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Biological Activity of *Bacillus thuringiensis* (Berliner) Strains on Larvae and Adults of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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ABSTRACT

The objective of this study was to evaluate the efficiency of Moroccan Bt strains against neonate larvae, third instar larvae and emerged adults of Ceratitis capitata. This Mediterranean fruit fly causes serious damages to Argan forest and other agricultural plants. There is no successful control program of this pest fly in the endemic Argan forest in Morocco. A single-dose test was performed on neonate larvae (25 *µL/g*) *and adult* (333.33 *µL/g*), *when three doses of Bt toxins* (50 *µL/g,* 100 *µL/g and* 150 *µL/g*) *were tested against third instar of C. capitata. Among the twenty-six Bt strains examined, local Bt* 13.4 *and Bt A*7 *strains showed highest toxicity levels against larvae and adults, when compared to the reference strain, Bacillus thuringiensis subsp. israelensis HD*567 "*code* 4*Q*1", *and commercial product "Skeetal". One hundred percent mortality was observed against neonate larvae after* 7 *days of application by Bt* 13.4 *toxin. Third instar larvae were very susceptible to Bt A*7 *and Bt M-Ag* 21.6 *strains with* 68% *mortality* (*Lethal Concentration*: LC_{50} = 1.115) *at a dose of* 150 *µL/g. The Bt A*7 *strain was also highly toxic to adults with* 81.66% *of mortality after* 7 *days of application. This study demonstrated that some of our collection Bt strains can contribute to integrated C. capitata management system with strong biological control components.*

Keywords**:** *Argan Forest*, *Bacillus thuringiensis*, *Biological Control*, *Ceratitis capitata*, *Diptera*

1. Introduction

Control of the Mediterranean fruit fly or medfly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae); one of the world's most destructive insect, continues to rely on the use of broad-spectrum chemical insecticides in bait sprays [1]. While these chemical products are generally effective, they are controversial because of concerns for adverse effects on human health and non-target organisms, environmental pollution and development of insecticide resistance [2]. Therefore there is a need for alternative strategies such as bacterial entomopathogenic toxins: *Bacillus thuringiensis*.

Commercial preparations of Bt have been shown to be successful biological control products worldwide [3,4]. Bt insecticidal activity primarily lies in its parasporal crystals. They are predominantly composed of one or more types of proteins, known as δ-endotoxins: the Cry proteins and the Cyt (cytolitic) proteins [5]. Nevertheless, other factors may contribute to Bt toxicity, such as the spore itself and extracellular soluble factors like β-exotoxins, VIP (vegetative insecticidal proteins) toxins, phospholipases, proteases and chitinases [6].

Cry toxins are highly specific to their target insects, are harmless to humans, vertebrates and plants, and are easily biodegradable [7]. To date, several invertebrates have been described to be susceptible to Bt strains, mostly insects from Lepidoptera, Diptera and Coleoptera, but also from other orders (Hymenoptera, Homoptera, Orthoptera and Mallophaga) and also species from nematodes, mites and protozoa. However, for many crystal-bearing Bt strains, no toxic activity has been detected yet [8]. Thus, in recent decades, there has been a great interest in the screening of Bt collections to find isolates useful for pest control [9-12]. Many dipteran species have been found to be susceptible to Bt; these

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include mosquitoes, blackflies, chironomids, tipulids, muscids, sciarids, drosophilids [13] and tephritidis; such as the olive fly *Bactrocera oleae* [14,15], the Mexican fruit fly *Anastrepha ludens* [16,17] and the Mediterranean fruit fly *C. capitata* [18-20].

According to our knowledge, there is no available data on the effect of Moroccan Bt strains isolated especially from the endemic Argan forest which is strongly infested by *C. capitata*.

In the present study, we evaluate the biological entomopathogenic activity of Bt strains isolated from Marrakech region and Argan region in Morocco. We test the insecticidal effects of Bt strains against neonate larvae, third instar larvae and emerged adults of *C. capitata*.

2. Materials and Methods

2.1. Insect Rearing

The insects used in the bioassays came from the laboratory colony of *C. capitata,* maintained at the Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech (Morocco).

The laboratory conditions used for rearing and bioassays were 25 ± 1℃, 60 ± 10% relative humidity (RH) and 12 h of light and 12 h of darkness. Eggs laid through a net placed on one side of an insect-rearing cage were collected in a plastic container that was filled with distilled water. Larvae from 0.5 ml of eggs were raised on an artificial diet containing 250 g wheat bran, 70 g sucrose, 36.3 g brewer's yeast, 2.8 g (methyl 4-hydroxybenzoate; Sigma), 2.8 g Nipasol (propyl 4-hydroxybenzoate; Sigma) nipagin, 2.5 g Benzoic acid and 600 mL distilled water.

Third-instar larvae were transferred into a pupation chamber with sand and were maintained there for at least 1 week before they were collected using a sieve. Adult flies were fed an artificial diet comprised of 20% (wt/wt) yeast autolysate and 80% (wt/wt) sucrose. Water was provided to the flies via a damp yellow sponge.

2.2. *B. thuringiensis* Strains

Samples were collected from different areas of Marrakesh region and endemic argan forests (southwest of Morocco) (**Table 1**). Sampling was performed from heterogeneous sources: soils underground surface (to avoid spore inactivation caused by UV radiation), *Citrus* Phyllplane, Farm animal excrement, wastewater and sludge of wastewater treatment system.

Bt isolation was carried out as described previously by Bel *et al.* [21]. Colonies with characteristic Bt morphology and presence of parasporal inclusions in the sporangium were selected and plated again for single colony isolation. After a second microscopic observation to verify presence of protein inclusions, strains were cultured in CCY (Casein Casein Yeast) liquid medium [22], for 2 days until sporulation. Vegetative cells were eliminated by heating the sample at 70℃ for 20 min. Aliquots were taken and stored in 25% glycerol at −20℃.

Table 1. Local Bt strains isolated from different habitats in Morocco.

Localisation	Habitat	No. of samples	No. of Bt isolates
Agadir	Argan soils	8	13
Essaouira	Argan soils	2	4
Marrakech	Olive-cultivated soil	1	3
	Bean-cultivated soil	1	1
	Farm animal excrement	1	1
	Citrus phylloplane	1	1
	Wastewater	1	1
	Sludge of wastewater treatment system	2	2
	Total	17	26

The standard strains, other than those isolated in this study were *Bacillus thuringiensis* subsp. *israelensis* HD 567 (code 4Q1, Bacillus Genetic Stock Center, B.G.S.C., Ohio State University, Columbus, OH, U.S.A.) and Skeetal, a commercial product based on *B. thuringiensis* subsp. israelensis (Valent Biosciences Corporation, Libertyville, U.S.A.).

2.3. Culture Conditions and Production of Spore – Crystal Biomass

A loopful of cells from a single colony of each strain grown in CCY agar medium were inoculated in 4.5 ml of liquid CCY medium (pre-culture) and grown for 48 h at 28℃ and agitated at 200 rpm. An aliquot was taken to verify spore and crystal formation (over 90% sporulation is optimum), and the pre-culture was incubated for 20 min at 70℃ to eliminate vegetative cells (synchronization).

The main culture (40 ml) was inoculated with 1/1,000 volumes of synchronized pre-culture and grown as mentioned above. Optimal crystal formation was checked by phase-contrast microscopy (DM2500, Leica Microsystems, Germany). The total number of cells in a culture was determined by plating 10-fold serial dilutions on CCY plates. Then the whole culture was centrifuged at 9,000 ×g for 10 min.

The pellet was washed once with ice-cold 1 mol/l NaCl, 10 mmol/l EDTA solutions. Finally, the pellet was suspended in 1 ml of 10 mmol/l KCl. Optical Density

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(OD) was measured at 600 nm and the suspensions were stored at −20℃ until bioassay. All steps after centrifugation were done on ice to limit proteolysis.

2.4. Bioassays

In order to determine and optimize the toxicity of 26 Bt strains, three kinds of bioassays were carried out:

2.4.1. Activity against First Instar Larvae

A single-dose test was performed; about 50 μL of the culture of each strain corresponding to $1.9 \cdot 10^8$ UFC/mL added onto 2 g of the artificial diet surface contained in 24-well polystyrene plates. One-first instar larvae of *C. capitata* was added per well, and the mortality was recorded after 7 days of incubation at 25℃ in dark conditions. Two replicates of 24 larvae were conducted for each Bt strain.

B. thuringiensis subsp. *israelensis* HD567 strain (Bti) (B.G.S.C. code 4Q1) and Skeetal, a commercial product based on Bti and reported to be active against dipterans, were used as standard controls. Sterile distilled water was used as a negative control.

2.4.2. Activity against Third Instar Larvae

Five third instar larvae were transferred to plastic recipients (30 mm diam × 70 mm depth) containing 10 g of diet (see insect rearing) mixed with the toxin, except for the control group, which had just received water with the diet. Three doses of Bt spore-crystals (50 μL/g, 100 μL/g and 150 μL/g) were used in five repetitions for each strain to confirm the results. The recipients were covered with nylon mesh, and fixed around the edges with a rubberband.

After 48 h of exposure, the number of dead larvae was counted and the pupae were separated from diet and then transferred to Petri dish (9 cm), until adult emergence. Bioassay tests were carried out to determine LC_{50} values of the Bt strains against *C. capitata* larvae.

2.4.3. Activity against *C. capitata* Adults

20 adults newly emerged (1-2 days old) were kept in the cage (25 cm by 25 cm) containing artificial diet (see insect rearing) mixed with 333.33 μL/g of spores and crystals mixture. The sweet substances (hydrolyzed protein and sugar) was used in the bioassay to attract the adults recently emerged to feed Bt spore-crystal. Water was provided to the flies via a damp yellow sponge. For each toxin, a negative control was prepared with distilled water and fly feeding. Three replicates per assay were carried out. Fly mortality was recorded daily for 7 days.

2.5. Statistical Analysis

The mortality assay was calculated according to the Abbot's formula:

Mortality corrected % = (1 − n in T after treatment/n in Co after treatment) * 100

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

The LC_{50} regression equation and the 95% confidence limit were calculated by using Probit analysis. Percentages of mortality obtained from bioassay tests were analyzed using one-way analysis of variance (ANOVA, $p < 0.05$). Tukey's test ($p < 0.05$) was used to analyze f significant differences among the Bt strains tested against *C. capitata*.

3. Results

3.1. Activity against First Instar Larvae

Results of the larvicidal effectiveness of 26 Bt strains are shown in **Table 2**.

The spore-crystal mixture of selected strains showed significantly larvicidal activity (df: 27, F: 10.42, $p < 0.005$ at 95%). The mortality rate was ranging from 8.33 to 100%, obtained after 7 days of treatment with single dose 25 μL/g. The toxin produced by Bt 13.4 strain showed the highest effect, with 100% of corrected mortality. The effect of Bt 2.1 strain was the lowest, with 8.33% as percentage of larval mortality. The reference strain 4Q1 (45.83%) and Skeetal (60.83%) caused mortality to the same degree with some of our strains or low like Bt M-Ag 2.7 (47.92%).

3.2. Activity against Third Instar Larvae

Screening for toxicity against *C. capitata* adults was performed using spore-crystal mixture. In **Table 3**, the results of bioassays on the biological activity of Bt strains tested against *C. capitata* third instar larvae are presented.

The susceptibility of third instar of *C. capitata*, evaluated as percentage of larvae that did not survive to adulthood, varied significantly between 0-8%, 8-48% 20-68% at dosages of 50 μL/g, 100 μL/g and 150 μL/g respectively (**Table 3**). At 150 μL/g, Bt A7 and Bt M-Ag 21.6 strains proved most effective activity (68%) whereas Bt A10 strain showed the lowest corrected mortality (20%).

The LC_{50s} determined after 48 h of application were ranging between 1.115 μL/g for Bt A7 and 3.199 μL/g for Bt A14. Significant differences were noted with tested doses (df: 2, F: 49.97, $p < 0.005$ at 95%).

3.3. Activity against *C. capitata* Adults

Table 4 shows biological activity of the spore-crystal mixture of Bt strains in newly emerged adults of *C.*

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Table 2. Entomopathogenic activity of the spore-crystal mixture of Bt strains against newly emerged larvae of *C. capitata*.

Bt strains	Mortality[a] (%) ± SE	95% Confidence Limits	
		Lower	Upper
Skeetal	60.83 ± 0.04824^{bcd}	0.778	0.6725
4Q1	45.83 ± 0.05924^{abd}	0.3316	0.6228
Bt A7	95.83 ± 0.02915^{a}	0.8997	0.8997
Bt M-Ag 4.1	85.42 ± 0.05148^{abc}	0.7506	0.9577
Bt 32.3	87.50 ± 0.04824^{abc}	0.778	0.972
Bt A14	66.67 ± 0.06876^{hi}	0.528	0.805
Bt A10	77.08 ± 0.0613^{1i}	0.6475	0.8942
Bt M-Ag 2.2	85.42 ± 0.03531^{abc}	0.8665	1.0085
Bt M-Ag 26.4	77.08 ± 0.06131^{bcd}	0.6475	0.8942
Bt M-Ag 21.6	77.08 ± 0.06131^{bc}	0.6475	0.8942
Bt M-Ag 2.4	87.50 ± 0.04824^{abc}	0.778	0.972
Bt A9	75.00 ± 0.06316^{cde}	0.6229	0.8771
Bt A11	66.67 ± 0.06876^{efg}	0.5283	0.805
Bt M-Ag 2.1	8.33 ± 0.04031^{fgh}	0.0022	0.1644
Bt M-Ag 2.7	47.92 ± 0.07287^{ghi}	0.3326	0.6258
Bt 7.16	62.50 ± 0.07062^{efg}	0.4829	0.7671
Bt 11.3	66.67 ± 0.0719^{cde}	0.4387	0.728
Bt 32.7	75.00 ± 0.06316^{cde}	0.6229	0.8771
Bt 10.3	95.83 ± 0.02915^{abc}	0.8997	1.017
Bt 13.4	100.00 ± 0.0000^{abc}	1.000	1.000
Bt 16.1	79.17 ± 0.05924^{bcd}	0.6725	0.9108
Bt B1	81.25 ± 0.05693^{bcd}	0.698	0.927
Bt B6	83.33 ± 0.04824^{fgh}	0.778	0.972
Bt B9	83.33 ± 0.05436^{fgh}	0.724	0.9427
Bt M-Ag 1.7	66.67 ± 0.06876^{abc}	0.5283	0.805
Bt M1	79.17 ± 0.04824^{abc}	0.778	0.972
Bt M5	68.57 ± 0.06761^{hi}	0.5515	0.8235
Bt M12	87.50 ± 0.04824^{cdi}	0.778	0.972

a: [a,b] Mean values from the same row with different letters in superscript are significantly different according to Tukey test ($p < 0.05$). Means followed by the same letter in columns are not different from each other by the Tukey's test at 5% significance.

Table 3. Entomopathogenic activity of the spore-crystal mixture of Bt strains against last instar larvae of *C. capitata*.

Strains	Mortality (%) ± SE			LC_{50}	95% Confidence Limits
	50 µL/g	100 µL/g	150 µL/g		
Skeetal	0 ± 0.0000	36 ± 1.30384	52 ± 1.14018	1.328	1.143 ± 1.650
4Q1	0 ± 0.0000	28 ± 0.89443	40 ± 0.0000	1.351	1.144 ± 1.833
Bt A7	8 ± 54772	48 ± 54772	68 ± 54772	1.115	0.938 ± 1.370
Bt M-Ag 4.1	1 ± 0.0000	40 ± 0.0000	60 ± 0.0000	1.250	1.067 ± 1.554
Bt 32.3	4 ± 0.44721	36 ± 0.44721	60 ± 0.0000	1.268	1.028 ± 1.833
Bt A14	4 ± 0.44721	12 ± 0.54772	24 ± 0.83666	3.199	1.738 ± % 100000002.000E + 12*
Bt A10	0 ± 0.0000	8 ± 0.54772	20 ± 0.70711	2.394	1.656 ± 4815083.000
Bt M-Ag 2.2	4 ± 0.44721	44 ± 0.44721	60 ± 0.0000	1.212	1.007 ± 1.592
Bt M-Ag 26.4	8 ± 0.54772	28 ± 0.54772	40 ± 0.0000	1.852	1.298 ± 11.893
Bt M-Ag 21.6	0 ± 0.0000	44 ± 0.44721	68 ± 0.54772	1.164	1.001 ± 1.381
Bt M-Ag 2.4	8 ± 0.54772	36 ± 0.44721	60 ± 0.70711	1.268	1.028 ± 1.833
Bt A9	0 ± 0.0000	28 ± 0.54772	40 ± 0.70711	1.604	1.297 ± 2.886
Bt A11	0 ± 0.0000	24 ± 0.44721	32 ± 0.54772	1.847	1.413 ± 5.420
Bt M-Ag 2.1	0 ± 0.0000	28 ± 0.54772	32 ± 0.54772	1.164	1.001 ± 1.381
Bt M-Ag 2.7	0 ± 0.0000	16 ± 0.44721	32 ± 0.54772	1.872	1.458 ± 6.085
Bt 7.16	0 ± 0.0000	20 ± 0.70711	36 ± 0.44721	1.743	1.390 ± 3.959
Bt 11.3	0 ± 0.0000	16 ± 0.44721	52 ± 0.54772	1.464	1.273 ± 2.005
Bt 32.7	0 ± 0.0000	16 ± 0.44721	53 ± 0.54772	1.464	1.273 ± 2.005
Bt 10.3	4 ± 0.44721	24 ± 0.83666	60 ± 0.70711	1.360	1.137 ± 1.877
Bt 13.4	0 ± 0.0000	32 ± 0.54772	64 ± 0.44721	1.264	1.094 ± 1.532
Bt 16.1	4 ± 0.44721	16 ± 0.44721	52 ± 0.54772	1.548	1.260 ± 2.603
Bt B1	4 ± 0.44721	20 ± 0.70711	52 ± 0.54772	1.515	1.231 ± 2.490
Bt B6	0 ± 0.0000	8 ± 0.54772	44 ± 0.44721	1.574	1.376 ± 2.400
Bt B9	0 ± 0.0000	12 ± 0.54772	40 ± 0.70711	1.654	1.391 ± 3.203
Bt M-Ag 1.7	0 ± 0.0000	36 ± 0.83666	56 ± 0.44721	1.313	1.117 ± 1.695
Bt M1	0 ± 0.0000	28 ± 0.54772	56 ± 0.44721	1.362	1.169 ± 1.768
Bt M5	0 ± 0.0000	8 ± 0.54772	28 ± 0.54772	1.947	1.529 ± 18.077
Bt M12	4 ± 0.44721	12 ± 0.54772	52 ± 0.54772	1.580	1.288 ± 2.723

a: [a,b] Mean values from the same row with different letters in superscript are significantly different according to Tukey test ($p < 0.05$). Means followed by the same letter in columns are not different from each other by the Tukey's test at 5% significance. *: Upper limits greater than or equal to 1.E20 are really infinite.

Table 4. Entomopathogenic activity of the spore-crystal mixture of Bt strains against emerged adults of *C. capitata*.

Strains	Mortality (%) ± SE	95% Confidence Limits	
		Lower	Upper
Skeetal	48.33 ± 0.2801^{bc}	0.7967	1.9652
4Q1	33.33 ± 0.25332^{bc}	0.424	1.4808
Bt A7	81.66 ± 0.52834^{a}	1.4217	3.6259
Bt M-Ag 4.1	80 ± 0.43799^{a}	1.2292	3.0565
Bt 32.3	70 ± 0.371^{c}	1.3213	2.8691
Bt A14	68 ± 0.55838^{c}	1.2162	3.5457
Bt A10	60 ± 0.347ca	1.1333	2.581
Bt M-Ag 2.2	80 ± 0.53282^{a}	1.3647	3.5876
Bt M-Ag 26.4	70 ± 0.4555^{c}	1.4784	3.3787
Bt M-Ag 21.6	80 ± 0.4555^{a}	1.4784	3.3787
Bt M-Ag 2.4	80 ± 0.59074^{a}	0.9106	3.3751
Bt A9	60 ± 0.4555^{ca}	1.4784	3.3787
Bt A11	56.66 ± 0.347^{d}	1.1333	2.581
Bt M-Ag 2.1	68.66 ± 0.32819^{ca}	1.1249	2.4941
Bt M-Ag 2.7	78.66 ± 0.371^{a}	1.3213	2.8691
Bt 7.16	75 ± 0.56625^{a}	1.1522	3.5145
Bt 11.3	70 ± 0.45848^{c}	1.3293	3.2421
Bt 32.7	75 ± 0.59074^{a}	0.9106	3.3751
Bt 10.3	81.66 ± 0.30971^{a}	1.6397	2.9318
Bt 13.4	80 ± 0.53282^{a}	1.3647	3.5876
Bt 16.1	80 ± 0.4555^{a}	1.4784	3.3787
Bt B1	80 ± 0.4555^{a}	1.4784	3.3787
Bt B6	75 ± 0.40908^{a}	1.4324	3.139
Bt B9	80 ± 0.26342^{a}	1.8791	2.978
Bt M-Ag 1.7	81.66 ± 0.4555^{a}	1.4784	3.3787
Bt M1	81.66 ± 0.53282^{a}	1.3647	3.5876
Bt M5	60 ± 0.347^{ca}	1.1333	2.581
Bt M12	80 ± 0.33503^{a}	1.7297	3.1274

a: ^{a,b} Mean values from the same row with different letters in superscript are significantly different according to Tukey test ($p < 0.05$). Means followed by the same letter in columns are not different from each other by the Tukey's test at 5% significance.

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capitata.

The difference of fly's mortality was significantly higher between those exposed to the spore-crystal mixtures and the ones exposed to the water control.

The majority of Bt strains killed more than 50% of *C. capitata* adults. The mortality values varied significantly between strains. They produced significant mortalities from 56.66 to 81.66% (df: 27, F: 0.68, $p < 0.005$ at 95%); when using both spore-crystal suspensions of Bt toxin after 7 days exposure time. The more toxic Bt strains caused adult mortality starting from the 2nd day or the 3rd day of ingestion.

The commercial product based on Bti (Skeetal) and the reference Bti strain (code 4Q1) were also tested against *C. capitata* adults. They were in general less efficient, showing corrected mortality about 48.33% and 33.33% respectively (**Table 4**).

4. Discussion

During sporulation, the Gram-positive bacterium, Bt forms crystalline protein inclusions, which possess insecticidal activity. These parasporal inclusions differentiate this species from other related species such as *Bacillus cereus* and *Bacillus mycoides* [23]. The crystals are generally smaller than the spores and can represent ±30% of the dry weight of the cell [24].

A total of 26 Bt strains were tested on both larvae and adults; and it was found that 100% of strains were toxic against adults of *C. capitata*, 92% on neonate larvae on adults, and 60% on third instar larvae. Neonate larvae and adults were more susceptible than third instar larvae of *C. capitata*; in fact, several strains produced high mortality only after 3 days of exposure. The toxicity of spore-crystal mixtures against third larvae was seldom high but some strains yielded 68% mortality after 48 hours of application at the doses used in these experiments.

The biological activity of Bt strains has been studied by many authors, who determined the insecticide potential of obtained toxins using both spore and crystal. Our results are similar to that reported by Gingrich [18]. This author have tested 94 strains of Bt and found that 15 of them killed at least 80% of *C. capitata* adults that fed pellets for 9 days.

Hassani and Gaouar Benyelles [19] have tested the effect of the preparation of Bti on wild third instar larvae and adults of *C. capitata* isolated from *Citrus* fruit orchards in Algeria, and observed toxicity in high doses (100 mg/g) with a reduction in average emergence (84.62%), concluding that the stage L_3 and adults of the pest are very susceptible at this dose of Bti product.

Molina *et al.* [25] have tested sporulated cultures of 115 bacterial strains of *Bacillus pumilus* (4.65×10^8 to 1.45×10^7 CFU/mL) against adults and neonate larvae of *C. capitata*. None of these strains were caused significant mortality of *C. capitata* adults compared with the negative controls. The mortality rates with the 115 bacterial strains at the end of the experiment ranged from 0% to 40%, while the average mortality rates with the negative controls ranged from 5% to 30%. Similar results were obtained in bioassays with larvae, the maximal corrected mortality rates with the 115 bacterial isolates ranged from 0% to 36%, while the average mortality rates with the negative controls ranged from 1% to 12% after 15 days, at the end of the experiment. After this toxicity screening, they obtained a novel *Bacillus pumilus* strain, which is highly toxic to *C. capitata* larvae. The mortality rate for *C. capitata* larvae ranged from 68 to 94% depending on the conditions under which the culture was kept before the bioassay.

Karamanlidou *et al.* [14] and Yamvrias and Anagnou [26] have reported a mortality > 80% when they used various Bt strains against old larvae of the dipteran olive fruit fly, *B. oleae* (Gmelin). Robacker *et al.* [16] evaluated the action of 55 Bt strains on larvae of Mexican fruit fly, *Anastrepha ludens* (Loew). Only 7 strains were found toxic on adults; the centrifuge pellets or precipitates of Bt strains have killed > 50% of larvae. The mortality rates varied between 4 to 62% after application of the pellets of these strains against adult flies. Only 5 of them killed 65-80% of adults in 10 days compared with 2.7% mortality in controls. The other killed 40% of adults in the same experiment.

Alberola *et al.* [15] studied the Bt activity against second instar of *B. oleae* larvae and new emerged adults. These authors have reported that both spore-crystal mixtures (10^9/mL) caused 70% mortality for larvae in 72 hours and 80% of mortality for adult flies in 6 to 10 days of application.

The obtained results during our study showed that some Bt strains were most toxic against adults than on larvae and vice versa. For example, the insecticidal activity of Bt M-Ag 2.1 against larvae of *C. capitata* is 8.33, whereas the activity of this strain towards *C. capitata* adults is 68.66 (**Tables 2** and **4**). The genes which coding for insecticidal activity against the two stages of the medfly, could be different [15,16]. Alberola *et al.* [15] have also reported that there are some different chemical conditions in the gut of the two stages of the insect that can control the activation or activity of the protoxin.

On the other hand, some Bt strains expressed high activities against the both stages of *C. capitata* such as Bt

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A7, Bt 13.4, Bt M-Ag 4.1, Bt M-Ag 2.2, Bt M-Ag 21.6 (**Tables 2** and **4**).

5. Conclusions

Sustainable agriculture will rely increasingly to biological control of pests such as *C. capitata* that is considered as a quarantine pest over the world and particularly in the Argan area. No control program has been undertaken until now against *C. capitata* in the Argan forest. The use of biopesticides is environmentally friendly and reduces the contact of human to chemical pesticides. The present study provides evidence for the insecticidal activity of Moroccan Bt strains against *C. capitata*. Some Bt strains showed a great activity against neonate larvae, third instar larvae and especially towards adults of *C. capitata*. A number of our collection Bt strains showed high insecticidal activity against *C. capitata* in comparison to that noted for the Bti HD567 strain and the commercial product Skeetal previously used in the control of harmful fruit flies. Some of our Bt strains can be used in the biological control system to fight against *C. capitata* and may contribute to reduce the use of chemical insecticides harmful to the consumers and the environment.

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