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A Method to Determine Lethal and Sublethal Concentrations of Recombinant Bioinsecticides Derived from *Bacillus thuringiensis* Against Larvae of *Spodoptera litura*

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ABSTRACT

The increased awareness of environmental pollution have led to the growing interests of the application of biological agents to control insects such as lepidopteran family .Thus , this study aimed to produce genetically improved bioinsecticide agents to be used to control *Spodoptera litura* which would be useful in reducing environmental pollution ,preventing resistance to bioinsecticides and for safe food production .Four *Bacillus thuringensis* strains were used as recipients ' while ' *Seratia marcescens* was used as a doner strain in the conjugation to induce new recombinants in *Bacillus thuringiensis* over expressed chitinase.Four matings were conducted in this study between *Serratia marcescence* and the four *Bacillus thuringiensis* strains which having opposite genetic markers.Two efficient transconjugants based on chitin hydrolysis on agar medium were selected from each conjugation to be evaluated for toxicites against the larvae of cotton leafworm in relation to their parents .The efficient LC_{50} was appeared by Tr_4 (1.93 $x10^5$ ppm) followed by Tr_6 and Tr_7 ($2x10^5$ ppm). However , more than 995% toxicity index was recorded by Tr_4,Tr_6 and Tr_7. Higher toxicity index observed by these transconjugants may be the related to the overexpression of chitinase genes.Therefore , the lethal concentration values obtained was necessary to kill 50% of larval population from *Spodoptera litura* in a certain period .The virulence of *Bt* strains showed that a lower concentration from Tr_4 was required to kill 100% of larval population .It must be pointed out that higher toxicity index obtained by transconjugants reflected the interaction between crystal genes from *Bt* and chitinase genes from *Seratia marcescens* which enhanced the possibility of larval mortality as a genetic helpful tool in integrated pest management in cotton fields.

Keywords : Conjugants , *Spodoptera litura* , *Bacillus thuringiensis* ,LC_{50} , *Serratia marcescens* .

INTRODUCTION

Cotton considered to be one of the most important crops and a major source of the national economy in Egypt. The Egyptian cotton leaf worm , *Spodoptera littoralis* is the most harmful cotton pests (Korrat *et al.* 2012). It is also a considerable cause of economic loss of many vegetables and field crops (Isman *et al.* 2007). Different kinds of insecticides are used to control this pest which cause a great damage and pollutions to the environment. Thus, the use of microbial pesticides for protecting crops from insect pests has assumed greater importance in recent years replace the harmful applications of the chemical pesticides (Chari *et al.* 1990). Thus, control of crop pests by the use of biological agents holds great promise as an alternative to the use of chemicals. Crude enzyme from different types of microorganisms have been used to control crop pest populations (Kramer *et al*. 1997). *Bacillus thuringiensis* (*Bt*) is a ubiquitous gram − positive, spore forming bacterium that forms a parasporal crystal during the stationary phase of its growth cycle. *Bt* was initially characterized as an insect pathogen and its insecticidal activity was attributed largely or completely to the parasporal crystals and also to the vegetative insecticidal proteins that producing by *Bt* during its vegetative growth stage (Fang *et al*. 2009). *Bt* is now the most widely used biological produced pest control agent and the foliar sprays from it plays a role in the integrated pest management strategies (Crickmore, 2006). Commercial preparations from *Bt* are based essentially on a mixture of spores and crystals (Moore and Navon, 1973).

Genetic transfers such as conjugation are important phenomena affecting evolution and have been used in genetic engineering laboratories to transfer genes of interest to allow their expression in target organisms (Somkiat *et al*. 2007). Bacterial conjugation is a horizontal gene transfer process from a donor cell bearing one or more conjugative plasmids to the recipient cell plasmid – free .Conjugative plasmids in most bacteria can even be transferred to distantly related or even unrelated microorganisms (Biedendieck *et al*. 2007). Selectable genetic markers are an important tool in the construction of bacterial transconjugants .Ideally, the genetic markers allow for efficient selection without affecting any cellular functions (Hentges *et al*. 2005). Resistance to antibiotic can be conferred by chromosomal or mobile genetic element (plasmids) (Jain *et al*. 2009). In nature plasmids increase bacterial genetic diversity and promote bacterial adaptation by horizontal gene spread (Gogerten *et al*. 2002).

Chitin is a long unbranched polysaccharide of an amino sugar (Chuan, 2006). It is abundant in nature as a structural in cuticle and integument of animals, especially in insects (Arakane and Muthukrishnan, 2010). Insect growth and development are strongly dependent on the construction and remodeling of chitinous structures. Chitinase induced damage to the peritrophic membrane in the insect gut causes a significant reduction in nutrient utilization and consequently in insect growth (Merzendorfer and Zimoch, 2003). Chitinase present in insect diet can decrease insect growth (Fitches *et al*. 2004). Chitinolytic bacteria such as *Serratia marcescense* have many potential applications as biocontrol agents (Wang *et al*. 2006). Over – expression of chitinase in entomopathogenic organism can increase insect mortality (Fan *et al*. 2007). This resarch aimed to calculate the lethal and the sublethal concentrations of recombinant biological control agents produced from the conjugation between *Serratia marcescence* and *Bacillus thuringiensis* and to select the effective doses

which would be used for biological control against cotton leaf worm.

MATERIALS AND METHODS

Bacterial strains : Bacterial strains used in this study, as well as, their references, or sources are listed in Table 1.

Table 1. Bacterial strains used in this study.

Strains	Source or reference	Designation
Serratia marcescens	Microbiology Dept., Soil, water and Environmental Research Institute, Agricultural Research Center (ARC).	*Sm*
Bacillus thuringiensis	Microbiology Dept., Soil, water and Environmental Research Institute, Agricultural Research Center (ARC).	Bt_1
Bacillus thuringiensis	*Bacillus* Genetics Stock Center, Biochemistry Dept., Ohio, University, Columbus, USA	Bt_2
Bacillus thuringiensis	*Bacillus* Genetics Stock Center, Biochemistry Dept., Ohio state, University, Columbus, USA	Bt_3
Bacillus thuringiensis NRRL-HD110	National Center for Agriculture Utilization Research , USA	Bt_4

Media : *Bacillus* strains were maintained on TGY medium as a complete medium according to Harris *et al*. (2004). This medium was used for genetic marking bacterial strains against antibiotics.

Peptone yeast extract medium (PWYE): This medium was used for separation of crystals and endospores according to Karamanlidou *et al*. (1991).

Peptone glycerol medium (PGM) : This medium was used to enhance pigmentation. according to Harris *et al*. (1992).

Luria-Bertani medium (LB) : This medium was used in mating experiments according to Ausubel (1987).

Mineral Medium (MM) : This medium was used for screening chitinase producing bacteria which was performed on colloidal chitin agar medium and incubated at 37°C according to Someya *et al*. (2011).

Minimal Sporulation Medium (MSM) : The conditions for growth and sporulation were as previously described (Ellar and posgate, 1974), with the exception of a modified sporulation medium used by Gordon *et al*. (1981).

Antibiotics used : However, antibiotic resistance markers are alternative to auxotrophic markers. .Thus, 14 antibiotics were used in this study with different concentrations (µg/ml) for genetically marking bacterial strains as shown in Table 2.

Target insect : A wild type strain of *S. littoralis* used in this study was collected from the Experimental Farm of Faculty of Agriculture inside the Camps of Mansoura University in June 2015. This strain was collected as a colony of eggs on cotton and *Ricinus communis* leaves which were not previously exposed to any insecticides. Egg masses were kept in glass jars (500ml) covered with cotton cloth. The jars were daily supplemented with fresh castor bean leaves as a source of food till hatching. Newly hatched larvae at six days old were offered castor bean leaves treated with bioinsecticides via a dipping technique, although leaves dipped in water served as control.

Table2. Different antibiotics and their concentrations used for genetic marking bacterial strains.

Antibiotics	Abbreviations	Concentration (µg/ml)
Chloramphenicol	*Cm*	35
Ampicillin	*Ap*	50
Tetracycline	*Tc*	20
Penicillin	*Pc*	150
Neomycin sulphate	*Nm*	800
Erythromycin-ethlsuccinate	*Eryth*	20
Rifampicin	*Rif*	150
Vancomycin	*Vc*	150
Hibiotic	*Hb*	400
Amoxycillin	*Am*	400
Ceftazidime	*Ce*	400
Cefotaxime	*Cf*	400
Cefoperazone	*Cp*	150
Genamycin	*Gm*	20

Insects were reared on castor bean leaves in laboratory under constant conditions of 27 ± 2ºC, photoperiod of 14 hrs light and 10 hrs dark .Larvae of *S. littorails* were put in glass jar (250ml) and feeding on treated castor bean leaves added daily .

Genetic marking : Susceptibility to antibiotics was measured by plate diffusion method according to Collins and lyne (1985) using cultures grown to logarithmic growth phase in TGY broth. The plates were incubated for 48hrs at 28° C and the diameter of resulting clear zones of inhibition was measured according to Toda *et al*. (1989).

Plasmid transfer via conjugation process : Mix 5 ml of the donor strain with 5 ml of the recipient strain in a test tube and incubated at 28° C for five, ten and fifteen days. At the end of mating time, the mating cells were plated on LB agar medium supplemented with selective antibiotics and incubated at 28° C for 48 h. Single colonies appeared on selective medium were picked up and grown on LB slant agar medium according to Grinsted and Bennett (1990).

Preparing of colloidal chitin : Colloidal chitin was prepared from chitin flakes by the method of Mahadevan and Crawford (1997). The chitin flakes were ground to powder, added slowly to 10 N HCl and kept overnight at 4°C after vigorous stirring. The suspension was added to cold 50% ethanol with rapid stirring and kept overnight at 25°C. The precipitate was collected and washed with sterile distilled water until the colloidal chitin became neutral (pH 7.0) and stored at 4°C until further use.

Screening chitinase producing bacteria : For enrichment chitinase-producing bacteria, a mineral medium (MM) containing colloidal chitin as a sole carbon and energy source was used. Chitinolytic activity was measured by observing the size of the halo zones formed around the colonies after seven days of incubation at 30°C according to Someya *et al*. (2011).

Separation of crystals and endospores : Bacteria were grown in petri dishes. The spores were collected from nutrient agar plates washed three times in ice-cold distilled water. Pellets (spores and crystals) were resuspended in small volumes of distilled water. The bacterial suspension cultures were prepared as follows. Loopfuls from bacterial colonies with spores and crystals were transferred to 1 ml of distilled water. Heat-shocked (70°C for 30 min) suspensions were transferred to 250 ml of PWYE medium and incubated at 30°C for 8 to 15 h with shaking at 180 rpm. Two milliliters of the PWYE culture was used to inoculate 1 liter of minimal sporulation medium (MSM) and was incubated at 30°C for 3 to 4 days with shaking at 180 rpm; at least 90% of bacterial cells were lysed releasing spores and crystals after this incubation. Spores and crystals were collected by centrifugation (10,000 x g for 10 min). Pellets were washed three times with ice-cold distilled water, and final pellets were resuspended in 20 ml of water and stored in refrigerator freezer (Karamanlidou *et al*. 1991).

Bioassay techniques

Toxicity tests in laboratory experiment : This study was conduced in Microbial Genetics Laboratory , Faculty of Agriculture , Mansoura University through the academic years of 2013 / 2014 , 2014 / 2015 and 2015 / 2016 . To assess the activity of bioinsecticides a series of five concentrations were prepared in distilled water which were as follows; 0.0, 2 x 10^5, 4 x 10^5, 6 x 10^5 and 8 x10^5 ppm. The dipping technique was adopted as described by Tabashnik *et al*. (1991), where fresh clean castor bean leaves (*Ricinus communis*) were immersed in each of the tested concentration for 30 sec . Nine bioinsecticides were used including control with three replicates were used for each concentration. Castor oil leaves were first washed with distilled water then dipped in bioinsecicide suspension of each concentration for 30 sec and allowed to air dry for one hour at room temperature before being offered to *S. littoralis* larvae.These leaves were used to feeding cotton leaf worm.

Bioassay experiment was conducted in plastic clean jars (250ml), each jar contained 15 larvae. A similar number of larvae were considered as a control in which larvae were offered castor been leaves immersed in distilled water .The bioassay jar was covered by a cotton tissue fixed with rubber band to prevent larvae from escaping. After dryness the bioinsecticide film on the leaves, the larvae were transferred into clean jars individually .The effects of bioinsecticides were evaluated against six – days – old larvae of cotton leaf worm, *Spodoptera littoralis* (mean body weight = 53 mg) at 25 °C under laboratory conditions. Larvae were fed for 24 hr on three grams of treated leaves added daily to a new breeding bottle. After 24hr , the survived larvae were transferred to clean jars and supplemented with treated leaves expect the control supplemented untreated leaves until pupation. The leaves were removed after 24 hr and replaced by another treated ones after the jars were cleaned and dryed .Larval mortality was recorded daily up to pupation developed .Mortality percentage was corrected by abbott formula (Abbott, 1925) as follows:

Abbott's formula: -

$$\text{Mortality\%} = \frac{\textit{Control survival} - \textit{Treatment survival}}{\textit{Control survival}} \times 100$$

The survived larvae were pooled and counted daily, then transferred to clean jars supplemented with treated leaves until pupation (Karamanlidou *et al*. 1991).

Determination of Lethal Concentration (LC) values : The LC_{30}, LC_{50}, LC_{70} and LC_{90} values were determined from leaf dipping technique at which five different concentrations of the tested bioinsecticides were used .The lethal concentration values were statistically estimated by milligrams per liter from regression lines according to Finney (1971).

Statistical analysis : Lethal concentration fifty (LC_{50}) of larval population was calculated from regression curves which were diagrammed according to Snedecor and Cochran (1955).

RESULTS AND DISSCUTION

Antibiotic resistance test : The results of 14 antibiotics tested by disc diffusion method on nutrient agar plates against five bacterial strains are shown in Table 3 and Figure 1 .The resistance pattern of these strains showed multiple resistance and multiple sensitive to the antibiotics used. The result of drug resistance pattern on nutrient agar showed that *Seratia marcescens* was resistant to pencillin, erythromycin, ceftazimide, whereas sensitive to other antibiotics. All bacterial strains tested were resistant to penicillin and ceftazimide , whereas sensitive to cefoperazone , rifampicin and vancomycin .The resistance pattern of bacterial strains tested in this study was ranged between resistant to three antibiotics to eight . These results agreed with Luna *et al*. (2007), who found that *B.thuringiensis* isolates were resistant to amoxicillin , ampicillin , ceftriaxone , pencillin and oxacillin , while susceptible to the remaining antimicrobials used .A large variety of specific biochemical functions such as resistance to antimicrobial drugs , production of bacteriocins and production of toxins ,have been attributed to some plasmids Bernhard *et al*. (1978).These antibiotics all have a similar mechanism of action ,stopping bacteria from multiplying by preventing it from forming the walls that surround them (Bautista and Teves , 2013) . The number of plasmids in *Bt* are variable from one to more than six (Carlson *et al*.1994).The *Bt* strains have different patterns of plasmids and show different toxicities against insects (Ren *et al*. 1995).

Conjugation and hydrolysis of colloidal chitin : Conjugation between *Seratia marcescens* as a donar strain and different strains of *Bacillus thuringiensis* as a recipients was done depending on the opposite genetic markers between both strains in each conjugation. Transconjugants appeared on selective medium

containing the opposite markers of antibiotics were picked up (Figure 2).

Ten transconjugants were taken to be used for select the efficient ones showing clear hydrolysis zone on colloidal chitin .On the basis of colloidal chitin degradation colonies were selected depending on the zone of clearance on colloidal chitin agar (CAA) plates (Figures 4 and 5) . On the bases of maximum chitinase production, two potential isolates from esch conjugation were selected for further studies against *Spodoptera litura*. Therefore, conjugation might provide means of transferring DNA between strains from both genus.Today, conjugation techniques have been used in virtually all cultured Gram-negative bacteria and have been reported in several Gram-positive bacteria (Schroder and Lanka, 2005). The results obtained herein agrees with Domingues and O'Sullivan (2013), who found that conjugation efficiencies between *E.coli* and *Bifidobacterium* observed initially ranged from 10^{-4} to 10^{-6} transconjugants per recipient , which are similar to reports in other *Actinobacteria*. The result indicated that *Serratia marcescens* which used as a donar strain in this study have the ability to produce a huge amount of chitinase in short time because of complete hydrolysis of colloidal chitin agar (Figure 3). Thus, this strain was used in this study as a donar strain to transfer chitinase gens to *Bacillus thuringiensis* strains for increasing the control of insect pests via chitinase producing transconjugants because biocontrol efficiency have been correlated with chitinase production (Wu *et al*.2010). However, microbes producing chitinases have received much attention regarding their potential development as biopesticides (Aggrawal *et al*. 2015) .In addition, found that the semi- synthetic diet containing sublethal doses of *S.marcescens* caused a dose – dependent inhibition of growth of first instar larvae .Among bacteria , *S. marcescens* have been reported to be a good producer of chitinases (Aggrawal *et al*. 2015). These bacteria enzymatically cleave the chitin present in the peritrophic membrane of the insect gut causing perforations ,leading to disease and subsequent death of the infected larva (Chandrasekaran *et al*. 2012) . Previous studies revealed that chitinase could enhance the insecticidal activity of *Bacillus thuringiensis* and it has been used in combination with *B. thuringiensis* widely. However, Hu *et al*. (2004) found that the expression of *Bt* chitinase in rather low and needs induction by chitin ,which limits its field applications .Thus, this study taken sense to costitutively express the *Bt* chitinase at a sufficiently high level via conjugating *Bt* strains with *S. marcescens* to offer advantages in biological control of pests using recombinant isolates of *Bt*.Biochemical and histochemical studies demonstrated that the insect peritrophic membrane consists of a chitin fibril network embedded in a protein – carbohydrate matrix.Pathogens have to cross the chitin – rich barrier to exert their virulence (Nation 2001).

Table 3 . Antibacterial activity of antibiotics on nutrient agar plates against *Bacillus thuringiensis* and *Seratia marcescens.*

Antibiotics	SM	Bt_1	Bt_2	Bt_3	Bt_4
Cm	-	-	-	+	-
Ap	-	+	+	+	+
Tc	-	-	-	-	+
Pc	+	+	+	+	+
Nm	-	-	+	-	-
Eryth	+	-	-	-	-
Rif	-	-	-	-	-
Vc	-	-	-	-	-
Hb	-	+	+	+	+
Am	-	+	+	+	+
Ce	+	+	+	+	+
Cf	-	+	+	+	+
Cp	-	-	-	-	-
Gm	-	-	+	-	-

Abbreviations used :

+ =Resistant — **- =Sensitive**
Cm = Chloramphenicol — ***Ap*= Ampicillin**
***Tc*= Tetracycline** — ***Pc*= Penicillin**
***Nm*= Neomycin sulphate** — ***Rif*= Rifampicin**
***Eryth*= Erythromycin-ethlsuccinate** — ***Vc*= Vancomycin**
***Hb*= Hibiotic** — ***Am*= Amoxycillin**
***Ce*= Ceftazidime** — ***Cf*= Cefoperazone**
***Gm*= Genamycin**

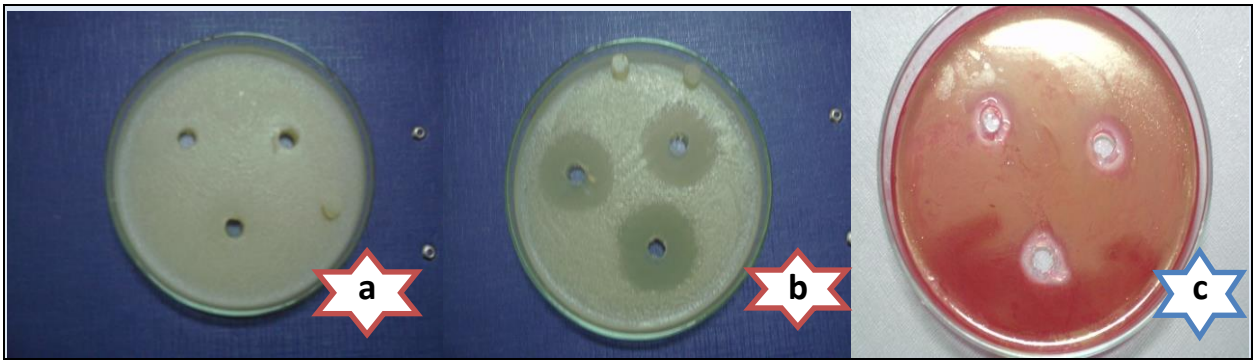

Figure 1. Bacterial colony of Bt_3 (b) , *Seratia mercescens* (c) in relation to uninoculated control (a) showing clear hydrolysis zone on antibiotic agar medium in plates number b and c .

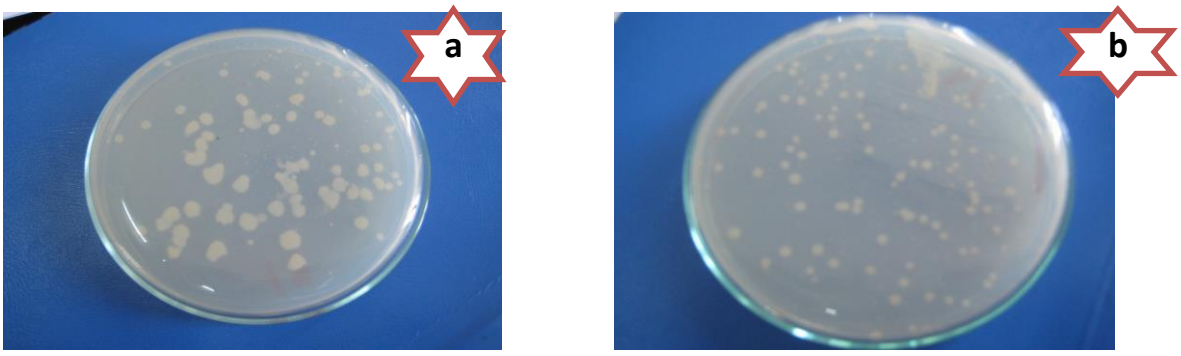

Figure 2. Bacterial colonies of recombinants resulted from conjugation between *Seratia marcescens* x Bt_4 (a) and *Seratia marcescens* x Bt_3 (b).

Similar studies on the effect of different *B. thuringiensis* strains on *Spodoptera littoralis* have shown great variability in toxicities, depending on whether insects were fed crystals, solubilized crystals or *in vitro* activated crystals (Aronson *et al*. 1991).

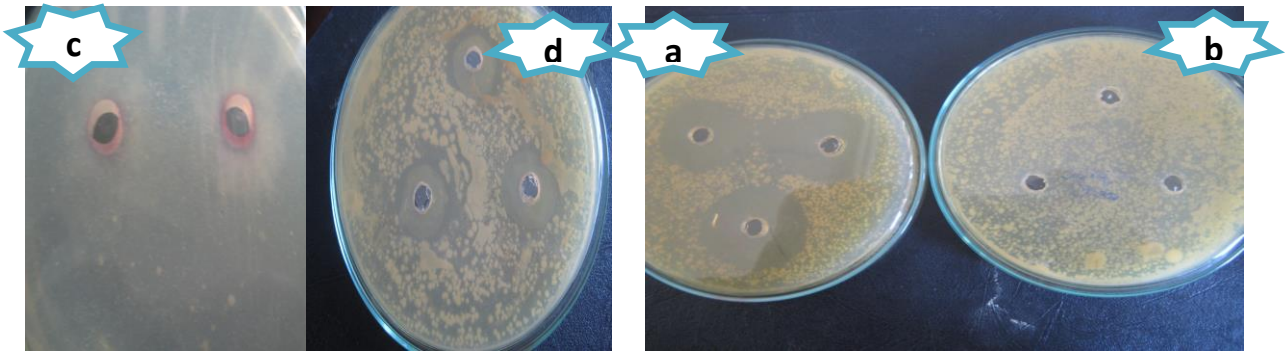

Figure 3. Colonies of Bt_2 (a) , *Seratia marcescens* (c) and Tr_3 in relation to uninoculated control plate containing chitin (b) showing clear hydrolysis zone on colloidal chitin agar medium.

Lethal concentration of *Bt* bioinsecticide: Preliminary assays in Table 4 and in Figures from 4 to 16 indicated that the efficient estimated LC_{50} value of *Bt* against *Spodoptera littoralis* larvae was appeared by Tr_4 (1.93 x 10^5 ppm) followed by Tr_6 and Tr_7 (2 x 10^5 ppm) . More than 95% toxicity index was recorded by Tr_4, Tr_6 and Tr_7. The LC values of *Bt* are dependent on type of recombinant crystal protein and geographical variation of the parental *Bt* strains.

These results agreed with Nguyen *et al*. (2005), who found that larvae of *Diadegma insulare* did not have enough time to complete their development in the *Bt* – infected hosts. Chilcutt and Tabashinik (1997a) found that *C. plutellae* larvae were killed in hosts infected by *Bt* .

The results obtained herein are in harmony with Ebrahimi *et al*. (2012), who found that LC_{50} of *Bt* against diamondback moth (DBM) larvae was 210 ppm , as well as , more than 80% mortality was recorded at 450 ppm of *Bt* concentration . Higher toxicity index observed by Tr_4, Tr_6 and Tr_7 may also be due to the gene expression of all three types of chitinases, viz exochitinase, endochitinase and chitobiosidase transferred from *Serratia marcescens* to *Bt* via conjugation. This agreed with Aggarwal *et al*. (2015) , who found that *serratia marcescens* produced all three types of chitinases and caused mortality in all developmental staged of *S.litura* larvae with LC_{50} ranging from 7.02 x 10^3 to 7.29 x 10^7 cfu ml $^{-1}$, as well as , LT_{50} for the different larval instars ranged from 3.1 days to 5.5 days . In addition , chitinase production is an important virulence factor contributing significantly to their potency as entomopathogenes Aggarwal *et al*. (2015).The genus *Serratia* contains several entomopathogenic species from which *S.marcenscens* has mostly been studied (Tan *et al*. 2006) . In bacteria, *S. marcescens* has been demonstrated as a good producer of chitinases. Wang *et al*. (2013) reported that pathogenicity of *S. marcescens* towards insects was attributed mainly to their production of chitinases and other hydrolytic enzymes.

The results obtained in this study appeared that transcojugant Tr_4 is higher pathogenic causing 100% toxicity index than other bioinsecticides used in this study .The same trend was obtained by Hernadez (1988) on subspecies *Bt aizawai* , *B. thuringiensis* and *Bt Kurstaki*, who observing mortality of 80% , 100 % and 70 % respectively , using 3 x10^7 cells / ml . The virulence assays showed that transconjugant Tr_4 was the most active recombinant isolate with an LC_{50} of 1.93x10^5 ppm followed by Tr_6, Tr_7 Tr_1 and Tr_5 with an LC_{50} of 2x 10 ^{5} , 2x10^5 , 2.3 x10^5 , 2.3 x10^5 ppm

,respectively. The LC values obtained was necessary to kill 50% of *S. littura* larval population in a certain period. The virulence of strains showed that Tr_4 require a lower concentration was needed to be lethal 100% of larvae population. Aranda *et al*. (1996) reported that *Bt aizawai* HD68 has two genes [cry 1A (a) , cry 1D] related to toxicity , while *Bt thuringiensis* 4412 has only one (cry 1B) . The same authors observed LC_{50} value of 77 mg / cm^2 for cry 1D and above 2.000 mg / cm^2 for cry 1A (a, b, c) . On the other hand, Chak *et al*. (1994) described a new strain of *Bt* with cry1 (a,b) , cry 1C and cry 1D genes , and emphasized that the high activity of *Bt aizawai* strains to *S . frugiperda* may be related to the interaction between cry1A and cry 1D genes .It must be pointed out that the mortality obtained by the efficient transconjugants may be due to the interaction between crystal genes from *Bt* and chitinase genes from *Serratia marcescens* which enhanced the possibility to use moderated toxic proteins as a helpful tool to control *Spodoptera littoralis* population in integrated pest management systems in cotton fields. The sublethal effects of *Bt* on *S. littoralis* were pointed out by Regev *et al*. (1996) , who determined the sublethal doses which caused reaction in consumption and delay in larvae and pupae development . Meanwhile, these effects were temporary and the intensity decreased with the growth of larvae. The mechanism by which gut bacteria mediate *B. thuringiensis* induced killing requires further elucidation, as do the different responses to gut bacteria in various insect species. From a pest management perspective, the ability of *B.thuringiensis* – induced mortality of other lepidopteran species may provide opportunities for increasing susceptibility or preventing resistance.

Table 4. Lethal concentrations of recombinant bioinsecticides against cotton leaf worm under laboratory conditions.

Bioinsecticides	LC_{30}		LC_{50}		LC_{70}		LC_{90}	
	ppm†	TI	ppm†	TI	ppm†	TI	ppm†	TI
Bt_3	0.025	88.00	02.22	86.90	04.41	87.30	06.61	87.14
SM	0.028	78.57	02.45	78.77	04.87	79.00	07.30	78.50
Mp	0.026	83.28	02.33	82.83	04.64	83.15	06.95	83.02
Tr_1	0.122	18.00	02.30	83.90	04.50	85.50	06.70	85.97
Tr_2	0.294	07.48	02.69	71.70	05.09	75.60	07.49	76.90
Bt_2	0.250	08.80	2.75	70.18	05.25	73.30	07.75	74.30
SM	0.028	78.57	2.45	78.77	04.87	79.00	07.30	78.50
MP	0.138	46.04	2.54	76.50	04.94	78.20	07.35	78.66
Tr_3	0.704	03.13	2.75	70.18	04.79	80.40	06.84	84.20
Tr_4	0.022	100.00	1.93	100.0	03.85	100.0	05.76	100.0
Bt_4	0.074	29.70	2.29	84.27	04.51	85.40	06.74	85.45
SM	0.028	78.57	2.45	78.77	04.87	79.00	07.30	78.50
Mp	0.050	56.49	2.31	83.55	04.57	84.27	06.84	84.23
Tr_5	0.209	10.50	2.30	83.90	04.39	87.69	06.48	88.88
Tr_6	0.062	35.48	2.00	96.50	03.96	97.20	05.90	97.60
Bt_1	5.000	00.44	9.73	19.80	14.47	26.60	19.21	29.98
SM	0.028	78.57	2.45	78.77	04.87	79.00	07.30	78.50
Mp	2.500	41.86	6.03	51.30	9.46	54.87	13.08	56.50
Tr_7	0.023	95.65	2.00	96.50	3.97	96.97	05.95	96.80
Tr_8	0.150	14.66	2.83	68.19	5.52	69.75	08.21	70.16

TI = Toxicity index %. **† = ppm x 10^5.** **MP = Mid – parents.**

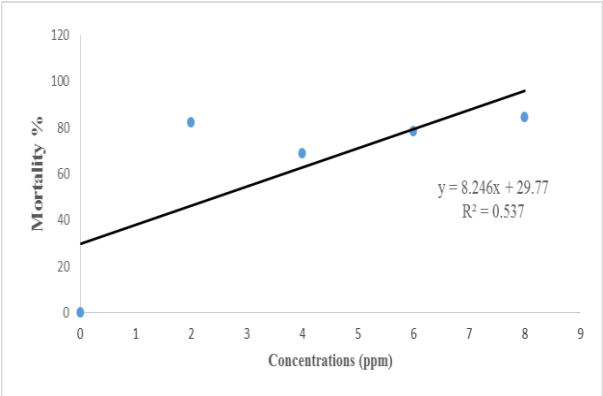

Figure 4. Toxicity regression lines of *Serratia marcescens* (*Sm*) against *S. littoratis* larvae treated with bioinsecticide for six days.

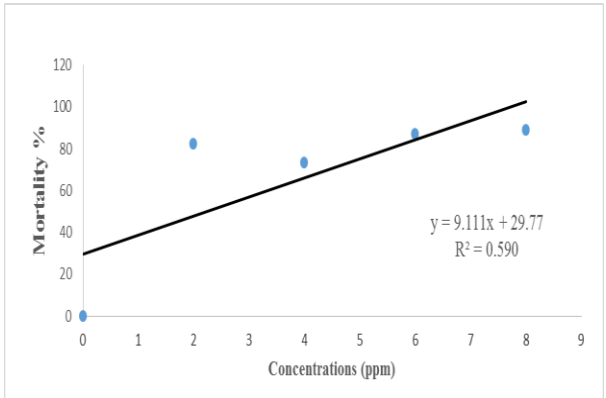

Figure 5. Toxicity regression lines of *Bacillus thuringiensis* (*Bt_1*) against *S. littoratis* larvae treated with bioinsecticide for eight days.

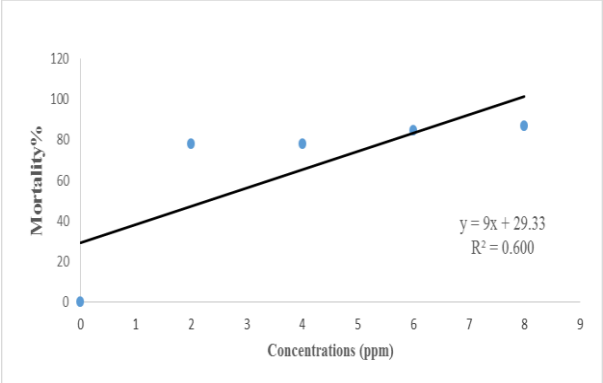

Figure 6. Toxicity regression lines of *Bacillus thuringiensis* (*Bt*$_2$) against *S. littoratis* larvae treated with bioinsecticide for eight days.

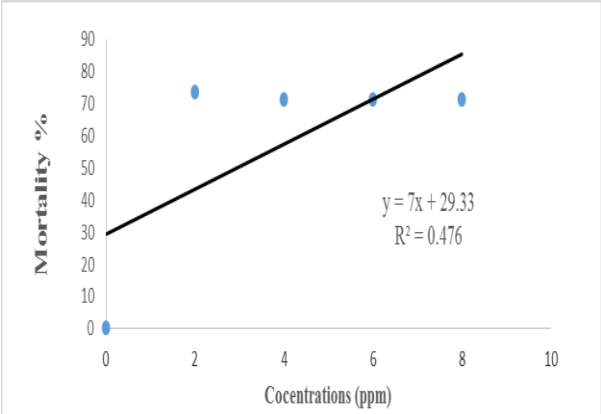

Figure 7. Toxicity regression lines of *Bacillus thuringiensis* (*Bt*$_3$) against *S. littoratis* larvae treated with bioinsecticide for six days.

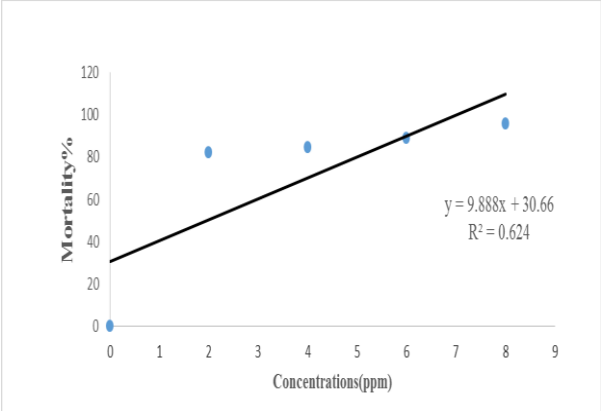

Figure 8. Toxicity regression lines of *Bacillus thuringiensis* (*Bt*$_4$) against *S. littoratis* larvae treated with bioinsecticide for six days.

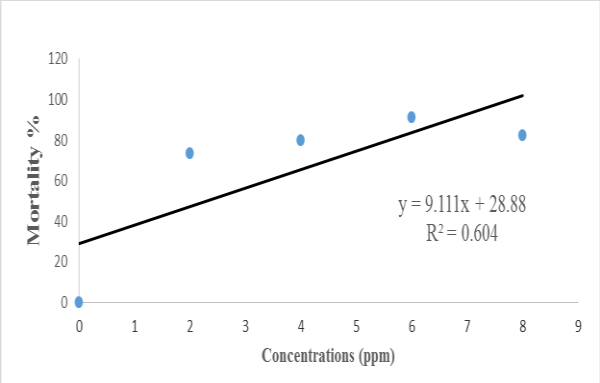

Figure 9. Toxicity regression lines of Tr$_1$ resulted from conjugation between *Bacillus thuringiensis* (*Bt*$_3$) X *Serratia marcescens* (*Sm*) against *S. littoratis* larvae treated with bioinsecticide for six days.

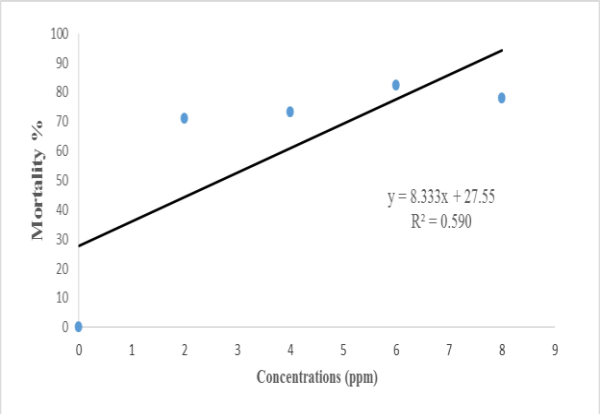

Figure 10. Toxicity regression lines of Tr$_2$ resulted from conjugation between *Bacillus thuringiensis* (*Bt*$_3$) X *Serratia marcescens* (*Sm*) against *S. littoratis* larvae treated with bioinsecticide for five days.

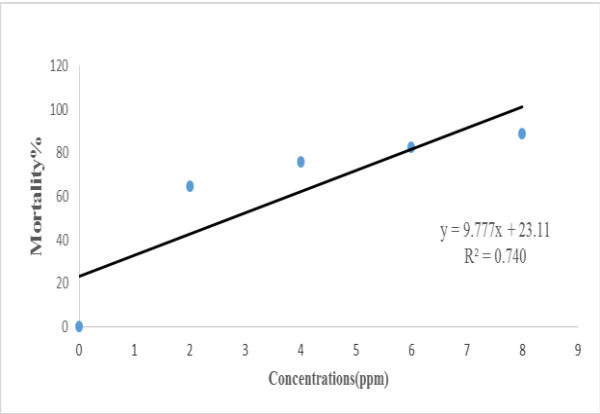

Figure 11. Toxicity regression lines of Tr$_3$ resulted from conjugation between *Bacillus thuringiensis* (*Bt*$_2$) X *Serratia marcescens* (*Sm*) against *S. littoratis* larvae treated with bioinsecticide for six days.

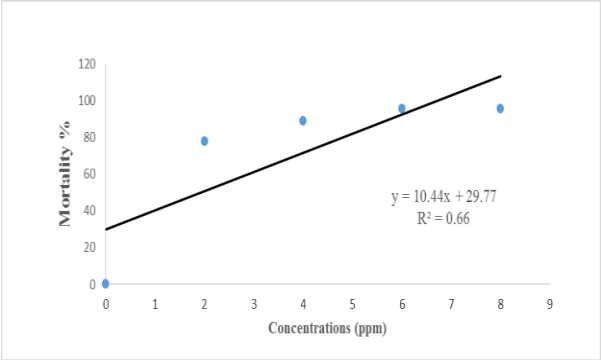

Figure 12 . Toxicity regression lines of Tr_4 resulted from conjugation between *Bacillus thuringiensis* (Bt_2) X *Serratia marcescens* (*Sm*) against *S. littoratis* larvae treated with bioinsecticide for eight days.

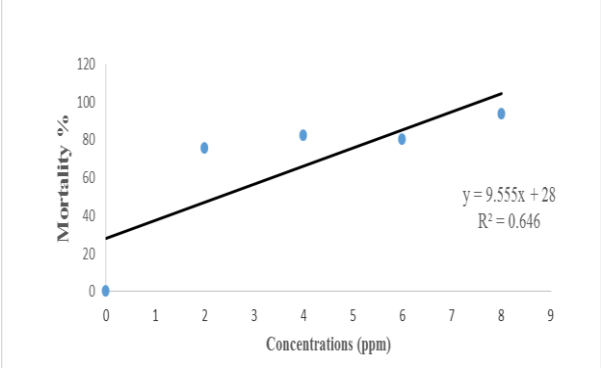

Figure 13. Toxicity regression lines of Tr_5 resulted from conjugation between *Bacillus thuringiensis* (Bt_4) X *Serratia marcescens* (*Sm*) against *S. littoratis* larvae treated with bioinsecticide for six days.

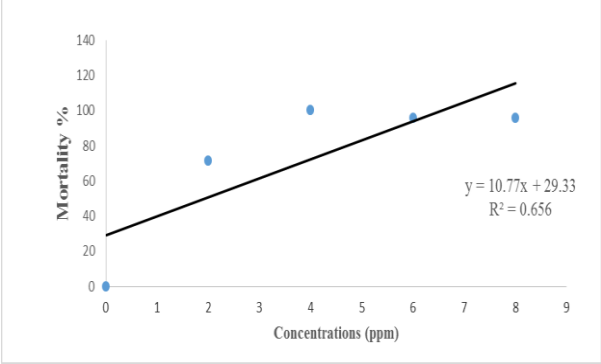

Figure 14. Toxicity regression lines of Tr_6 resulted from conjugation between *Bacillus thuringiensis* (Bt_4) X *Serratia marcescens* (*Sm*) against *S. littoratis* larvae treated with bioinsecticide for eight days.

This contribution of *Bt* caused host mortality suggested that toxin feeding caused a transition of therwise bening bacteria into opportunistic pathogenes in some, but not all hosts (Broderick *et al*. 2009). In this study bacterial transconjugants were constructed between *Bt* and *Serratia* to obtain recombinant isolates expressed crystal and chitinase genes for increasing susceptibility of cotton leafworm populations to recombinant bioinsecticide , as well as, preventing resistance to bioinsecticides.

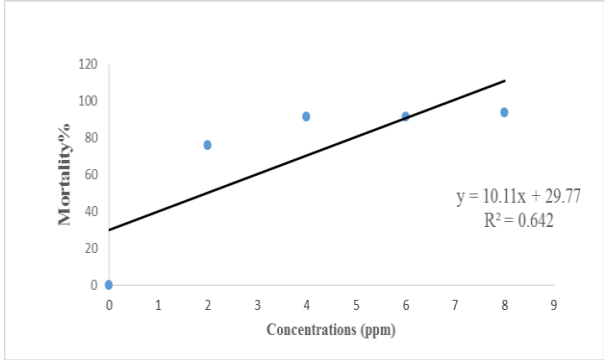

Figure 15. Toxicity regression lines of Tr_7 resulted from conjugation between *Bacillus thuringiensis* (Bt_1) X *Serratia marcescens* (*Sm*) against larvae *S. littoratis* larvae treated with bioinsecticide for ten days.

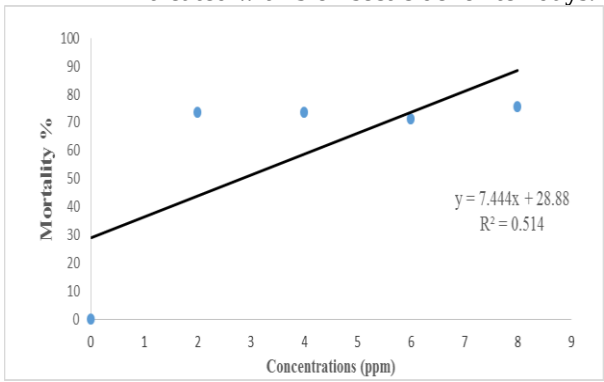

Figure 16. Toxicity regression lines of Tr_8 resulted from conjugation between *Bacillus thuringiensis* (Bt_1) X *Serratia marcescens* (*Sm*) against *S. littoratis* larvae treated with bioinsecticide for three days.

In conclusion, increasing toxicity of *Bt* strains via genetic techniques as seen in this study reduce the dosage of microbial product needing in pest control and saving plant protection expenses. Some of bioinsecticides induced in this study caused 100% mortality within eight days such as transconjugants Tr_2 , which appeared significant mortality in relation to the mid parents.Thus,recombinants isolates provides interesting aspect in integrated pest management .

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حساب التركيزات المميتة وتحت المميتة من الإتحادات الوراثية الجديدة للمبيدات الحيوية المشتقة من الباسيلس ثيرونجينسز المستخدمة ضد يرقات دودة ورق القطن

على ماهر محمد العدل [1] ، خليفه عبد المقصود زايد [1] ، كوثر سعد قش [1]، أحمد إبراهيم السيد [2] و ميرفت إبراهيم كمال [1]
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أدت زيادة معدلات التلوث البيئى إلى تنامى الإهتمام بوسائل المكافحة الحيوية المستخدمة ضد الحشرات بصفة عامة و رتبة حرشفية الأجنحة بصفة خاصة . لذلك كان الهدف من هذه الدراسة هو تحسين الكفاءة الوراثية للمبيدات الحيوية المستخدمة ضد يرقات دودة ورق القطن لإستخدامها فى برامج مكافحة الحشرات ، وذلك للحد من معدلات التلوث البيئى الناتجة عن إستخدام المبيدات الكيمائية و بغرض الإنتاج الآمن للغذاء . إستخدمت فى هذه الدراسة أربع سلالات من الباسليس ثيرونجنسز كمستقبلات فى عملية التزاوج مع بكتريا السراتيا كسلالة معطية لإستحداث إتحادات وراثية جديدة من الباسليس ثيرونجنسز بها تعبير جينى مرتفع لإنتاج إنزيم الشيتينيز الذى يقوم بتحليل الشيتين الموجود فى الهيكل الخلوى للحشرات مؤدياً إلى تحللها وموتها ومن الإتحادات الوراثية الجديدة تم إنتخاب أكفأ سلالتين فى تحليل الشيتين من المتحولات التزاوجية الناتجة عن كل تهجين بالنسبة لسميتهم ضد يرقات دودة ورق القطن مقارنة بالسلالات الأبوية الداخلة فى كل تهجين . تم تقدير أكفأ تركيز قاتل لـ50% من اليرقات بواسطة المتحولة التزاوجية Tr_4 (10^5x1,39) جزء فى المليون) ، متبوعاً ذلك بالمتحولات التزاوجية Tr_6 ,Tr_7 (10^5x2 جزء فى المليون) . سجلت المتحولات التزاوجية Tr_6 ,Tr_7 ,Tr_4 دليل سمية يعادل أكثر من 95% . أوضحت النتائج المتحصل عليها حدوث تعبير جينى مرتفع لإنزيم الشيتينيز فى هذه المتحولات التزاوجية . لذلك فإن عملية حساب التركيز القاتل تعتبر عملية مهمة فى مكافحة الحشرات للوصول إلى التركيز الذى يسبب قتل لـ 50% من عشائر يرقات دودة ورق القطن فى فترة زمنية معينة . ولقد تبين أن إستخدام تركيز منخفض من المتحولة التزاوجية Tr_4 قد يؤدى إلى موت 100% من عشائر اليرقات . من هنا يجب إلقاء الضوء على أن دليل السمية المرتفع الذى تم الحصول عليه بواسطة المتحولات التزاوجية يعكس مدى تفاعل جينات الكريستالز كمواد بروتينية سامة من الباسليس ثيرونجنسز مع الجينات المنتجة لإنزيم الشيتينيز من السراتيا واللذين يعززا معاً من زيادة إحتمال موت اليرقات كوسائل وراثية فعالة فى برامج مكافحة الحشرات فى حقول القطن .