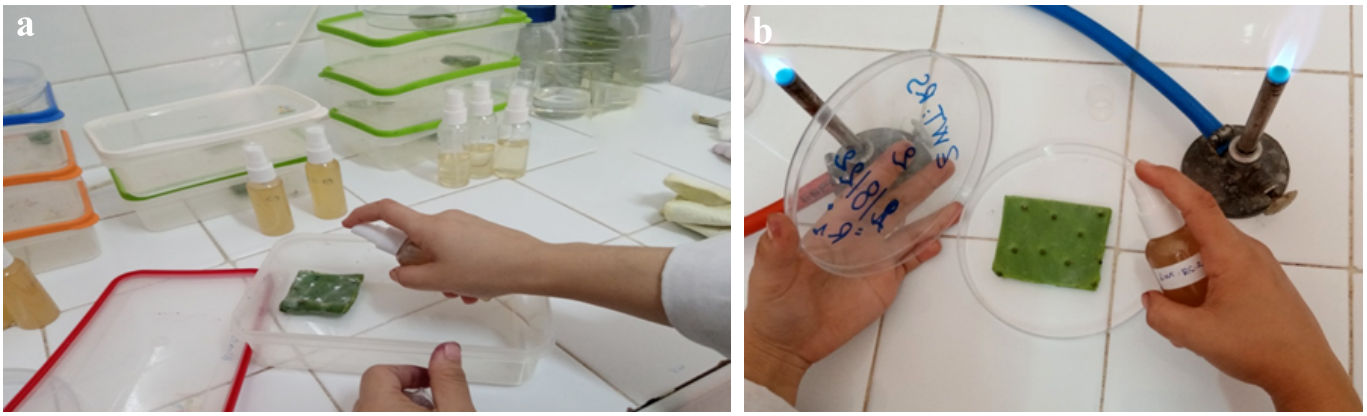

Figure 1. Bacterial application to adult females (**a**) and first-instar nymphs (**b**) of *D. opuntiae* under laboratory conditions.

Table 1. Percentage of first-stage nymph mortality after treatment with the selected bacteria. Within a column, values with the same letters are not significantly different according to the Newman & Keuls test at 5%. Values are the average of three replications; values represent the mean and standard deviation.

Isolate	Exposure period	
	48 h	72 h
Control	10.00 ± 10.00[a]	33.33 ± 5.77[a]
ZTRS 2	33.33 ± 15.28[ab]	70.00 ± 10.00[a]
TRD 16	53.33 ± 20.82[b]	80.00 ± 26.46[a]
BRS 35	30.00 ± 10.00[ab]	53.33 ± 23.09[a]

Table 2. Percentage of adult female mortality after treatment with the selected bacteria. Within a column, values with the same letters are not significantly different according to the Newman & Keuls test at 5%. Values are the average of three replications; values represent the mean and standard deviation.

	Time of exposure	
	6 days	9 days
Control	21.62 ±7.30[a]	44.78±5.59[a]
ZTRS2	36.71±6.03[a]	83.63±12.50[bc]
TRD16	26.42±13.64[a]	67.02±21.36[ab]
BRS35	70.16±13.10[b]	97.62±4.12[c]

a broad spectrum of insects (Burges, 1982; Dowling and Waterfield, 2007; Ruiu, 2020). Entomopathogenic bacteria occupy a special place in the natural control of many insects, particularly relevant are *Bacillus* species that effectively control pests and offers encouraging prospects. Bacterial antagonistic activities have been attributed to their capability to produce enzymes like chitinase and cellulase (Veliz *et al.,* 2017; Qessaoui *et al.*, 2022). Perhaps these cell wall degrading enzymes can affect *D. opuntiae* disrupting the insect's internal structures and functions, and ultimately impairing its survival. Chitinase targets chitin, a major component of the insect's exoskeleton, thereby weakening it and rendering the insect more vulnerable to environmental stress or predation (Paschapur *et al.,* 2021). Cellulase, on the other hand, has the capacity to break down cellulose in the insect's gut, which in turn affects its ability to digest food (Barbosa *et al.,* 2021). The results of the present study demonstrate that the application of bacteria can be used for the bio-control of *D. opuntiae*, providing a favorable alternative to chemical pesticides. However, further research is required towards taxonomically identify the selected bacteria and their mechanisms of action. Laboratory investigations should be accompanied by field experiments to identify the optimal application of the most effective bacterial isolates on the wild cochineal. Researchers also need to establish suitable bacterial formulations, explore their compatibility with other biopesticides (such as botanical extracts or oils) and determine the most suitable method of application under field conditions.

Conclusions

The results of this study demonstrate that bacterial isolates from the cactus pear rhizosphere, particularly TRD16 and BRS35, exhibit potential as biocontrol agents against *D. opuntiae* nymphs and adult females. This research highlights the promising role of bacteria in integrated pest management, offering an eco-friendly alternative to chemical pesticides. Further investigation about bacterial formulations and their secondary effects on plants is necessary to fully explore their practical application in *D. opuntiae* control.

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