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EFFICIENCY OF INTERGENERIC RECOMBINANTS BETWEEN *BACILLUS THURINGIENSIS* AND *BACILLUS SUBTILIS* FOR INCREASING MORTALITY RATE IN COTTON LEAF WORM

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ABSTRACT

In this study , two strains of *Bacillus* belonging to two serotypes and four of their transconjugants were screened with respect to their toxicity against lepidopterous cotton pest. . Bacterial transconjugants isolated from conjugation between both strains were evaluated for their transconjugant efficiency caused mortality in *Spodoptera littoralis* larvae . Two groups of bioinsecticides ; crystals , crystals and spores have been isolated from *Bacillus* strains and their transconjugants . Insecticidal crystal protein (ICP) was specific for lepidopteran insects because of the toxin sufficient both for insect specificity and toxicity . The toxicities of these two groups against larvae of *Spodoptera littoralis* was expressed as transconjugant efficiency , which related to the mean number of larvae died expressed as mortality percentage . The results showed transconjugant efficiency in reducing the mean number of *Spodoptera littoralis* larvae feeding on leaves of *Ricinus communis* sprayed with bioinsecticides of *Bt* transconjugants . Most values of positive transconjugant efficiency related to increasing mortality percentage are due to toxicological effects appeared in response to the treatments with crystals + endospores than that of crystals alone . This indicated that crystals + endospores was more effective for increasing mortality percentage than that resulted by crystals . Higher positive transconjugant efficiency in relation to the mid parents and better parent was appeared at 168 h of treatment . The results indicated that recombinant *Bacillus thuringiensis* are important control agents for lepidopteran pests , as well as , susceptibility decreased with larval development . The results also suggested a potential for the deployment of these recominant entomopathogens in the management of *Spodoptera. littoralis* larvae .

Key words : Mortality percentage , Recombinant bioinsecticides , *Spodoptera littoralis* , Transconjugant efficiency .

1. INTRODUCTION

Bacterial pathogens used for insect control are spore – forming , rod - shaped bacteria in the genus *Bacillus* . They occur commonly in soils , and most insecticidal strains have been isolated from soil samples . Bacterial insecticides must be eaten by target insects to be effective ; they are not contact poisons. Insecticidal products composed of a single *Bacillus* species or subspecies may be active against an entire order of insects , or they may be effective against only one or a few species . For example, products containing *Bacillus thuringiensis* var. *kurstaki* kill the caterpillar stage of a wide array of butterflies and moths . In contrast, *Bacillus popilliae* var. *popilliae* (milky disease) kills Japanese beetle larvae but is not effective against the closely related annual white grubs (masked chafers) that infest lawns in much of the Midwest. The microbial insecticides most widely used in the United States since the 1960s are preparations of the bacterium *Bacillus thuringiensis* (*Bt*) . *Bacillus thuringiensis* products are produced commercially in large industrial fermentation tanks . The bacterial cells usually produce a spore and a crystalline protein toxin - called an endotoxin - as they develop. Most commercial *Bt* products contain the protein toxin and spores, but some contain only the toxin component . When *Bacillus thuringiensis* is ingested by a susceptible insect , the protein toxin is activated by alkaline conditions and enzyme activity in the insect's gut . If the activated toxin attaches to specific receptor sites, it paralyzes and destroys the cells of the gut wall, allowing the gut contents to enter the insect's body cavity. Poisoned insects may die quickly from the activity of the toxin or may stop feeding and die within two or three days from the effects of septicemia (blood – poisoning) . *Bacillus thuringiensis* does not reproduce and persist in the environment in sufficient quantities to provide continuing control of target pests . The bacteria may multiply in the infected host , but because few spores or crystalline toxins are produced , few infective units are released when a poisoned insect dies . Consequently , *Bacillus thuringiensis* products are applied much like synthetic insecticides . *Bacillus thuringiensis* treatments are inactivated within one to a few days in many outdoor situations, and repeated applications may be necessary for some crops and

pests [1] .

The fall armyworm (*Spodoptera frugiperda*) , that attacks various cultures is one of the most important pests of maize in the Americas [2] , causing about 20% production losses in Brazil [3] . Although the use of chemicals is the prevailing method to control this pest, problems such as ecological disequilibrium, pollution, risks during application and high costs are present [4] . Furthermore, the insecticides kill the fall armyworm natural enemies, favoring rapid reinfestation with serious damage to the culture [3] . In fact, all these questions increased the interest in alternative strategies to manage this pest .

Nowadays, some methods, solely or together, get the satisfactory control of *Spodoptera frugiperda*. Among the entomopathogenic agents used in biological control of lepidopterous pests the *Bacillus thuringiensis* Berliner bacterium (*Bt*) has gained special attention as an alternative method [5] . This microorganism acts in the insect gut due to crystals, composed by protoxins, discharged in the gut due to the alkaline pH that causes solubilization. These protoxins, in presence of digestive enzymes, are converted in toxic polypetides (delta - endotoxins). The activated toxins cross the peritrofic membrane, join to specific receptors in apical membrane of columnar cells of midgut, and insert themselves into the membrane [6] . The formation of pores disrupts the ionic gradients and osmotic balance in the apical membrane, resulting in cell swelling and lysis. This phenomenon leading to massive destruction of epithelium, causing death of larvae [7] .

Some times ago, the efficacy of this microorganism against *Spodoptera frugiperda* was considered questionable, but more recently the increment in researches on the use of *Bacillus thuringiensis* against this lepidopterous brought some interesting results [**8**] . Whitlock *et al.* [9] , found that both the standard *Bacillus thuringiensis kurstaki* (HD – 1) and the formulated commercial product resulted from this strain have shown limited pathogenicity against the tobacco cutworm (*Spodoptera litura*) . However, two new isolates of *Bacillus thuringiensis* (K-2074 and K-2178) isolated from Taiwan have been identified through an active screening program to be highly pathogenic against the tobacco cutworm . The biological activities of two species of bacteria isolated from soil of cotton fields identified as *Bacillus subtilis* strain NRC313 (*BS* NRC313) and *Bacillus thuringiensis* strain NRC335 (*BT* NRC335) were evaluated by Abd El-Salam *et al.* [10] against the third larval instar of the cotton leafworm, *Spodoptera littoralis* (Boisd.). They found that the different entomopathogenic bacteria of *BS* NRC313 and *BT* NRC335 contained 10 × 10^8 cell / ml , caused mortality of 100 and 97.3% for the above mentioned strains , respectively .

Concentrations of 2.5 × 10^8 to 10 × 10^8 cell/ml of strains *BS* NRC313 and *BT* NRC335 were applied to the larvae : LC_{50} were 3.3 × 10^8 and 3.9 × 10^8 cell/ml respectively. The influence of exposure to toxin concentrations manifested in terms of decreasing the adult emergence and prolongation of the generation period. The percentage of larvae that survived and succeeded to pupate increased by decreasing the concentration . The longevity of adult emergence that resulted from larvae treated with *Bacillus subtilis* were 6.0 ± 0.51 and 9.0 ± 0.63 days at 5 × 10^8 and 2.5 × 10^8 cell/ml, respectively compared with 9.8 ± 0.47 in control . Their results indicated that *Bacillus subtilis* was more potent than *Bacillus thuringiensis*. Field applications of *B. thuringiensis, B. subtilis* and Reldan achieved 55.6, 67.4 and 89.4% reduction of the cotton leafworm larvae , *Spodoptera littoralis* , in clover plants under field conditions . *Bacillus thuringiensis*, or simply *Bt*, is a naturally occurring soil bacterium that, when sprayed on plants, is toxic to certain pest insects . For years, farmers and home gardeners have used *Bacillus thuringiensis* as a microbial spray pesticide to control caterpillars, certain types of beetles, as well as mosquitoes and black flies . More recently, scientists have developed techniques by which traits from the *Bt* bacterium, including its ability to resist pests, can be introduced into a plant. Specifically, scientists have identified the gene that produces the toxin in *Bacillus thuringiensis* and through the use of biotechnology, have incorporated it into the genetic material of several plants . These *Bt* plants, which include corn, cotton, and potatoes, now synthesize their own bacterial protein to kill pests . Thus, farmers need not rely on external spraying . For example, use of conventional pesticides recommended for control of the European corn borer has dropped by about one -third since *Bt* corn was introduced .

Since the independent discovery of *Bacillus thuringiensis* in two lepidopteran species, *Bombyx mori* (L) and *Ephestia kuehniella* (Zeller), at the beginning of the 20th century [11] , Lepidoptera have served as the leading insect model for elucidating the mode of action and specificity of *B. thuringiensis* and its associated insecticidal toxins. A variety of lepidopteran whole-animal models, as well as , cell lines and membrane preparations derived from Lepidoptera , have been used to identify factors that enhance or inhibit *Bacillus thuringiensis* activity and define the cellular and molecular responses to *Bacillus thuringiensis* toxin . In particular, the use of lepidopteran cell culture and brush border membrane vesicle preparations to dissect the complex interactions between toxin and midgut receptors have generated a substantive understanding of the mechanisms of pore formation and defined the processes that lead to disruption of larval gut integrity [12] . In combination with studies on resistant insects, studies in these models have also

identified the specific midgut receptors involved in toxin binding, including cadherin - like proteins, aminopeptidases, and additional GPI-anchored proteins such as alkaline phosphatase [13] . These studies have also demonstrated that susceptibility to *Bacillus thuringiensis* varies among different species of Lepidoptera, in the amount of toxin required to cause mortality, the speed of mortality, and the response to toxin following ingestion [14] **.** However , *Bacillus thuringiensis* is considered ideal for pest management because of its specificity to pests and because of its lack of toxicity to humans or the natural enemies of many crop pests . This leading to induce new recombinant biopesticides via conjugation between different *Bacillus* strains . Though , this work aimed to evaluate transconjugant efficiency of recombinant *Bt* isolates with potential impact to control cotton leaf worm , *Spodoptera littoralis* , via increasing mortality of cotton leaf worm , as well as , reducing leaf damage .

2. MATERIALS AND METHODS

2.1 Microbial strains :

Bacillus thuringiensis serovar *Kurstaki* (NRRL HD-1) and *Bacillus subtilis* (NRRL NRS-744) were obtained from Dr. L.K. Nakamura, U.S. Department of Agriculture, Agricultural Research Service, U.S. Department of Agriculture, Peoria, I Ilinois. The strains were maintained on L.B. Slape medium, containing of ; 1% tryptone , 0.5% yeast extract and 0.5% NaCl , pH 7.5 [15] **.**

2 .2 Mass culturing of *Spodoptera litura*

The field collected egg masses of *Spodoptera litura* were used to initiate the mass culturing under laboratory conditions. The egg masses were kept in the egg cage. After emergence, first instar larvae were weighted and transferred to *Bt* and non *Bt* sprayed leaves in the bottles . The leaves were weighted , changed daily and the faecal pellets removed from the container every 24h . Grown up larvae were weighted daily .

2.3 Host plants

Fresh leaves of *Ricinus communis* were collected daily , squares and middle leaves were used for the experiments . Leaves were cleaned and three grams were weighted and placed in each container daily .

2.4 *Bacillus thuringiensis* formulations used in the experiment

Crystals + endospores , as well as , crystals isolated from *Bacillus thuringiensis* were used in liquid formulations using 200 µl of the suspension . The bio – insecticide were applied on 250 ml bottles , as well as , mixed with 3 grams of leaves as diet for larvae .

2.5 Antibiotic susceptibility assays

Antibiotic susceptibility was measured by plate diffusion method, according to Collins and Lyne [16] with cultures grown to logarithmic growth phase in nutrient broth of LB medium. Bacterial suspension (0.2 ml) was mixed with 10 ml of LB agar medium in petri dishes. Wells (8 mm diameter) were punched in the agar, using a stainless steel borer, and were filled with 0.1 ml of the antibiotic concentration . The plates were incubated overnight at 37°C and the diameter of resulting zones of inhibition was measured . Three replicates were used for both each bacterial strain, and concentration of antibiotics used [17]. Different antibiotics were used with the concentration of 400 µg/ml, according to Roth and Sonti [18] **.**

2.6 *rfa* mutation

Strains having the deep rough (*rfa*) character should be tested for crystal violet sensitivity [19] . For the test, nutrient agar plates are seeded with cultures of the strains to be tested and a sterile filter paper disc containing crystal violet is placed on the surface of each seeded plate by pipette 10 µl of a 1 mg/ml solution of crystal violet to the center of sterile filter paper discs (1/4 inch) . Invert the plate and incubate at 37°C. After 12 h incubation, a clear zone of inhibition (approximately 14 mm) appears around the disc indicating the presence of the *rfa* mutation which permits large molecules such as crystal violet to enter and kill the bacteria. Wild-type strains or strains containing the *gal* deletion are not inhibited because the crystal violet cannot penetrate the cell.

2.7 Conjugation

Nutrient broth cultures, in the late - exponential growth phase, were used. Quantitative spot mating of conjugal transfer was carried out, according to Lessl *et al*. [20] , by inoculating 10 µl samples of the donor (*Bt*) cultures onto the surface of selective medium, previously seeded with 100 µl of the recipient (*Bs*) culture. A single colony of transconjugants was picked up and transferred to LB slant agar medium .

2.8 Separation of crystals and endospores

Crystals and endospores were collected and purified according to Karamanlidou *et al*. [21] . Bacteria were grown in petri dishes and the spores were collected from nutrient agar washed three times in ice - cold distilled water . Pellets (spores and crystals) were resuspended in small volumes of distilled water . Bacterial cells were lysed to releasing spores and crystals and then collected by centrifugation (10000 x g for 10 min.) . Pellets were washed three times with ice - cold distilled waters and final pellets were resuspended in 20 ml of water and stored at -5°C. To

purify crystals from spores and cellular debris, samples were sonicated and centrifuged on discontinuous sucrose density gradients (67 to 72 to 79% [wt/vol] sucrose) at 15000 xg for 2 h. Crystal bands and spore pellets were purified by three centrifugations and washed with distilled water. Final pellets were resuspended in small volumes of distilled water and stored at -5°C.

2.9 Bioassay of toxicity

Toxicity was bioassayed against *Spodoptera littoralis* second instar larvae (mean body weight = 10 mg) according to Klanfon and DeBarjac [22] with some modifications. Bacterial cell component of *B. thuringiensis* was approximately 10^9 crystals and/or spores per milliliter was used with the dilution of 1:1 . Larvae of *Spodoptera littoralis* were exposed to the appropriate dose of the component of *Bacillus thuringiensis* using a micropipette to dispense 200 µl of the suspension on 2-3 grams of diet surface of *Ricinus communis* [23] **.** Then this drop was evenly distributed over the diet surface with a sterile glass rod, and the surface was air – dried . Mortality was recorded daily after 24 h for 6 - 7 days . Surviving larvae from each replicate were pooled and numbered daily [24] **.**

2.10 Measuring transconjugant efficiency

Transconjugant efficiency was calculated according to Winfridus Bakker [25] using the following formula ;

TE (Mid parents) = Average P_{F1} - Average P_P/ Mid parents , measured in units of the trait

TE (Better parent) = Average P_{F1} - Average Better parent / Better Parent , measured in units of the trait

P_{F1} = average performance of crossbreds .

P_P = average performance of parents lines = $P_1 + P_2$ / 2 .

3. RESULTS AND DISCUSSION

3.1 Genetic markers in conjugation :

A commercial *Bt* product was first registered in the United States in 1958 ; by 1960 it was cleared for use on food crops and in 1961 it was registered for use in Canada. It is now the most widely used naturally occurring pest control product in the world [26] .

Both *Bacillus thuringiensis* and *Bacillus subtilis* used in this study were tested for the antibiotic (hiconcil) and drug resistance (Crystal violet) as shown in Table (1) . *Bacillus thuringiensis* was found to be more resistant to Hiconcil and sensitive to crystal violet . In addition , *Bacillus subtilis* was found to be sensitive to hiconcil and resistant to crystal violet . This have often relied upon resistance as a genetic marker to identify bacterial strains [27] . Crystal violet resistance in *Bacillus subtilis* is also similar to that of hiconcil in *Bacillus thuringiensis* and provides a second potential marker to be use as an opposite genetic markers in conjugation process , as well as , to be use for isolating bacterial transconjugants . The results obtained herein agreed with Stuart *et al* [28] , who examined forty - eight clinical isolates of *Streptococcus suis* for antibiotic sensitivity and the presence of plasmid DNA . It was determined that isolates showed a substantial increase in resistance to erythromycin (*ery*) , clindamycin, and tetracycline (*tet*) . Eleven of the 48 isolates contained plasmid DNA as revealed by DNA isolation and gel electrophoresis . Plasmid DNA from four strains resistant to the above three antibiotics was tested for the ability to transform an antibiotic sensitive recipient .

Table 1 . Genetic markers of antibiotic drugs and crystal violet as measured by the presence (+) or absence (-) of inhibition zone .

Marking agents	Bacterial strains	
	B. thuringiensis	*B. suBtilis*
Hiconcil	+	-
Crystal violet	-	+

After the above strains were genetically marking , conjugation process was done between *Bacillus subtilis* (*Hico⁻ rfa⁺*) with *Bacillus thuringiensis* serovar *Kurstaki* (*Hico⁺ rfa⁻*), depending upon the opposite genetic markers determined in this study . The results obtained herein agreed with that found by Campbell [29] **,** who reported that genes located on a circular strand of DNA called an R-plasmid may contain several antibiotic-resistant genes. Through a process called conjugation an antibiotic - resistant bacterium can transfer the antibiotic resistance genes from an R-plasmid to a non-resistant bacterium . This allows a species of bacteria to possess enough genetic variability for adapting to a changing environment and to compete with its neighbors .

3.2 Transconjugant efficiency of recombinant bioinsecticides for reducing the number of *Spodoptera littoralis* larvae

The results summarized in Table 2 showed transconjugant efficiency of *Bacillus thuringiensis* transconjugants . The mean number of *Spodoptera littoralis* larvae feeding on leaves of *Ricinus communis* (gram / day) sprayed with recombinant *Bt* bioinsecticides were reduced . It is of interest to note that most values of positive transconjugant efficiency related to toxicological effects was due to treatment of cry + endospores than that treated with crystals

alone . This indicated that crystals + endospores was more effective than crystals alone for increasing mortality . Higher positive transconjugant efficiency in relation to the mid parents and better parent was appeared at 168 h of treatment . To be effective , *Bt* must be eaten by insects during their feeding stage of development , when they are larvae. *Bacillus thuringiensis* is ineffective against adult insects . More than 150 insects, mostly lepidopterous larvae , are known to be susceptible in some way to *Bacillus thuringiensis* . During the process of spore formation, *Bt* also produces unique crystalline bodies . When eaten, the spores and crystals of *Bt* act as poisons in the target insects . *Bt* is therefore referred to as a stomach poison. *Bt* crystals dissolve in the intestine of susceptible insect larvae. They paralyze the cells in the gut, interfering with normal digestion and triggering the insect to stop feeding on host plants . *Bacillus thuringiensis* spores can then invade other insect tissue, multiplying in the insect's blood, until the insect dies . Death can occur within a few hours to a few weeks of *Bt* application, depending on the insect species and the amount of *Bt* ingested. Typical agricultural formulations include wettable powders, spray concentrates, liquid concentrates, dusts, baits, and time, release rings .

Table 2 . Transconjugant efficiency of accumulated mortality larvae referred to crystals, crystals and endospores isolated from *Bacillus thuringiensis* against *Spodoptera littoralis* .

Bioinsecticides		Treatment time (h)					
		24		48		72	
		Cry	Cry + End	Cry	Cry + End	Cry	Cry + End
***Bacillus** thuringiensis*		0	0.67	0.33	2	0.33	2
***Bacillus** subtilis*		0.33	1.33	0.67	2	1	2.67
Mid Parents		0.2	1.0	0.5	2.0	0.7	2.3
TA	TEMP	-100	-33.0	166.0	-16.5	151.1	-28.5
	TEBP	-100.0	103.0	0.0	149.3	-16.5	67.0
TB	TEMP	100.0	100.0	-34.0	66.5	-50.4	42.6
	TEBP	0.0	50.4	-50.7	66.5	-67.0	24.7
TC	TEMP	306.1	100.0	34.0	50.0	0.8	42.6
	TEBP	103.0	50.4	0.0	50.0	-33.0	24.7
TD	TEMP	-100.0	167.0	-100.0	66.5	0.8	71.3
	TEBP	-100.0	100.8	-100.0	66.5	-33.0	49.8

Table 2 . Continued .

Bioinsecticides		Treatment time (h)						
		96		120		144		168
		Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry + End
***Bacillus** thuringiensis*		0.33	3	1	3.33	1.67	5.67	5.33
***Bacillus** subtilis*		1.67	2.67	3	4.67	3.33	4.67	2.67
Mid Parents		1.0	2.8	2.0	4.0	2.5	5.2	4.0
TA	TEMP	166.0	17.5	50.0	41.8	46.8	22.4	75.0
	TEBP	-0.4	99.4	0.0	21.4	10.2	11.6	31.3
TB	TEMP	100.0	41.1	166.5	33.3	153.2	29.0	75.0
	TEBP	19.8	33.3	77.7	14.1	90.1	17.6	31.3
TC	TEMP	-33.0	41.1	-33.5	25.0	-33.2	-3.3	50.0
	TEBP	-59.9	33.3	-55.7	7.1	-49.8	-11.8	12.6
TD	TEMP	0.0	52.7	100.0	41.8	100.0	16.1	66.8
	TEBP	-40.1	44.3	33.3	21.4	50.2	5.8	25.1

Cry = Crystals End = Endospores. T = Transconjugant
TEMP = Transconjugant efficiency related to mid – parents .
TEBP = Transconjugant efficiency related to better parent .

The results indicated that *Bacillus thuringiensis* is not a synthetic chemical. *Bacillus thuringiensis* products contain the highly specialized protein crystals and dormant spores of bacteria. These are only activated when they are eaten by a susceptible species of insect. Unlike broad spectrum insecticides, *Bacillus thuringiensis* is highly specific - that is, it affects only certain species of insects and has no effect on others . The toxicity of *Bacillus thuringiensis* appeared due to *Bt* begins to work after a caterpillar eats a piece of

leaf with *Bt* crystal proteins and spores on it (caterpillars are the immature stage of butterflies and moths) . Susceptible caterpillars have a strongly alkaline digestive tract (in contrast, humans and other animals have acidic digestive tracts) . When the crystals reach the caterpillar's gut, they dissolve in the alkaline conditions and release the proteins contained in the crystal. Through a series of complex biological processes, the proteins disrupt the lining of the gut, which causes the caterpillar to starve. Infected caterpillars may not die for several days, but they usually stop feeding immediately because their digestive tract is paralyzed by the activity of the crystal proteins .

Moreover, the contribution of enteric bacteria to host mortality as seen in this study suggests that toxin feeding causes a transition of otherwise benign bacteria into opportunistic pathogens , in some but not all hosts. These associations between *Bacillus thuringiensis* toxin and the gut microbiota of Lepidoptera may provide a useful model with which to identify the factors involved in the induction of adverse effects by normally beneficial or benign bacteria .

The results obtained in this study indicated that enteric recombinant bacteria played an important roles in killing Lepidoptera across a range of feeding and relative susceptibility to *Bacillus thuringiensis* . This impact of enteric bacteria differs among species . The obtained results agreed with Ashfaq *et al* . [30] , who studied the effects of transgenic *Bacillus thuringiensis* - cotton cultivar (DPL 32) on three instars of soybean looper, *Pseudoplusia includens* (Walker) , in the laboratory . The authors fed the first, third, and fifth instars using field collected *Bt* - cotton leaves for one , two , four and seven days or until pupation, and then transferred to artificial diet. Mortality during the larval stage increased linearly in response to an increase in the length of feeding time on *Bt*- cotton by first and third instars. The maximum mortality of about two out of three larvae occurred for first instars fed on *Bt* - cotton until pupation. For the fifth instar, there was no significant response to feeding time; however, most of these larvae reached pupation before four days of feeding on *Bt*-cotton. The length of the larval developmental period also increased linearly with an increase in feeding time on Bt-cotton in first and third instars; again, there was no significant response in the fifth instars. For both mortality and larval developmental time, the linear trend lines for the first and third instars were quite similar. Pupal weight declined linearly in the first and fifth instars in response to feeding time on *Bt*-cotton . Although pupal weight also declined for third instars, the response was not linear. The effect of *Bt*-cotton appears not to extend past pupation in that there were no significant responses in mortality and developmental time of pupae during the pupal stage.

The results also agreed with Carrieri *et al* [31] , who evaluated toxicity persistence of *Bacillus thuringiensis* var. *israelensis* (*Bti*) in laboratory and field trials to develop a new protocol for *Aedes albopictus* monitoring . Their results showed a good performance of all tested formulations (> 97% mortality at day 14 , for all the formulations) , but only Vectobac™ 12AS at the concentration of 1 ml / liter showed an efficacy of 100% for 2 weeks . The same authors designed a field study to test the effect of *Bti* on the ovitrap check interval or influence of ovipositional response of gravid *Ae. albopictus* females , using three different ovitrap treatments : ovitraps with tap water checked weekly ; ovitraps with tap water checked every 2 weeks ; ovitraps with *Bti* (Vectobac 12AS, dose of 1 ml/liter) checked every 2 weeks . Their study demonstrated that in the ovitrap, the toxic action of a 1% solution of *Bti* was maintained for at least 14 days with mortality of 100% and that rainfall did not seem to negatively influence the residual action of *Bti*. Therefore the probability that the larvae may complete the developmental cycle in ovitraps with *Bti* seems to be very low. The oviposition activity index showed that *Bti* enhances the oviposition rate of *Ae. albopictus* by 17.4% .The data obtained by the same authors indicated that larvae surviving *Bt* - cotton are adversely affected in several ways, which should be considered in evaluating *Bt*-cotton suppression of *Spodoptera littoralis* .

3.3 Transconjugant efficiency of recombinant bioinsecticides in increasing mortality percentage of larvae :

The results presented in Table 3 appeared transconjugant efficiency of recombinant bioinsecticides in increasing mortality percentage . Transconjugant efficiency was achieved by transconjugant A in relation to better parent at the treatments of cry + endosopores than that treated with crystals alone . In contrast , transconjugant A appeared higher values of transconjugant efficiency in mortality percentage in relation to the mid parents at the treatments of crystals than that treated with crystals + endospores at 48 h to 144h . The same trend was also achieved by transconjugant B in relation to the midparents from 96h to 144h of treatments . However , transconjugant B appeared higher values of transconjugant efficiency in mortality percentage related to better parent when the larvae was treated with crystals + endospores than that treated with crystals alone at the times from 24h to 96h . Moreover , transconjugant C appeared higher values of transconjugant efficiency in mortality percentage from 24h to 144h , in relation to the midparents , when the larvae was treated with

crystals + endospores than that treated with crystals alone . The same trend in relation to midparents , as well as to better parent , was also achieved by transconjugant D from 24h to 96h .

Table 3 . Transconjugant efficiency in mortality percentage of *Spodoptera littoralis* neonate larvae fed on leaves of *Ricinus communis* sprayed with *Bacillus thuringiensis* preparations .

Bioinsecticides		Treatment time (h)					
		24		48		72	
		Cry	Cry + End	Cry	Cry + End	Cry	Cry + End
***Bacillus** thuringiensis*		0	8.33	4.17	25	4.17	25
***Bacillus** suBtilis*		4.17	16.67	8.33	25	12.5	33.33
Mid Parents		2.09	12.5	6.3	25.0	8.3	29.2
TA	TEMP	-100	-33.4	166.7	-16.7	149.9	-28.6
	TEBP	-100	99.8	0.0	150.1	-16.7	66.6
TB	TEMP	100.0	100.0	-33.3	66.7	-50.0	42.9
	TEBP	0.0	50.0	-49.9	66.7	-66.6	25.0
TC	TEMP	299.5	100.0	33.3	50.0	-0.1	42.9
	TEBP	99.8	50.0	0.0	50.0	-33.4	25.0
TD	TEMP	-100.0	166.6	-100.0	66.7	-0.1	71.4
	TEBP	-100.0	99.9	-100.0	66.7	-33.4	50.0

Table 3 . Continued .

Bioinsecticides		Treatment time (h)						
		96		120		144		168
		Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry + End
***Bacillus** thuringiensis*		4.17	37.5	12.5	41.67	20.83	66.67	70.83
***Bacillus** subtilis*		20.8	33.33	37.5	58.33	41.67	58.33	62.5
Mid Parents		12.5	35.4	25.0	50.0	31.3	62.5	66.7
TA	TEMP	166.6	17.7	50.0	41.7	46.7	26.7	31.3
	TEBP	0.0	100.0	0.0	21.4	10.0	18.7	23.5
TB	TEMP	100.0	41.2	166.7	13.3	153.3	33.3	31.3
	TEBP	20.0	33.3	77.8	-2.8	90.0	25.0	23.5
TC	TEMP	-33.4	41.2	-33.3	25.0	-33.3	0.0	12.5
	TEBP	-60.0	33.3	-55.5	7.1	-50.0	-6.3	5.9
TD	TEMP	0.0	53.0	100.0	41.7	100.0	20.0	25.0
	TEBP	-40.0	44.5	33.3	21.4	50.0	12.5	17.6

Cry = Crystals End = Endospores.
TEMP = Transconjugant efficiency related to mid – parents .
TEBP = Transconjugant efficiency related to better parent .

Meanwhile , transconjugant D appeared higher values of transconjugant efficiency in mortality percentage in relation to the midparents and better parent at 120h and 144h when the larvae was treated with crystals than that treated with crystals + endospores . The results indicated that recombinant bioinsecticides recorded the highest mortality than their parents . However , crystals + endospores exhibited higher mortality percentage than crystals . The results obtained in this study suggest a potential for the deployment of these recominant entomopathogens in the management of *S. littoralis* larvae , as well as , usceptibility decreased with larval development .

The results obtained in this study agreed with Sneh *et al* [32] , who found that among more than 50 isolates of *Bacillus thuringiensis* BERLINER (*Bt*) tested , 7 incited 100% mortality when 2nd instar larvae of *Spodoptera littoralis* BOISDUVAL were fed on alfalfa leaves dipped in a spore - crystal suspension of 10^8 colony forming units / ml . Among those isolates , *Bt* 24 demonstrated the highest activity . Larvae of instars 1 and 2 were the most susceptible to *Bt* . However, larvae of all instars were killed by isolate *Bt* 24 . Larvae that survived after feeding with *Bt* 24 were retarded and fed less . Their weight relative to the controls was lower as the spore concentration on the leaves on which they fed was higher .

The results obtained in this study are also in accordance with Moore and Navon [33] , who tested

the effect of ten different laboratory strains of *Bacillus thuringiensis* and of the thermostable exotoxin on larvae of *Spodoptera littoralis* Boisduval , and found that endotoxin poisoning was not observed and whenever mortality rose substantially above the controls , it seemed due to the thermostable exotoxin and not to the parasporal crystal . Treatment of a commercial preparation with substances dissolving the crystal did not elicit acute toxemia, nor did abrasives administered with this preparation bring about fulminating septicemia . The results indicated that recombinant *Bacillus thuringiensis* are important control agents for lepidopteran pests [34] , [35] , [36] .

4. In conclusion , this work demonstrated that recombinants of *Bacillus thuringiensis* - induced killing in lepidopteran pests . Recombinant bioinsecticides increased mortality in *Spodoptera littoralis* larvae . This work represents an intriguing model at which genetically modified bacteria may play a significant role in controlling lepidopteran pests .

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