BIOLOGICAL ACTIVITY OF SOME NATIVE *BACILLUS THURINGIENSIS* BERLINER STRAINS AGAINST *EUTETRANYCHUS ORIENTALIS* KLEIN (ACARI: TETRANYCHIDAE)

ALAHYANE, H.[1,2*] – EL ALAOUI, A.[2] – ABOUSAID, H.[1,2] – AIMRANE, A.[1] – ATIBI, Y.[3] – OUFDOU, K.[2] – EL MESSOUSSI, S.[1]

[1]*Laboratory of Molecular & Ecophysiology Modeling, Faculty of Sciences Semlalia, University Cadi Ayyad, Marrakech, BP 2390, 40000 Morocco*

[2]*Laboratory of Biology and Biotechnology of Microorganisms, Faculty of Sciences Semlalia, University Cadi Ayyad, Marrakech, BP 2390, 40000 Morocco*

[3]*Laboratory of Biodiversity and Ecosystem Dynamics, Faculty of Sciences Semlalia, University Cadi Ayyad, Marrakech, BP 2390, 40000 Morocco*

**Corresponding author*
e-mail: alahyanerh@gmail.com; phone: +212-654-828-298

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Abstract. Ten *Bacillus thuringiensis* strains (Bt) isolated from Moroccan habitats were screened for their acaricidal proprieties against the Citrus brown mite, *Eutetranychus orientalis* Klein (Acarina: Tetranychidae) under laboratory conditions. Results of bioassay indicated that larvae are more susceptible to the tested Bt strains than adult females at different concentrations (0.5, 1, 2, 4 and 8 mg/ml). Among the Bt strains examined, Bt 26.4 (LC_{50} = 1.533 mg/ml) and *Bt* 13.4 (LC_{50} = 1.385 mg/ml) showed respectively the highest mortalities to adults and larvae when compared with others and with the commercial product of Bt. Concerning fecundity, the effect of all tested strains showed highly significant reduction within adult females, whereas no significant effect was observed in eggs hatching for all strains.
Keywords: *Bacillus thuringiensis, spore-crystal mixtures, Moroccan habitats, biological control, Eutetranychus orientalis, citrus*

Introduction

The Citrus brown mite, *Eutetranychus orientalis* (Klein), is principally a pest of citrus (Jeppson et al., 1975) that attacks a wide range of agricultural, ornamental, and medicinal plants (Rasmy, 1978). It has been found on about 70 host plants of important economic value (Jeppson et al., 1975; Smith-Meyer, 1987; EPPO, 1990). The damage engendered by *E. orientalis* can cause from one side direct effects, including change of leaves' color that become yellowish-brown following chlorophyll depletion, little webbing, leaf fall and branches dieback leading to trees defoliation. From another side, it set off indirect effects such as a decreased photosynthesis process and a retarded grow of plants (Yousef et al., 2006). Actually, the application of synthetic acaricides is commonly applied in a wide agricultural range to manage this invasive mite. Consequently, the use of these acaricides cause negative effects to human health and environment (Cavalcanti et al., 2010; Kumral et al., 2010). However, to achieve a sustainable management, alternative controls are necessary to reduce the use of conventional pesticides (Isman, 2001; Choi et al., 2003), one of which an

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entomopathogenic bacterial toxins, *Bacillus thuringiensis,* may have interesting substitutive managerial effect.

Bacillus thuringiensis (Bt) preparations are one of the successful biological control products (van Frankenhuyzen, 2009) that can be used as alternative to achieve integrated pest management (IPM) program. Several forms of Bt preparations such as liquid and solid are now available on the market for application as biological control agents against many pests (George and Crickmore, 2012). Currently, over than 400 Bt preparations are used as bio-insecticide product which represents approximately 2% of the total insecticidal sold (Bravo et al., 2011).

The insecticidal activity characteristic of Bt is mainly due to its crystalline proteins (δ-endotoxins) produced concomitantly with endospore at the stationary phase of its growth cycle (Schnepf et al., 1998). Though δ-endotoxins are highly specific to their target, they are safe to humans, plants and vertebrates, and are completely biodegradable (IPSC-WHO, 1999). They are indeed toxic to several insects belonging to dipteran, lepidopeteran, and coleopeteran insects, as well as pests from other orders such as fluke, protozoa, nematodes, and mites (Jisha et al., 2013). To our knowledge, few studies have demonstrated the efficacy of Bt against mites, among which Hall et al. (1971) found that thuringiensin could be used to control the citrus red mite, *Pananychus citri* (McGregor). Similarly, the efficiency of thuringiensin was revealed on the adults of *T. urticae* (Royalty et al., 1990; Payne et al., 1993; Chapman and Hoy, 2009). Furthermore, some recent studies have suggested also that Bt preparations are effective to control *Tetranychus macfarlanei* (Neethu et al., 2015), *Aceria guerreronis* (Smitha et al., 2015) and *E. orientalis* (Jisha et al., 2017).

To our knowledge, no study has investigated the acaricidal effects of native Bt strains isolated from Moroccan habitats. Therefore, in the present study, we evaluate the biological activity of ten Bt strains isolated from Marrakech region and Argan field in Morocco. In addition, we test the effects of those Bt strains against eggs, larvae and adult females of *E. orientalis.*

Materials and methods

Rearing of E. orientalis

The Citrus brown mite, *E. orientalis*, was collected from citrus orchards of Agafay, latitude 31°30’01.6”N, longitude 8°14’55.4”W in May 2018. Collected mites were reared continuously on bean plants (*Phaseolus vulgaris* L.) at Laboratory of Molecular and Ecophysiology Modeling, Faculty of Sciences Semlalia, University Cadi Ayyad, Marrakech (Morocco). The rearing conditions used were 29 ± 2 °C and 70 ± 5% R.H. The photoperiod was 16 L: 8 D using fluorescent lamps.

Bacterial strains

10 Bt strains were selected from 83 Bt strains previously isolated from Moroccan habitats (Aboussaid et al., 2010, 2011). The selection of strains was based on the morphological, biochemical and genetic characters (*Table 1*).

Production of spore-crystal biomass

A loopful of bacterial from a colony of each selected strain grown in CCY agar medium were used to inoculate a small tube containing 4.5 ml of liquid CCY medium

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(pre-culture), then the pre-culture was left to grow for 48 h at 28℃ and agitated at 200 rpm (Edmund Bühler GmbH KS-15, Shakers). An aliquot was taken from the pre-culture to verify the formation of spore and crystal (over 90% sporulation is optimum). To eliminate vegetative cells, the pre-culture was heating at 70℃ for 20 min (synchronization). 40 ml of main culture was inoculated with 1/1000 volumes of synchronized pre-culture and incubated as mentioned above (Aboussaid et al., 2011). From this culture, serial dilutions10-fold (10^{-1} to 10^{-5}) has been realized to determine the total number of cells by plating 0.1 ml of each dilution on CCY plates. Then the whole culture was centrifuged for 10 min at 9 000 × g (Universal 320R, centrifuge Hettich).

Table 1. *Morphological characterization (phase-contrast microscopy), biochemical (SDS-PAGE) and genetic (SDS-PAGE and PCR) of the selected Bt Moroccan strains (Aboussaid et al., 2010, 2011)*

N°	Strains	Sampling site	Habitat	Crystal form	Gene *cry* (PCR)
1	Bt A1	Marrakech	Wastewater -Sludge of wastewater treatment system	Spherical	*cry 7/8 + cry 9*
2	Bt A4			Irregular	*cry 7/8 + cry 9*
3	Bt A10			Spherical	*cry 11*
4	Bt A14			Spherical	*cry 11*
5	Bt A-Mg Mg2.7	Taroudant	Argan soils	Spherical	*cry 11*
6	Bt 21.6	Essaouira	Argan soils	Irregular	*cry 11*
7	Bt 26.4			Irregular	*cry 4*
8	Bt 32.3			Crystal > 1	*cry 4*
9	Bt 13.4			Irregular	*cry 4*
10	Bt B9	Beni-mellal	Bean-cultivated soil	Spherical	*cry 4*

The supernatant was discarded and the pellet was washed one time with ice-cold 1 mol/l NaCl, 10 mmol/l EDTA solutions (Ethylenediaminetetraacetic acid). Then the pellet was concentrated by lyophilisation to express the mixtures spore and crystal in mg. Finally, the pellet lyophilized was suspended in 1 ml of 10 mmol/l KCl (Aboussaid et al., 2011). Optical Density (OD) was measured by spectrophotometry at 600 nm (Spectrophotometer "VR-2000", P-Selecta) and the suspensions were stored at -20 °C until bioassay. To limit proteolysis after centrifugation, all steps were done on ice.

Doses preparation

Doses of spore-crystal of each Bt strain were prepared by mixing 10 microliters of Triton-X100 (G-Labrogos), with 80 mg of each Bt strain, then 10 ml of distillate water was added to obtain 8 mg/ml. A series of dilution were prepared from the stock solution (8 mg/ml) using distillate water.

Distillate water and Triton-X100 at a rate 0.01% were used as absolute control, and Bt commercial product (DELFIN®, *Bacillus thuringiensis* sp. *Kurstaki*) and Triton-X100 were used as positive control (Bt. sp. *Kurstaki,* 2 mg/ml + 0.01% Triton-X100).

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Acaricidal activities of Bt strains against E. orientalis

Acaricidal effect of Bt strains on adult females and larvae

Toxicity of spore-crystal mixture of Bt strains to *E. orientalis* was evaluated following the method of Royalty et al. (1990) with slight modifications. Bean plant leaf discs were immersed during seven seconds in five concentrations 0.5, 1, 2, 4 and 8 mg/ml of each selected Bt strains. We have followed the same method to prepare the absolute control and the positive control. Treated leaf discs were placed on the lower surface in a petri dish surrounded with moist cotton wool and then allowed to completely dry at ambient temperature. Twenty *E. orientalis* adult females were introduced by using fine brush into the center of each leaf discs. Five replicates were made for each concentration. The same protocol was conducted for the larvae; eggs were placed on treated leaf discs, then the newly emerged larvae were used for the bioassay.

For all assays, after being exposed to leaf discs treated with *B. thuringiensis*, the larval and the adult females' mortalities were daily evaluated within the 96 h following the treatment. Furthermore, the number of oviposited eggs on the leaf was recorded at the 96th post-treatment hour.

Ovicidal effects of Bt strains

To determine an ovicidal activity of spore-crystal mixture of Bt strains, 20 adult females of *E. orientalis* were introduced on bean plant discs for oviposition and kept overnight. These discs were placed, the lower surface in petri-dish lined with moist cotton wool. After 24 h, the adult females were removed and the leaf discs were kept with 20 eggs on it; the excess of eggs was removed using a fine brush. Eggs laid on leaf discs were sprayed with five concentrations of spore-crystal mixture of each Bt strains. The leaf discs of absolute control and positive control were also prepared. Hatchability was determined 9 days after treatment and the eggs that did not hatch after this time was considered dead.

Statistical analysis

Mortality observations were subjected to one-way ANOVA by using SPSS program, version 11.5. Tukey's test was used for comparisons of means mortality. Probit analysis was used to determine lethal concentrations (LC_{50}) and the control mortalities were corrected by using Abbott's formula (*Eq. 1*).

$$\text{Corrected mortality } \% = \frac{1 - \text{n in T after treatment}}{\text{n in Co after treatment}} \times 100 \qquad \text{(Eq.1)}$$

where: n = Insect population, T = treated, Co = control.

Results

Effect of spore-crystal mixture of Bt strains on adult females of E. orientalis

Acaricidal activity of spore-crystal mixture of 10 selected Bt strains in female adults of *E. orientalis*, is shown in *Table 2*. There were significant differences between the corrected mortality of *E. orientalis* adults treated with five concentrations of each Bt

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strains ($p < 0.05$). Furthermore, a positive correlation was observed revealing an increase of the corrected mortality with the increase of toxins concentrations.

The corrected mortality values varied significantly between strains and the acaricidal rates were ranging from 6.43 to 28.73% (df: 10, $F = 17.96$, $p < 0.0001$) at 0.5 mg/ml, from 21.84 to 43.67% (df: 10, $F = 5.49$, $p < 0.0001$) at 1 mg/ml, from 40.23 to 62.07% (df: 10, $F = 4.23$, $p < 0.0001$) at 2 mg/ml, from 45.97 to 71.26% (df: 10, $F = 3.02$, $p < 0.005$) at 4 mg/ml and from 56.32 to 83.91% (df: 10, $F = 2.45$, $p < 0.020$) at 8 mg/ml.

At 96 h after treatment within the screened strains, Bt 26.4 was found to be the most toxic with a caused mortality reaching 83.91% for the highest dose (LC_{50} = 1.533 mg/ml). The effect of Bt 13.4 was significantly lower compared to the others in every tested concentration (LC_{50} = 4.228 mg/ml). Moreover, significant difference was noted between corrected mortality caused by positive control (71.01%) and those caused by our strains with the concentrations 0.5, 1 and 2 mg/ml, whereas in higher concentrations, 4 and 8 mg/ml, no significant difference was observed (*Table 2*).

Table 2. *Effect of spore-crystal mixture of Bt strains on adult females of E. orientalis 96 h after treatment*

Bt strains	Corrected mortality ± standard error [a,b]					LC_{50}	95% confidence limits
	0.5 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml		
Bt A1	14.94±2.14abA	43.67±3.81cB	51.72±3.89abcB	54.02±1.81abB	67.81±2.92abC	2.457	1.611±3.652
Bt A4	8.73±2.53abA	29.88±6.65abcAB	52.87±6.13abcBC	55.17±8.79abBC	71.26±4.81abC	2.424	1.804±3.203
Bt A10	28.73±1.4bA	37.93±8.41abcAB	56.32±3.89abcB	66.66±2.81abC	77.01±1.81abC	1.703	1.122±2.385
Bt A14	11.72±7.25abA	21.84±6.94aAB	40.23±5.32aB	49.42±5.57abCD	65.51±3.63abCD	3.778	2.706±5.304
Bt A-Mg Mg2.7	6.43±2.42aA	25.28±2.56abB	41.38±4.94abBC	64.36±3.35abD	70.11±3.35abD	2.795	2.104±3.624
Bt 21.6	11.03±3.77abA	28.73±5.33abcA	62.07±4.66bcC	66.66±6.89abB	70.11±8.41abB	2.171	0.145±7.380
Bt 26.4	27.58±3.89bA	37.93±8.01abcAB	56.32±2.29abcBC	67.81±5.91abCD	83.91±4.22bD	1.533	1.078±2.039
Bt 32.3	14.94±4.94abA	41.38±1.15bcB	51.72±3.89abcB	71.26±5.45bC	79.31±5.01bC	1.768	1.293±2.300
Bt 13.4	19.54±7.26abA	31.03±3.14abcAB	43.67±5.86abB	45.97±4.31aC	56.32±7.83aC	4.228	2.541±8.545
Bt B9	25.28±4.06abA	40.23±2.93bcAB	45.97±4.66abB	67.81±5.33abC	75.86±2.81abC	1.889	1.279±2.623
Positive control*	71.01±1.87c	71.01±1.87d	71.01±1.87c	71.01±1.87b	71.01±1.87ab	_	_
_	df: 10, F=17.96;	df: 10, F=5.49;	df: 10, F=4.23;	df: 10, F=3.02;	df: 10, F=2.45;	_	_
_	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.005	p < 0.020	_	_

[a]Treatments followed by the same lowercase letter each column are not significantly different at α = 0.05
[b]Treatments followed by the same uppercase letter each row are not significantly different at α = 0.05
*Commercial product of *Bacillus thuringiensis.* Applied dose: 2 mg/ml and Triton-X100 at the rate of 0.01%

Effect of spore-crystal mixture of Bt strains on larvae of E. orientalis

Results of larvicidal effectiveness of 10 Bt strains are presented in *Table 3*. The difference of mite's corrected mortalities was significantly higher between those exposed to the lower concentrations of spore-crystal mixtures and those exposed to higher concentrations. The corrected mortality was dose dependent and varied significantly within all strains. Larvicidal rates were ranging from 17.01 to 35.10% (df: 10, $F = 13.22$, $p < 0.0001$) at 0.5 mg/ml, from 20.21 to 44.68% (df: 10, $F = 8.29$, $p < 0.0001$) at 1 mg/ml, from 39.36 to 67.02% (df: 10, $F = 3.53$, $p < 0.002$) at 2 mg/ml, from 57.45 to 71.27% (df: 10, $F = 3.02$, $p < 0.842$) at 4 mg/ml and from 72.34 to 85.11% (df: 10, $F = 2.90$, $p < 0.007$) at 8 mg/ml.

The lethal concentrations to kill 50% of larvae (LC_{50}) were determined after 96 h following treatment and summarized in *Table 2*. Results showed that Bt 13.4 at 8 mg/ml

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cause maximum mortality (LC_{50} = 1.385 mg/ml) followed by Bt A-Mg Mg2.7 (LC_{50} = 1.472 mg/ml), Bt A10 (LC_{50} = 1.473 mg/ml), Bt 26.4 (LC_{50} = 2.191 mg/ml) and Bt A1 (LC_{50} = 2.263 mg/ml). Significant difference was noted between corrected mortality caused by positive control and those caused by our strains at lower concentrations 0.5 and 1 mg/ml, whereas no significant difference is revealed at the higher concentrations 2, 4 and 8 mg/ml (*Table 3*).

Table 3. *Effect of spore-crystal mixture of Bt strains on larvae of E. orientalis treated 96 h after treatment*

Bt strains	Corrected mortality ± standard error [a,b]					LC_{50}	95% confidence limits
	0.5 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml		
Bt A1	23.402±3.61abA	25.53±2.37abA	47.87±3.91abB	63.83±6.81aBC	77.65±2.61abC	2.263	1.737±2.907
Bt A4	28.72±2.71abA	34.04±2.71abA	52.13±4.75abB	63.83±5.42aBC	75.53±4.63abC	2.304	1.609±3.184
Bt A10	34.04±2.12abA	36.17±8.06abAB	54.25±4.93abBC	63.83±2.61aC	88.29±1.99bD	1.473	1.095±1.894
Bt A14	25.53±1.68abA	32.97±3.19abA	53.19±4.57abB	64.89±3.19aBC	72.34±3.91abC	2.286	1.627±3.129
Bt A-Mg Mg2.7	30.85±3.36abA	44.68±2.71bB	55.32±2.71abBC	63.83±6.15a C	80.85±3.61abD	1.472	1.028±1.976
Bt 21.6	26.59±4.87abA	34.04±7.25abA	39.36±5.72a A	65.95±7.99a B	85.11±2.61bB	2.419	1.794±3.130
Bt 26.4	25.53±7.89abA	44.68±2.71b B	52.13±2.37abBC	63.83±1.06aCD	74.46±2.61abD	2.191	1.482±3.097
Bt 32.3	22.338±2.71abA	34.04±1.31abAB	42.55±4.25a B	62.76±3.36aC	74.46±3.11abC	2.332	1.745±3.089
Bt 13.4	35.10±1.99bA	43.61±3.61bA	67.02±4.25b B	71.27±2.71aB	76.59±3.97abB	1.385	0.883±1.954
Bt B9	17.01±2.71aA	20.21±5.57aA	46.81±8.57abB	57.45±2.91aBC	73.41±4.75abC	2.829	2.195±3.648
Positive control*	68±2.54c	68±2.54c	68±2.54b	68±2.54a	68±2.54a	_	_
_	df: 10, F=13.22;	df: 10, F=8.29;	df: 10, F=3.53;	df: 10, F=3.02;	df: 10, F=2.90;	_	_
_	p < 0.0001	p < 0.0001	p < 0.002	p < 0.842	p < 0.007	_	_

[a]Treatments followed by the same lowercase letter each column are not significantly different at α = 0.05
[b]Treatments followed by the same uppercase letter each row are not significantly different at α = 0.05
*Commercial product of *Bacillus thuringiensis.* Applied dose: 2 mg/ml and Triton-X100 at the rate of 0.01%

Effect of spore-crystal mixture of Bt strains on fecundity of E. orientalis

The results of fecundity of treated adult females are presented in *Table 4.*

Table 4. *Fecundity of E. orientalis treated with Bt strains 96 h after treatment*

Bt strains	Mean fertility± standard error [a,b]				
	0.5 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml
Bt A1	2.01±0.19aB	0.56±0.06aA	0.49±0.09a A	0.42±0.11aA	0.11±0.04aA
Bt A4	5.54±0.55bB	1.44±0.39abcA	0.42±0.16a A	0.18±0.09aA	0.12±0.12aA
Bt A10	1.81±0.45aB	0.43±0.09aA	0.44±0.09a A	0.30±0.08aA	0.11±0.03aA
Bt A14	1.89±0.15aB	0.84±0.15abA	0.71±0.11a A	0.58±0.11aA	0.45±0.07aA
Bt A-Mg Mg2.7	1.83±0.32aA	1.00±0.17abA	0.69±0.13a A	0.39±0.09aA	0.53±0.04aA
Bt 21.6	3.91±0.56abA	2.48±0.65bcBC	1.44±0.38a AB	0.66±0.09aA	0.10±0.04aA
Bt 26.4	2.29±0.37aA	0.98±0.12abA	0.54±0.11aA	0.42±0.07aA	0.31±0.06aA
Bt 32.3	1.83±0.46aA	1.25±0.24abcA	0.68±0.11aA	0.51±0.06aA	0.25±0.03aA
Bt 13.4	2.32±0.31aB	1.21±0.22abcAB	0.78±0.06aA	0.32±0.07aA	0.28±0.08aA
Bt B9	2.11±0.35aA	0.35±0.11aA	0.51±0.07aA	0.48±0.27aA	0.08±0.03aA
Absolute control	9.63±0.61c	9.63±0.61d	9.63±0.61c	9.63±0.61c	9.63±0.61c
Positive control *	2.92±0.63a	2.92±0.63c	2.92±0.63b	2.92±0.63b	2.92±0.63b
_	df: 11, F=27.26;	df: 11, F=49.13;	df: 11, F=4.23;	df: 11, F=92.77;	df: 11, F=110.03;
_	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.00001	p < 0.0001

[a]Treatments followed by the same lowercase letter each column are not significantly different at α = 0.05
[b]Treatments followed by the same uppercase letter each row are not significantly different at α = 0.05
*Commercial product of *Bacillus thuringiensis.* Applied dose: 2 mg/ml and Triton-X100 at the rate of 0.01%

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The bioassay has shown a decrease of oviposition within the treated females with increasing concentrations of selected Bt strains. In the absolute control, the mean of oviposited eggs reached 9.63 per female, a fecundity which is highly significantly superior to others of every Bt treated females ($p < 0.0001$). Whereas, the comparison between fecundities of females treated with the positive control and those treated with Bt strains has shown no significant difference at low doses (0.5 and 1 mg/ml), while at high doses, the fecundity values of Bt treated females decreased significantly.

Effects of spore-crystal mixture of Bt strains on eggs hatching

The observed hatch rates at 9 days after treatment are presented in *Table 5*. No concentration of all the ten Bt strains was able to avoid eggs hatching. There was no significant difference in eggs hatchability within tested Bt strains, absolute control and positive control ($P < 0.05$).

Table 5. *The percentage of eggs hatching 9 days after treatment with spores-crystal mixtures of Bt strains*

Bt strains	Percentage of hatchability ± standard error[a]				
	0.5 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml
Bt A1	82±2.54ab	82±2.01ab	82±3.39ab	83±3.74abc	80±3.53a
Bt A4	89±1.87ab	83±2.54ab	77±2.54a	76±2.91ab	74±1.87a
Bt A10	87±4.06ab	79±2.91a	74±3.67a	74±1.87ab	73±2.54a
Bt A14	91±3.31ab	87±2.54ab	86±2.91ab	79±1.87ab	81±1.87ab
Bt A-Mg Mg2.7	91±2.91ab	85±4.47ab	79±3.67a	79±3.67ab	76±5.33a
Bt 21.6	84±3.67ab	83±2.54ab	79±3.67a	77±3.39ab	75±3.53a
Bt 26.4	88±2.54ab	84±2.91ab	82±3.74ab	81±4.01ab	79±4.31a
Bt 32.3	91±1.87ab	85±3.53ab	84±1.87ab	83±2.54abc	83±2ab
Bt 13.4	84±4.3ab	81±4.01ab	83±4.89ab	89±2.91abc	78±2.54a
Bt B9	83±2.54ab	79±3.31a	75±4.18a	72±2.54a	67±2a
Control	97±1.22b	97±1.22b	97±1.22b	97±1.22b	97±1.22b
Positive control[b]	77±6.04a	77±6.04a	77±6.04a	77±6.04ab	77±6.04a
_	df: 11, F=27.26;	df: 11, F=49.13;	df: 11, F=4.23;	df: 11, F=92.77;	df: 11, F=110.03;
_	p < 0.000	p < 0.000	p < 0.000	p < 0.000	p < 0.000

[a]Treatments followed by the same letter are not significantly different at $\alpha = 0.05$
[b]Commercial product of *Bacillus thuringiensis.* Applied dose: 2 mg/ml and Triton-X100 at the rate of 0.01%

Discussion

Bacillus thuringiensis is an entomopathogen able to product two proteins inclusions during sporulation. The parasporal crystalline inclusion containing Cry and Cyt (also known as δ-endotoxins) are toxic to different insect orders (Schnepf et al., 1998; Tsuchiya et al., 2002; Aboussaid et al., 2010). Many formulations of Bt toxins revealed high toxicity to mite (Vargas et al., 2001; Jisha et al., 2017). For instance, these toxins have been reported to have different influences on *Tetranychus urticae*, such as antifeedant effect and reduction of fecundity (Royalty et al., 1990, 1991). Such toxicity of Bts is principally due to the primary action of Cry toxins consisting of the lysis of the midgut epithelial cells in the target insect so that it forms pores in the apical microvilli membrane of the cells (Aronson and Shai, 2001; de Maagd et al., 2001; Bravo et al., 2007).

Our results showed a significant acaricidal effect of ten Bt strains spore-crystal mixtures against adult females and larvae of *E. orientalis.* For adult females, 100% of

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tested Bt are toxic and at the higher concentration (8 mg/ml) the mortality ranged between 65.51% and 83.91% after four days. These are corresponding with the data of Neethu et al. (2015) who studied the Bt activity against adults of *Tetranychus macfarlanei* and have reported that the culture pellet, mixtures of δ-endotoxin and crystals, (1-10 mg/ml) mixed with artificial diet caused mortality ranged from 40% to 80% four days after the treatment. Moreover, Jisha et al. (2017) have demonstrated the efficacy of the crude pellet of Bt var. *Kurstaki* (Btk) to combat *E. orientalis*. The results of this study revealed that the crude Btk-toxin eliminate completely the mite of infested plants after 12 days of direct spraying, an effect that has been established without supplementing any adhesive or surfactant. For larvae, all tested Bt strains are highly toxic and the mortality exceeds 80% for some strains. Similar results were reported by Vargas et al. (2001) in a study where the larvae of *Tetranychus urticae* and *Pananychus ulmi* have been highly susceptible to thuringiensin (β- exotoxin). Nevertheless, the results found by Alper et al. (2013) are in disagreement with our data. These authors have studied the effects of spore-crystal mixtures of 31 native *B. thuringiensis* isolates against *T. urticae* nymphs where they have found that 42% of the isolates caused mortality ranged between 16% and 30%. In addition to this, the same study has reported that 58% of the isolates resulted in less than 15% mortality. The difference observed between our results and those of Alper et al. (2013) on the larvicidal effect of the mixture may be linked to variability on the genes coding the Cry proteins.

The larvae of *E. orientalis* are more susceptible to spore-crystal mixtures of our Bt strains than adult females. However, the mortality of both stages increased with increasing concentration of Bt strains. These results agreed with the findings of Royalty et al. (1990) and Vargas et al. (2001) who reported that the application of thuringiensin affect more the immature stages than adults of *Tetranychus urticae* and *Pananychus ulmi,* with an observed dose dependent mortality for the both stages. Therefore, the adult mortality might be a result of the disruption of a diverse biochemical mechanism to that in immature stages (Beebee and Bond, 1973a, b).

Furthermore, the results presented in *Table 4* indicate that the spore-crystal mixtures of our Bt strains caused a reduction in the fecundity of adult female of *E. orientalis.* These are consistent with the results found by Royalty et al. (1990) and Vargas et al. (2001) who noted that the number of eggs laid by treated females of *Tetranychus urticae* is lesser than untreated ones.

Moreover, our finding indicates that spore-crystal mixtures of our strains did not show any ovicidal effect to eggs of *E. orientalis,* which is in agreement with Reza et al. (2011) who postulated that all concentration of thuringiensin did not revealed significant difference in the percentage of eggs hatching of *Tetranychus urticae*.

Conclusions

Based on the present research, Moroccan Bt strains provide the evidence for their acaricidal properties. The most Bt strains tested revealed a great activity against adult females and especially towards larvae and adults, also they reduced greatly the fecundity of female adults. All these results confirm that most of our Bt strains can be used as a good alternative to conventional pesticides harmful to consumers and environment.

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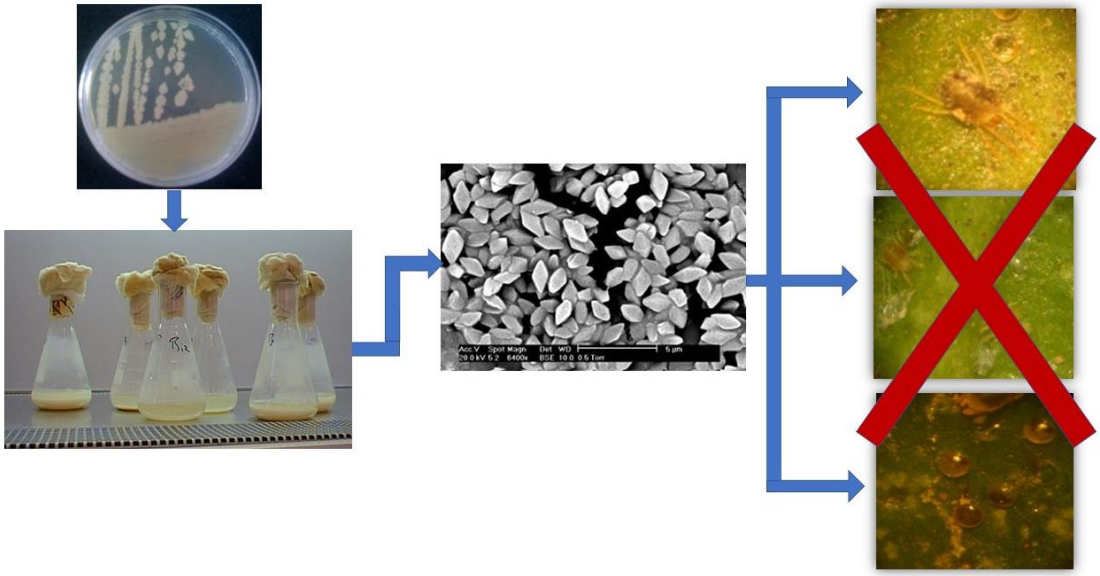
APPENDIX

Graphical abstract



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