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Efficacy of Toxins and Spores of a Local Rhizobacterial Strain *Bacillus clausii* (MT305787) against the Wax Moth *Galleria mellonella*

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Abstract

To reduce the dependence of the agricultural sector on chemical inputs, the use of biopesticides is becoming increasingly effective and advocated, the formulation of biocides based on microorganisms is a more efficient solution. Our work is based on exploiting the larvicidal potential of a bacterial strain (B8) of the genus Bacillus against the fifth instar larvae of the hive moth Galleria Mellonella. The bacterial strain used in the current study was isolated from medlar rhizosphere in the region of Boumerdes (located in the north of Algeria). 16s RNA sequencing and phylogenetic analysis affiliate it with the species Bacillus clausii.

The first part consists in highlighting the production of enzymes and toxins involved in the biocontrol of pests. Afterward, the insecticidal activity of the bacterial spores and toxins was evaluated against G. mellonella. The effect of the bacterial strain on the hemolymphatic metabolites (proteins, lipids, and carbohydrates) of the larvae was also studied. The insect was reared under controlled conditions, four concentrations of the bacterial spores were prepared and tested on the larvae (C1= 3.10^5 sp/ml, C2= 2.10^4 sp/ml, C3= 7.10^3 sp/ml, C4= 3.10^3 sp/ml) and three bacterial toxins concentrations were tested on the larvae (C1 = 15µl / ml, C2 = 30µl / ml and C3 = 60µl / ml). The obtained results indicate that the spores and toxins of the bacterial strain have a significant effect on G. mellonella larvae causing very remarkable symptoms (a strong decrease in appetite and malformations). The mortality rates vary according to the tested concentration. The toxin's acid extract gave a better effect than the toxin's alkaline extract and the bacterial spores. On the other hand, the determination of hemolymphatic metabolites revealed a decrease in protein, carbohydrate and lipid contents.

Keywords: *Bacillus clausii, entomopathogenic effect, Galleria mellonella, spore, toxin.*

1. INTRODUCTION

The Greater wax moth *Galleria mellonella* (Lepidoptera- Pyralidae) is a very common insect in the Mediterranean basin. It attacks beehives and damage the combs with in active bee colonies and in storage condition. Larvae feed on wax, pollen, honey and can bore a tunnel around the base of the wax comb. Larvae produce silk fibers that can trap bee brood cells. They can also transmit pathogenic diseases to bees, such as American foulbrood [1, 2, 3]. In addition, damage to the cappings, together with the presence of faeces and other debris, can reduce the yield and saleability of bee products, especially honey [4].

Indeed, the most effective method of protection against wax moth is the action of the bees themselves. However, this is not enough, so beekeepers need to manage their colonies using environmentally-friendly methods. Today, there is considerable interest in finding alternatives to the chemical pesticides to eliminate the pests and phytopathogens [2, 5, 6].

Moreover, the rhizosphere is an area that harbors many beneficial bacteria. These bacteria are of immense agronomic interest. On the one hand, they can help plants by producing growth-promoting phytohormones with other bioactive compounds [7]. On the other hand, many rhizobacteria agents of biocontrol have been identified, in particular bacteria of the genus Bacillus [8]. The genus *Bacillus* represents a large group of sporulating, Gram-positive bacteria belonging to the phylum Firmicutes, with optional aerobic or anaerobic metabolism [9].

From a biotechnological point of view, the most important feature of *Bacillus* species is their diverse secondary metabolism and their ability to produce a wide variety of structurally different antagonistic substances that may have a key role in biological control of phytopathogenic [7], another interest has emerged in recent years which is the entomopathogenic effect of these rhizobacteria belonging to the *Bacillus* genus.

Therefore, the purpose of the present study is the search for a rhizobacterial strain effective against the wax moth *G. mellonella.*

2. MATERIAL AND METHODS

A. Presentation of the bacterial strain

The strain used in this study is *Bacillus clausii* strain B8 (MT305787) isolated from the rhizosphere of medlar (*Mespilus germanica* L.- family Rosaceae) at the region of Boumerdes (coastal region of northern Algeria; latitude: 36° 76′ 75″ North, longitude: 3° 70′ 29″ East). According to a previous study [10], this bacterium that grow at pH7-8 with a growth temperature of 15-50 °C, presents positive reactions for hydrolysis of gelatin, casein, starch and nitrate reduction.

This strain presents very interesting agronomic traits, it is able to produce lytic enzymes (cellulases, phosphatase, phospholipase) with an intense production of siderophores and cyanhydric acid (HCN). These enzymes and molecules are very important for the biocontrol of phytopathogens and pests [10].

B. Search for enzymes involved in biocontrol Chitin hydrolysis enzyme

- Preparation of colloidal chitin: The preparation of colloidal chitin was performed according to the modified protocol of Hsu and Lockwood [11] and Gomez Ramirez et al. [12].

10 grams of ground chitin is treated with 75 ml of concentrated HCl (~12M). Hydrochloric acid is added gradually, with continuous stirring for 60 minutes in a chemical hood and at room temperature (25° C). The chitin-HCl mixture is left in the open air or in a chemical hood for half an hour. The clear filtrate obtained is then poured into two litters of cold distilled water to allow the precipitation of colloidal chitin. The whole is put at 4° C under static conditions to facilitate the precipitation of colloidal chitin. After 12 hours, the excess water is aspirated and the precipitate is passed through two layers of filter paper, housed in a funnel that is placed in a filtration flask. The filtrate retained in the paper is then washed extensively with distilled water (~3L) until the pH is neutralized (pH~7).

The resulting colloidal chitin was pressed between filter papers, then placed in a 100 mL glass beaker covered with two layers of aluminium and sterilized by standard temperature and pressure (STP) autoclaving (15 psi, 20 minutes, 121°C). Autoclaved colloidal chitin was stored at 4°C until further use in wet form.

- Detection of chitinase: In order to study the hydrolysis of chitin, 2 different media were prepared, one containing colloidal chitin and a medium containing non-colloidal chitin:

Medium 1: The medium used is composed in g/l of: colloidal chitin: (0.6 - 0.8); K2HPO4 (2.7); KH2PO4 (0.3); MgSO47H2O (0.7); NaCl (0.5); KCl (0.5); Yeast extract (0.13); Agar (15). After plating with spots, incubation lasts for a minimum of 7 days at 30°C.

Chitinase activity is manifested by the appearance of a transparent halo around the discs.

Medium 2: The test is performed on agar medium supplemented with 10g of non-colloidal chitin. Inoculation on medium is performed by depositing clusters of bacteria on the surface, after incubation for 72h at 30°C the

reaction is obtained. A positive reaction is manifested by the appearance of a white halo around the bacterial colonies [13, 14].

Lipides hydrolysis enzyme

For the detection of lipolytic enzyme production, Tween has been used as a lipid substrate in the medium [15]. The principle of this method is based on the precipitation of crystals of the calcium salt of the fatty acid under the influence of a lipase. After incubating for 24 hours at 30°C, the precipitation of crystals will be visible as an opaque halo around the colonies in case the strain has lipolytic activity.

C. Toxin extraction assay and thin layer chromatography

Toxin extraction undergoes goes through several steps [16]. From a young culture, bacterial colonies were collected and deposited in test tubes containing 9ml of sterile physiological water and incubated 24h at 37°C. Next 5ml of each suspension was placed in sterile bottles containing 150ml of sterile nutrient broth and closed with sterile cotton and aluminum foil and incubated for three days at a temperature of 30°C under permanent agitation (180rpm). After incubation, the cultures are centrifuged at 5000 rpm for 15 to 20 minutes. This operation allows the separation of the supernatant and the pellet.

Alkaline extraction

To 100ml of the sample 10ml of NaOH is added at a sufficient normality (10N). After shaking, the pH paper is used to test the alkalinity. An equivalent volume of dichloromethane is added.
After several gentle shakings for two minutes the organic phase (dichloromethane) (lower phase) is filtered on anhydrous sodium sulfate.

Acid (H+) extraction

To 100ml of the sample, 10ml of HCl is added at a sufficient normality (6N) to reach a pH close to 2. After shaking, we use the pH paper to test the acidity. Then we add an equivalent volume of ether. After shaking for 2min, the aqueous phase (sample) (organic phase) (upper phase) is recovered on anhydrous sodium sulfate.

Separation and revelation of toxins by thin layer chromatography (TLC)

- Preparation of the tank: the tank used contains 100ml of solvent system, which is filled with a mixture of solvents which contains: methyl acetate, isopropyl alcohol, chloroform, methyl alcohol methyl alcohol, 0.5% aqueous KCL solution (25, 25, 25, 10, 9 v /v/v/v), and its atmosphere is saturated for 2h before is saturated for 2h before the introduction of the silica gel plates.

- Plate preparation: the toxin's extracts are deposited as spots of 20 µl at 2 cm from the bottom edge of the plate and 2cm from the lateral edges. The deposit is done by small fractions with a micropipette while drying progressively under cold air stream.

- Elution: when the solvent front arrives at about 2 cm from the upper edge of the plate, the migration is stopped, the opaques are removed from the tank, the solvent front is then noted. The plate is dried at room temperature.

- Revelation: the revelation is carried out under UV at 350 nm, to localize the different spots. The results are read by calculating the characteristic migration constant of each chemical species in a given chemical species in a given stationary phase/mobile phase system is called the ratio (Rf). The frontal ratio (Rf) is calculated by the following formula:

$$Rf = \frac{\text{Distance traveled by the solvent (cm)}}{\text{Distance traveled by the stain (cm)}}$$

D. Treatment of G. mellonella larvae with bacterial spores and toxins

Rearing of the wax moth larvae

A *G. mellonella* larvae were recovered from infected frames and introduced into glass jars and maintained at 32°C. The larvae were fed with a mixture consisting of 300 ml of liquid honey, 150 ml of glycerin, 200 g of milk powder, 200 g of whole meal flour, 100 g of inactive brewer's yeast, 400 g of cornmeal and 250 g of wheat bran. To accelerate their development, they were also fed finely ground pollen mixed with a honey solution (proportion 2/3, 1/3) and made into balls [2]. After emergence, the butterflies are recovered and transferred to other glass jars that have been covered with pleated paper for mating and egg laying.

Preparation of sporulation broth

Pure colonies were inoculated into test tubes containing 5 ml of sporulating broth (Minimal Basal Salts, MBS - 0.68% KH_2PO_4,0.03% $MgSO_4$-$7H_2O$, 0.002% $MnSO_4$, 0.002%$Fe_2(SO_4)_3$, 0.002% $ZnSO_4$-$7H_2O$, 0.002%$CaCl_2$, 1% tryptone, and 0.2% yeast extract, pH 7.2) [17]. The tubes were then incubated at 30°C with agitation (200 rpm) for 48 h to allow sporulation and crystal formation [18, 19].

Bioassays

Concerning the biological treatments, the protocol followed is demonstrated in the two previous studies by Oulebsir-Mohandkaci *et al.* [3] and Benzina *et al.* [20].

Concerning sporal treatment, four concentrations were prepared, C1= 3.10^5 sp/ml, C2= 2.10^4 sp/ml, C3= 7.10^3 sp/ml, C4= 3.10^3 sp/ml. For toxins, three concentrations were prepared, C1= 100 µl/ml, C2=60 µl/ml and C3=20 µl/ml, dilutions were made in sterile distilled water.

The treatment took place by trophic exposure. Signs of infection and mortality were monitored daily for 7 days. Controls were treated with sterile distilled water. The tests were performed in triplicate.

The recorded mortality data were corrected using Abbott's formula and expressed as percentages [21]. The dose-mortality response was analyzed afterwards by probit analysis to give LC50 and LT50 values [22].

Quantitative determination of hemolymphatic metabolites in G. mellonela larvae

To introduce the bacterial spores, the individuals were injected at their abdominal extremity by the suspension. Hemolymph sampling was performed 2h, 6h and 12h after the application of the treatment following a light puncture applied at the level of the abdominal extremity of the insect.

Techniques for the determination of proteins, carbohydrates and lipids in the hemolymph are provided by Oulebsir-Mohandkaci *et al.* [3]. The proteins were determined according to the Bradford method using Coomassie blue. The carbohydrate dosage was performed using the anthrone and sulfuric acid. For the determination of hemolymphatic lipids, the assay uses the reagent of vanillin and orthophosphoric acid.

E. Statistical Analysis

The ANOVA and Tukey 's LSD test were used for insect mortality and value $p < 0.05$ was considered significant. This treatment was carried out using SPSS v. 25.0 programs.

3. RESULTS AND DISCUSSION

After incubation at 30°C for 24h, *Bacillus clausii* strain B8 (MT305787) showed visible distinct colonies with specific morphological criteria. Microscopic observation of cells (Gram staining) showed rod-shaped and Gram-positive bacteria, with central spores non-deforming the vegetative cell (fig. 1).

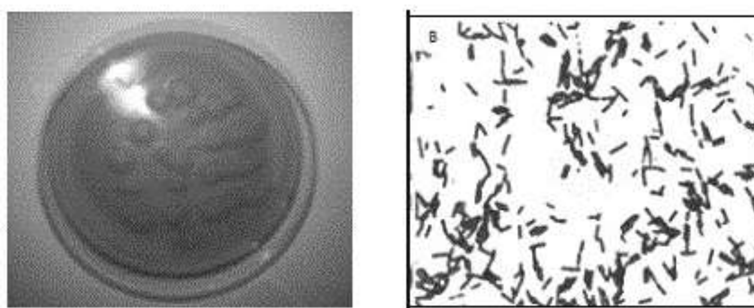

Fig. 1. Macroscopic and microscopic characteristics of the strain B8.

A. Highlighting chitin and lipide hydrolyzing enzymes

The hydrolysis of chitin results in the appearance of a clear halo around the colonies after plating on a minimal medium containing chitin as the only carbon and energy source, and incubation at 30°C. The results indicate that strain B8 is positive after plating on the medium containing colloidal chitin after 7 days of plating and on the medium containing non-colloidal chitin after 72h of plating.

For the lipide hydrolysis enzyme, strains that exhibit lipolytic activity will show up as crystal precipitation that will be visible as an opaque halo around the colonies. The result of lipase production by strain B8 indicates that it shows a positive reaction to the lipase test (fig. 2).

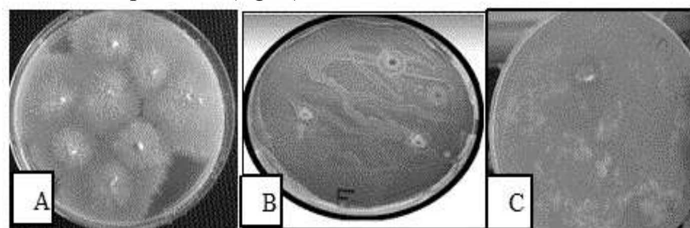

Fig. 2: Response of strain B8 to chitin degradation on medium containing colloidal chitin (A), and medium containing non colloidal chitin (B) and lipide degradation (C)

B. Extraction of toxins, separation and revelation by chromatography

The extracts of toxins obtained from alkaline medium and on acid medium are kept in eppendorf tubes in the refrigerator until they are used. Concerning the thin layer chromatography, the migration lasted approximately 5h. The results show that the toxins of the alkaline extract present an Rf= 1.00632 while the acid extract presents two spots corresponding to Rf1= 1.01273 and Rf2= 1.84883).

C. Study of the pathogenicity of the B8 strain on Galleria mellonella larvae

Effectiveness of bacterial spores

Mortalities were obtained in larvae treated with the high concentration ($3*10^5$ sp/ml) from the first day. This mortality rate evolves in time to reach 100% on the 8th day. On the other hand, for the low concentration of sporal treatment, a total mortality was recorded on the 13th day (fig. 3).

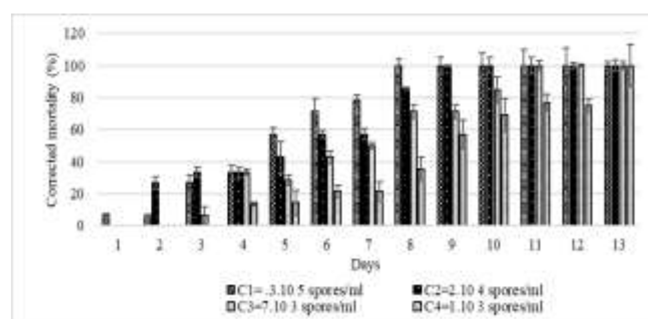

Fig. 3. Corrected mortality rate in *G. mellonella* larvae treated with B8*. *ANOVA reveals a significant difference at the 5% level (p<0.013674) between doses, as well as for time (p<0.003700). The tukey test divides the applied concentrations into three groups; (Control), (C1-C2) and (C3-C4).

Effectiveness of bacterial toxins (alkaline and acid extracts)

After treatment of *G. mellonella* larvae with both alkaline and acid extracts, the percentage of mortality varies and evolves according to concentration and time, we notice that the most important mortality rate is obtained by treating the larvae with the concentration C1 = 100 µl/ml of the toxin's acid extract with 77.77 % of corrected mortality after only 4 days. The same percentage was obtained with the same concentration of the toxin's alkaline extract after 6 days (fig4).

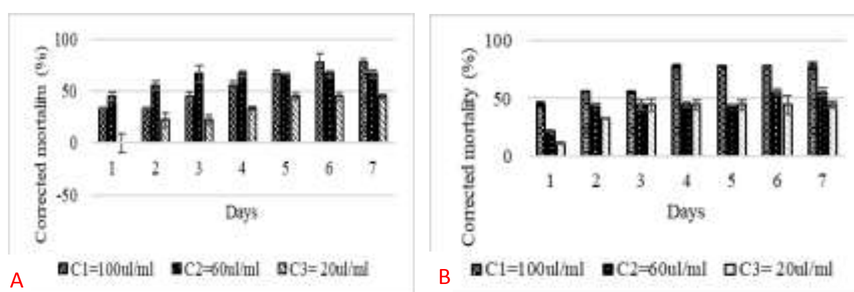

Fig. 4: Percentage of corrected mortality of *G. mellonella* larvae obtained 7 days after treatment with toxin's alkaline (A) and acidic (B) extract of B8 strain. *ANOVA reveals a highly significant difference at the 5% threshold (p<0.000 for concentration, and p<0.004 for time).

The dose-response effect is clearly marked in treated larvae by toxins. The results for C2 and C3 are close compared to C1.
This is confirmed by the tukey test which divides the applied concentrations into three groups; (Control), (C1) and (C2-C3) (fig. 5).

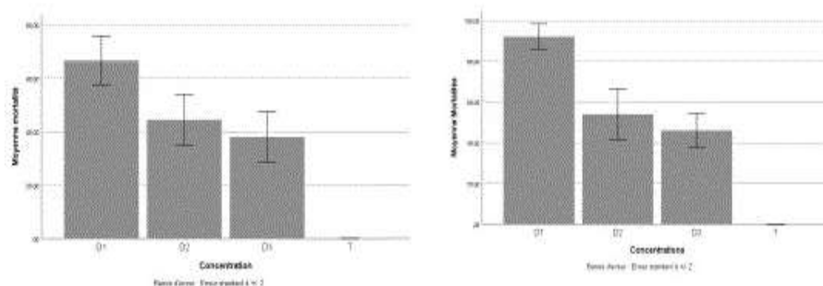

Fig. 5: Average mortalities obtained as a function of the concentration applied with alkaline (left) and acidic (right) extract of B8 strain toxins.

LT50
The lethal times after which 50% of the treated individuals of *G. mellonella* died increase with decreasing concentrations. The lowest value is 1.462 days for the C1 concentration (100µl/ml) of the toxin's acid extract. On the other hand, the highest value is 9.33 days obtained following the treatment with bacterial spores at the concentration C4= 1.10^3 (table I).

Table I: LT50 values of *G. mellonella* larvae treated with the two toxin extracts and the bacterial spores

LT50 (by days)		**Concentrations**		
Bacterial toxins		C1 = 100ul/ml	C2=60ul/ml	C3=20ul/ml
	Alkaline extract	2,371	2,7497	5,445
	Acid extract	1,462	5,0350	6,558
LT50 (by days)	Concentrations			
Bacterial spores	C1= 3.10^5sp/ ml	C2= 2.10^4sp/ml	C3= 7.10^3sp/ml	C4= 1.10^3sp/ml
	1.99	2.88	5.75	9.33

LC50
The effectiveness of bacterial treatments was expressed as LC50 values. We notice according to the results obtained, that the lethal concentration for 50% of the individuals is variable according to the time and the treatment; the LC50 decrease with the time. We note that the lowest LC50 values are obtained on day 6 for the three-treatment applied (table II).

Table II: LC50 values obtained in *G. mellonella* larvae treated with the two toxin's extracts and the bacterial spores.

LC50		**After 2 days**	**After 4 days**	**After 6 days**
Bacterial toxins (ul/ml)	Alkaline extract	162,03	45,49	26,78
	Acid extract	77,26	35,97	30,61
Bacterial spores (spores/ml)		$25{,}11.\ 10^3$	$1{,}48.10^3$	$7{,}41.10^2$

Evaluation of the effect of strain B8 on the hemolymphatic composition of G. mellonella
Concerning proteins, the comparative examination of the results obtained in the L5 larvae of *G. mellonella* (the control and the treated individuals) shows a clear increase in the controls compared to the treated ones, the dose-effect relationship is very marked.

It is also noted that the concentration of hemolymphatic lipids in treated larvae is significantly lower than in controls. This decrease is closely related to the concentration of treatment applied and is more marked after 12 hours. Lower concentrations of carbohydrates are recorded in the larvae treated with the three concentrations of strain B8 compared to the controls and the decrease is more marked after 6 and 12 hours (table III).

Table III: Strain B8-related changes in total protein, lipid and carbohydrate content (ug/ul) of *G. mellonella* larvae

Bacterial strain B8	Protein (ug/ul)			Lipid (ug/ul)			Carbohydrate (ug/ul)		
	2 h	6 h	12 h	2 h	6 h	12 h	2 h	6 h	12 h
Control	22	22±0,15	20,8	0,15	0,14±0.01	0,013	56,8	53,7	46,05
C1= 3.10^5 Sp/ml	20,5 ± 0,14	20,2± 0,14	18,25± 0,35	0,125± 0,01	0,075± 0,01	0,05± 0,01	56,41± 0,58	36,35± 0,49	29,26 ± 0,37
C2=2.10^4 Sp/ml	21,1± 0,14	20,25± 0,35	18,45± 0,64	0,09± 0	0,075± 0,01	0,055± 0,01	49,32± 0,45	31,145± 0,20	38,025± 0,03
C3=7.10^3 Sp/ml	21,9 ± 0,14	21 ± 0	20,25 ± 0,35	0,04 ± 0	0,035 ± 0,01	0,035 ± 0,01	48,055 ± 0,08	37,26 ± 0,37	31,085 ± 0,12

4. DISCUSSION

The involvement of rhizobacteria as biocontrol agents with a double beneficial effect, antagonistic and entomopathogenic, makes these microorganisms highly in demand today. Indeed, their entomopathogenic effect involves bioactive molecules that they produce such as toxins or hydrolytic enzymes directly targeting insects [23]. In fact, the current study aimed to evaluate entomopathogenic efficacy of a local rhizobacterial strain *Bacillus clausii* (MT305787) and its principal compounds against an important pest of the bee, *G. mellonella*.

The *Bacillus* genus is known to be a good candidate for the production of a wide range of enzymes of industrial interest. Similarly, the results we obtained show that the tested strain is a producer of the two sought enzymes, chitinase and lipase.

The results related to the chitinolytic and lipase activity show that the tested bacteria produce them. Chitinases are important enzymes involved in the control of fungi and insects. Lipases are widely distributed in Gram + bacteria, in particular in the genus *Bacillus*, the most important one. The interest in microbial lipases has been increasing due to their relatively simple manufacturing process and their stability against temperature [24].

The exploration of the relationship between extracellular enzymes activity and virulence of *Candida glabrata* isolates on *Galleria mellonella* larvae has been studied using various agar medium to detect the protease, phospholipase, and lipase activities of *Candida glabrata*. Pearson test shows that the activity of protease of *Candida glabrata* was positively correlated with the pathogenicity of *Galleria mellonella* larvae, on the contrary, phospholipase and lipase have no activity [25].

On the other hand, another molecule researched is the bacterial toxin, which plays a primary role in biocontrol especially in the insecticidal activity of some lepidopterans [20], such as the large wax moth, *Galleria mellonella*, which is a ubiquitous pest of the honey bee, *Apis mellifera* considered as one of the factors contributing to the decline of honey bee populations [26].

The extraction and characterization of the toxins of this bacterial strain indicated the presence of three spots for the acidic and alkaline extract in the chromatographic profile with different frontal ratios, which allows us to assume that they belong to different groups.

After that, healthy wax moth larvae were isolated and treated by four concentrations of bacterial spores (C1= 3.10^5 sp/ml, C2= 2.10^4 sp/ml, C3= 7.10^3 sp/ml, C4= 3.10^3 sp/m) and three concentrations of toxins (C1=100 µl/ml, C2=60 µl/ml and C3=20 µl/ml) by ingestion (oral route). For the sporal treatment. The results showed an important sensitivity to bacterial concentrations applied in particular with C1= 3.10^5 sp/ml which caused a total mortality after 8 days. We note also that the results obtained for the toxin's acid extract are better than those of the toxin's alkaline extract.

Indeed, some strains of *Bacillus thuringiensis* (B.t) have a spore and a parasporal inclusion composed of protein toxins. In the mesenteron of the insect, in the presence of a basic stomach pH (8.9), this crystal will disintegrate to release an endotoxin, which will cause paralysis of the digestive tract and an ion imbalance in the hemolymph [27, 28]. The same authors add that B.t is effective against certain species of Coleoptera, Lepidoptera and Diptera due to the basic intestinal pH. However, it would not be effective against locusts due to intestinal acidity.
The obtained results are not in agreement with these findings because the treatment of the larvae of the lepidopteran *G. mellonella* by the toxin's acid extract gave a better effect than the toxin's alkaline extract, which suggests that the toxins of *B. clausii* do not have the same properties than those of Bt.

Along with the results obtained for the mortality rate, the LT50 examination shows that the *G. mellonella* larvae treated with the toxin's acid extract of strain B8 are weaker than the toxin's alkaline extracts.

Several species of the genus *Bacillus* and related such as *Bacillus thuringiensis, Bacillus popillia, Bacillus alevi, Bacillus larvae, Bacillus lentimorbus* and *Bacillus sphaericus* have a particular capacity to induce mortality in certain insects [29]. However, *Bacillus clausii* is known as a probiotic [30] and only few studies proved its insecticidal activity ([31, 32].

Several studies indicated that the highest entomopathogenic bacterial activity were associated with production of metabolites acting as potent insecticides such as toxins, vegetative proteins, enzymes and antibiotics [33].

B. thuringiensis is the most commonly used entomopathogenic bacterium in biological control, acts by releasing toxins [27]. The interaction of *Bacillus* toxins with the receptors of the epithelial cells of the digestive system causes the death of insects following a disruption of the osmotic regulation of the cells [29, 34, 35].

Seven cyclic lipopeptide biosurfactants were isolated from the fermentation broth of endophytic *Bacillus clausii* DTM1. Bioassays showed interesting insecticidal activities of the tested molecules [36].

The composition of the hemplymph of the wax moth *G. mellonella* manifested a decrease of hemolymphatic carbohydrates, lipids and proteins concentrations in the treated larvae by the strain B8. This may be due to action of molecules produced by this bacterium especially during sporulation.

In a previous study, the production of toxins by a strain of *Aspergillus* by submerged fermentation was tested, the study indicates the production of Ochratoxin A and Fumagillin. Biotests using these mycotoxins against the wax moth have shown that they affect the pest and cause a disturbance in hemolymphatic metabolites content. The application of minimal inhibitory doses of biocontrol agents will prevent possible bee mortality [3]. Altuntas *et al.* [37] suggest that the plant growth regulator gibberellic acid (GA3) treatment affects negatively hemocyte physiology and cell immune responses inducing cell necrosis and apoptosis of *G. mellonella* larvae. In this insect, the extent of melanization of hemocytes showed noticeable differences by time.

Finally, safety assessment of *Bacillus clausii* UBBC07 were conducted in rats by Lakshmia *et al.* [38]. The acute toxicity study showed the oral LD50 to be> 5000 mg / kg (630 billion cfu / kg) body weight. These results suggest consumption of *B. clausii* UBBC07 is safe for humans.

5. CONCLUSION

The current study, showed an important biocontrol potential of the rhizobacterium, *Bacillus clausi* Strain B8(MT305787) against the larvae of the wax moth *Galleria mellonella*.

These findings further confirm the importance of using microbial compounds such as bacterial toxins and enzymes as biocontrol agents, as these compounds are able to cause severe damage to host epithelial cells, ultimately leading to the death of the insect. Furthermore, as it has been observed, they lower proteins, carbohydrates and lipids content of the hemolymph insect affecting the metabolism of the latter.

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