Insecticidal Efficiency of Intergeneric Recombinants between *Bacillus thuringiensis* and *Bacillus subtilis* Against Viability of Cotton Leafworm

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Abstract: *Bacillus thuringiensis* is the most widely applied biological insecticide and is used to manage insects that affect forestry and agriculture. This ubiquitous spore-forming bacterium kills insect larvae largely through the action of insecticidal crystal proteins and is commonly deployed as a direct bacterial spray. Moreover, this study aimed to measuring recombinant efficiency of recombinant bioinsecticides (*Bacillus thuringiensis* x *Bacillus subtilis)* against *Spodoptera litoralis.* For decades, the mechanism of insect killing has been assumed to be toxin-mediated lysis of the gut epithelial cells, which leads to starvation, or *B. thuringiensis* septicemia. In the present work, two strains of *Bacillus* strains belonging to two serotypes and four of their transconjugants were screened with respect to their insecticidal activity against lepidopterous cotton pest. Two strains of *Bacillus* were screened for their drug resistance to be used as a genetic markers to identify bacterial strains in the conjugation process. *B. subtilis* was found to be resistant to crystal violet and sensitive to hiconcil. Although, *B. thuringiensis* was found to be sensitive to crystal violet, except for it was resistant to hiconcil. Bacterial transconjugants isolated from conjugation between both strains were more resistant to both crystal violet and hiconcil. Two groups of crystals and spores have been isolated within *Bacillus* strains and their transconjugants. The results appeared a highly potent recombinant efficiency in reducing leaves consumption at 72h (crystals), 120h (crystals + endospores) and 144h (crystals + endospors). However, transcongugants B and c was more effective in reducing the rate of consumption at all different times of larval ages. The weight of surviving larvae feeding on leaves sprayed with recombinants of Cry, Cry + End. was markedly reduced especially at 48h of larval age. However, ransconjugants A, B and C appeared recombinant efficiency in reduced survival percentage of larvae at 96h, 120h, 144h and 168h in response to recombinants of both cry, cry + endospores. The same trend was also shown by transconjugant C at 48h and 72h in response to both recombinant bioinsecticides. The results indicated that recombinant endotoxin preparations containing crystals + endospores was more effective against *Spodoptera litoralis* than that containing crystals.

Keywords: Bioinsecticides, Recombinant efficiency, Insecticidal activity, Recombinants, *Spodoptera littoralis*.

INTRODUCTION

Bacillus thuringiensis (or Bt) is a Gram-positive, soil-dwelling bacterium, commonly used as a biological pesticide; alternatively, the Cry toxin may be extracted and used as a pesticide. *B. thuringiensis* also occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well as on the dark surfaces of plants [1]. During sporulation, many Bt strains produce crystal proteins (proteinaceous inclusions), called δ-endotoxins, that have insecticidal action. This has led to their use as insecticides, and more recently to genetically modified crops using Bt genes. There are, however, many crystal-producing Bt strains that do not have insecticidal properties.

B. thuringiensis was first discovered in 1901 by Japanese biologist Shigetane Ishiwatari. In 1911, *B. thuringiensis* was rediscovered in Germany by Ernst Berliner, who isolated it as the cause of a disease called *Schlaffsucht* in flour moth caterpillars. In 1976, Robert A. Zakharyan reported the presence of a plasmid in a strain of *B. thuringiensis* and suggested the plasmid's involvement in endospore and crystal formation. *B. thuringiensis* is closely related to *B.cereus*, a soil bacterium, and *B.anthracis*, the cause of anthrax: the three organisms differ mainly in their plasmids. Like other members of the genus, all three are aerobes capable of producing endospores. Upon sporulation, *B. thuringiensis* forms crystals of proteinaceous insecticidal δ-endotoxins (called crystal proteins or Cry proteins), which are encoded by *cry* genes. In most strains of *B. thuringiensis*, the *cry* genes are located on the plasmid [2, 3].

Cry toxins have specific activities against insect species of the orders Lepidoptera (moths and butterflies), Diptera (flies and mosquitoes), Coleoptera (beetles), Hymenoptera (wasps, bees, ants and sawflies) and nematodes. Thus, *B. thuringiensis* serves as an important reservoir of Cry toxins for production of biological insecticides and insect-resistant genetically modified crops. When insects ingest toxin crystals, the alkaline pH of their digestive tract activates the toxin. Cry toxin gets inserted into the insect gut cell membrane, forming a pore. The pore results in cell lysis and eventual death of the insect [4].

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Spores and crystalline insecticidal proteins produced by *B. thuringiensis* have been used to control insect pests since the 1920s. They are now used as specific insecticides under trade names such as Dipel and Thuricide. Because of their specificity, these pesticides are regarded as environmentally friendly, with little or no effect on humans, wildlife, pollinators, and most other beneficial insects. The Belgian company Plant Genetic Systems was the first company (in 1985) to develop genetically engineered (tobacco) plants with insect tolerance by expressing *cry* genes from *B. thuringiensis* [5].

B. thuringiensis-based insecticides are often applied as liquid sprays on crop plants, where the insecticide must be ingested to be effective. The solubilized toxins are thought to form pores in the midgut epithelium of susceptible larvae. Recent research has suggested the midgut bacteria of susceptible larvae are required for *B. thuringiensis* insecticidal activity [6].

Bacillus thuringiensis serovar *israelensis*, a strain of *B. thuringiensis* is widely used as a larvicide's against mosquito larvae, where it is also considered an environmentally friendly method of mosquito control.

In Lepidoptera, specificity is due in part to the extremely alkaline midgut environment that is required to solubilize the protoxin into the active form. Solubilized protoxins are activated by midgut proteases and bind to receptors on the epithelial surface. Then, by a process that remains unclear, the toxins appear to insert into the membranes of gut cells, where they form pores that lead to cell lysis [7]. It has been proposed that disruption of the midgut epithelium results in a prolonged cessation of feeding and eventual death by starvation. An alternative proposed mechanism of killing is that extensive cell lysis provides spores access to the more favorable environment of the hemocoel, where they germinate and reproduce, leading to septicemia and death [8]. Although these two models have been cited many times in the literature, neither is entirely consistent with experimental observations.

Conjugation is the direct transfer of DNA from one bacterial cell to another bacterial cell. The transferred DNA is a plasmid, a circle of DNA that is distinct from the main bacterial chromosome. The F plasmid is similar to a virus or a transposon in its ability to move independently of the main chromosome. The transfer of the plasmid take advantage of the complementary nature of double stranded DNA. One strand of the plasmid is transferred and the other remains in the original cell. Both strands have the complementary stranded added so that each cell ends up with a complete plasmid. In this study conjugation was done between two cultures of *Bacillus* strains belonging to two serotypes (*B. thuringiensis* x *B. subtilis)* and four of their transconjugants were screened for toxic activity against lepidopterous cotton pest.

Mediate transfer of genetic material (DNA) from one bacterial cell (donor) to the other (recipient) making possible the subsequent recombination events. The most obvious difference between three processes of DNA transfer is the mode of transfer of genetic material (DNA) from donor to recipient cell. In conjugation, donor cell transfers its DNA to a recipient cell only when both the cells are in physical contact through a specialized sex-pilus or conjugation tube. In transformation, there is transfer to cell-free or naked DNA from donor cell to recipient cell. In transduction, the transfer of genetic material (DNA) from donor cell to recipient cell is mediated by a bacteriophage.

The recombination in bacteria usually takes place (except for rare occurrence of complete DNA transfer during conjugation) forming partial normal diploids also called heterozygotes (heterogenotes) or merozygotes (merogenotes). Thus the recipient cells are converted to heterozygotes or merozygotes containing a fragment of the donor DNA, the exogenote, and a complete recipient DNA, the endogenote. This study aimed to evaluate recombinant efficiency of recombinants resulted from conjugation between *Bacillus thuringiensis* x *Bacillus subtilis* for their toxic effects of bioinsecticides in reducing feed consumption and decreased survival of *Spodoptera littoralis* larvae.

MATERIALS AND METHODS

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 5% peptone, 0.1% yeast extract and 0.5% NaCl, pH 7.5 [8].

Antibiotic Susceptibility Assays

Antibiotic susceptibility was measured by plate diffusion method, according to Collins and Lyne [9] 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 each bacterial strain, and concentration of antibiotics used [10]. Different antibiotics were used with the concentration of 400 µg / ml, according to Roth and Sonti [11].

rfa Mutation

Strains having the deep rough (*rfa)* character should be tested for crystal violet sensitivity [12]. 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.

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*. [13], by inoculating 10 µl samples of the donor cultures onto the surface of selective medium, previously seeded with 100 µl of the recipient culture. A single colony of transconjugants was picked up and transferred to LB slant agar medium.

Separation of Crystals and Endospores

Crystals and endospores were collected and purified according to (Karamanlidou *et al*., [14]. 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.

Bioassay of Toxicity

The toxicity was bioassayed with *Spodoptera littoralis* second instar larvae (mean body weight = 10 mg) according to Klanfon and DeBarjac [16] 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 *B. thuringiensis* using a Gentaur micropipette to dispense 200 µl of the suspension on 2-3 gram of diet surface of *Ricinus communis* [15]. 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 weighted daily [16].

Measuring Recombinant Efficiency

Recombinant efficiency (RE) was calculated according to Winfridus Bakker [17] using the following formula;

RE (Mid parents) = Average F_1-Average P_P / Mid parents, measured in units of the trait

RE(Better parent) = Average PF_1-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.

RESULTS AND DISCUSSION

Genetic Marking Due to Antibiotics

A brief look at an example of penicillin resistance reveals the increase in the frequency of antibiotic-resistant organisms since the time when antibiotic use became common. Penicillin is an antibiotic produced by the common bread mold Penicillium that was

discovered accidentally in 1929 by the British microbiologist, Alexander Fleming. By the 1940s, penicillin was available for medical use and was successfully used to treat infections in soldiers during World War II. Since then, penicillin has been commonly used to treat a wide range of infections. In 1967 the first penicillin-resistant Streptococcus pneumoniae was observed in Australia, and seven years later in the U.S. another case of penicillin-resistant S. pneumoniae was observed in a patient with pneumococcal meningitis. The increase in resistance among these organisms clearly indicates a change in the frequency of antibiotic resistance genes [2, 12].

Both *Bacillus thuringiensis* and *Bacillus subtilis* used in this study were tested for their antibiotic and drug resistance using 18 compounds (Table **1**). *B. thuringiensis* was found to be more resistant to Hiconcil, and intermediate resistant to streptomycin. In addition, *B. thuringiensis* was found to be sensitive to all other antibiotics and drugs (crystal violet) (Figure **1**). Similarly *B. subtilis* was found to be more sensitive to all antibiotics and drugs. *B. subtilis* was found to be more resistant to crystal violet (Figure **2**), for disappearance of inhibition zone. This have often relied upon resistance as a genetic marker to identify bacterial strains [18]. Resistance to antibiotics and chemical compounds has proved to be a stable marker with a high level of resistance available. Crystal violet resistance in *B. subtilis* is also similar to that of hiconcil in *B. thuringiensis* and provides a second potential marker for use in conjugation process to isolate bacterial transconjugants. The results obtained here are in agreement with those reported by Garg *et al*. [19], who screened more than 200 wild type isolates of chikpea for their drug resistance, they found that only 15 were resistant to one or more antibiotics at concentration of 5 µg/ml (units / ml). In addition, some isolates were found to be resistant to more than one antibiotic.The bacterium that acquires the antibiotic resistance genes still has the physical and metabolic qualities that distinguish it from other bacteria kinds and associates it with its own kind of bacteria. The observed increase in the frequency of antibiotic-resistant bacteria has resulted from the increased use of antibiotics in medicine and agriculture, resulting in the reduction of organisms that do not possess antibiotic resistance genes.

The results obtained herein agreed with Stuart *et al*. [20], who examined forty-eight clinical isolates of Streptococcus suis for antibiotic sensitivity and the

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***
Tetracide	-	-
Kanamycin	-	-
Streptomycine	-	-
Flucamox	-	-
Ampicillin	-	-
Duricef	-	-
Vibramycin	-	-
Flummox	-	-
Erythromycin	-	-
Velosef	-	-
Cidocteine	-	-
Cloxapen	-	-
Septazole	-	-
Hostacycline	-	-
Tetracycline	-	-
Hiconcil	+	-
Chloramphenicol	-	-
Crystal violet	-	+

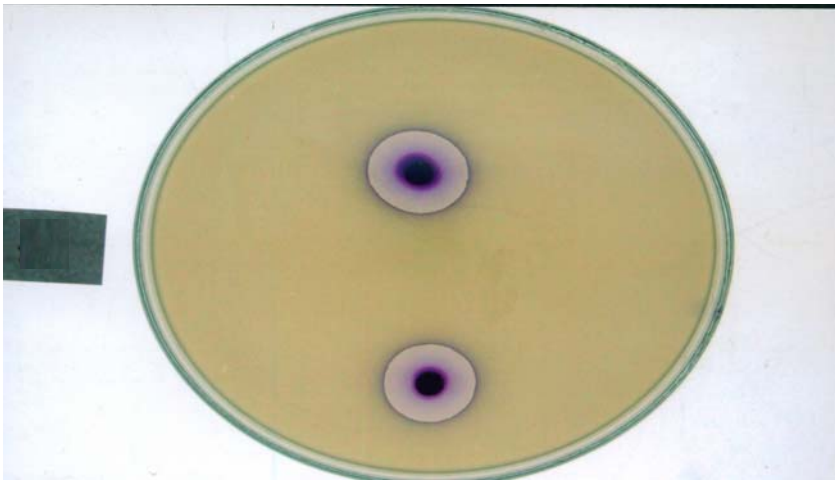


Figure 1: Sensitivity of *B. thuringiensis* to crystal violet.

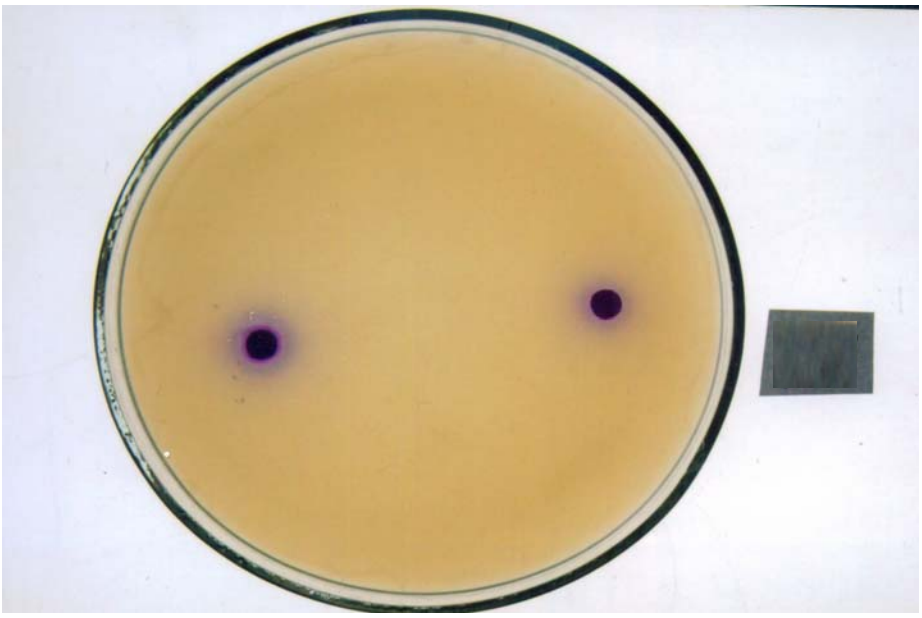


Figure 2: Resistance of *B. subtilis* to crystal violet.

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.

Conjugation

Conjugation process was carried out between *Bacillus subtilis* (*Hico*$^{-}$ *rfa*$^{+)}$ with *Bacillus thuringiensis* serovar *Kurstaki* (*Hico*$^{+}$ *rfa*$^{-}$*)*, which have the opposite genetic markers. The results obtained here are in accordance with that found by Campbell [21], 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 to adapt to a changing environment and to compete with its neighbors.

Effect of Bioinsecticides on the Rate of Consumption of *Spodoptera littoralis* Larvae

The results summarized in Table **2** and diagrammatic in Figure **3** showed Recombinant efficiency in reducing the average consumption of *Spodoptera littoralis* larvae feeding on leaves of

Table 2: Recombinant Efficiency (RE) Percentage in Reducing (-) the Average Consumption of *Spodoptera littoralis* Larvae Feeding on Leaves of *Ricinus communis* (gram/day) After Sprayed with *Bacillus thuringiensis* Bioinsecticides.

Bioinsecticides		Treatment time (h)												
		24		48		72		96		120		144		168
		Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry + End
B. thuringiensis		0.18	0.19	0.20	0.19	0.21	0.24	0.26	0.28	0.32	0.25	0.20	0.20	0.40
B. subtilis		0.19	0.22	0.22	0.23	0.22	0.19	0.32	0.23	0.34	0.45	0.42	0.40	0.32
Mid Parents		0.19	0.21	0.21	0.21	0.22	0.22	0.29	0.26	0.33	0.35	0.31	0.30	0.36
TA	REMP	-2.7	-7.3	9.5	-4.8	2.3	-30.2	44.8	5.9	0.0	-17.1	258.1	36.7	72.2
	REBP	-5.3	-13.6	4.5	-13.0	0.0	-37.5	31.3	-3.6	-2.9	-35.6	164.3	2.5	55.0
TB	REMP	2.7	-7.3	-9.5	38.1	-16.3	-7.0	17.2	2.0	45.5	-28.6	464.5	-73.3	-63.9
	REBP	0.0	-13.6	-13.6	26.1	-18.2	-16.7	6.3	-7.1	41.2	- 44.4	316.7	-80.0	-67.5
TC	REMP	2.7	-12.2	0.0	28.6	-34.9	-11.6	-10.3	-17.6	33.3	-28.6	-25.8	-30.0	86.1
	REBP	0.0	-18.2	-4.5	17.4	-36.4	-20.8	-18.8	-25.0	29.4	-44.4	-45.2	-47.5	67.5
TD	REMP	-8.1	31.7	-19.0	38.1	-2.3	2.3	-13.8	49.0	3.0	-28.6	122.6	-30.0	113.9
	REBP	-10.5	22.7	-22.7	26.1	-4.5	-8.3	-21.9	35.7	0.0	-44.4	64.3	-47.5	92.5

Cry = Crystals, End = Endospores, T = Transconjugant, REMP = Recombinant efficiency related to mid – parents, REBP = Recombinant efficiency related to better parent.

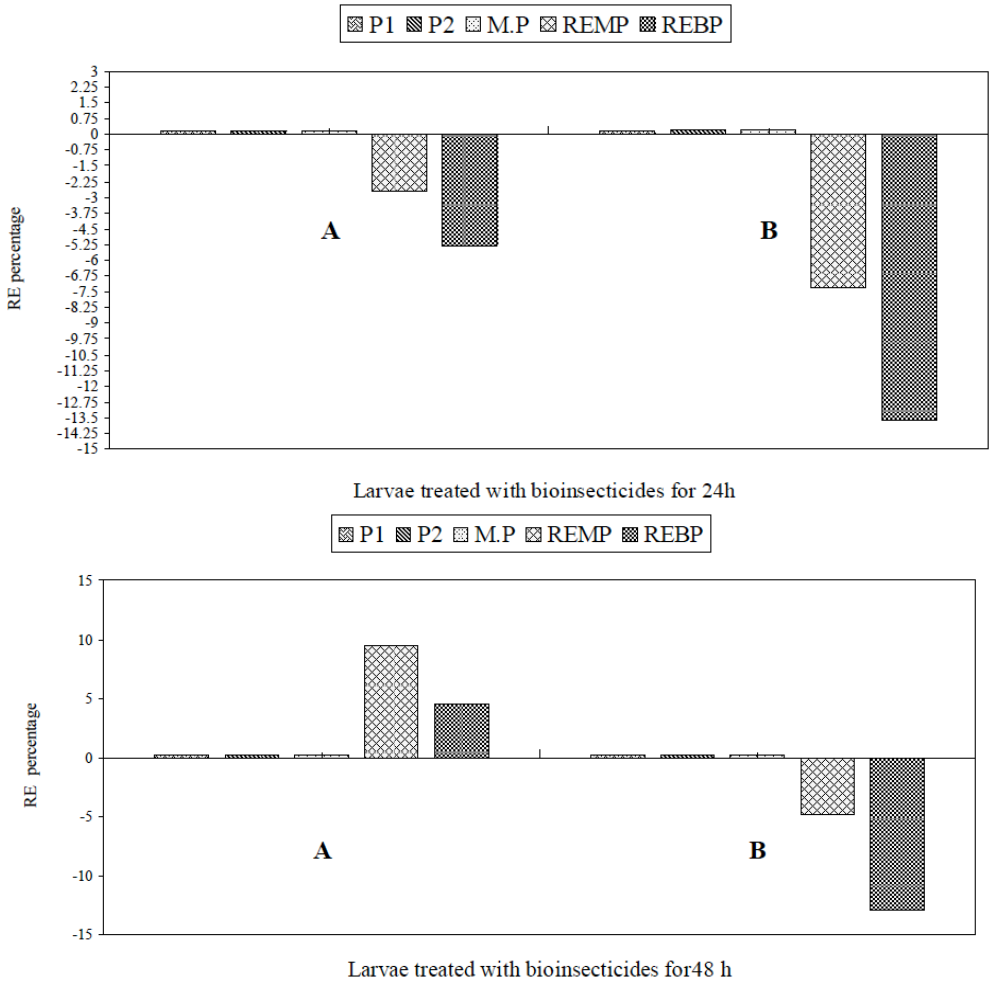
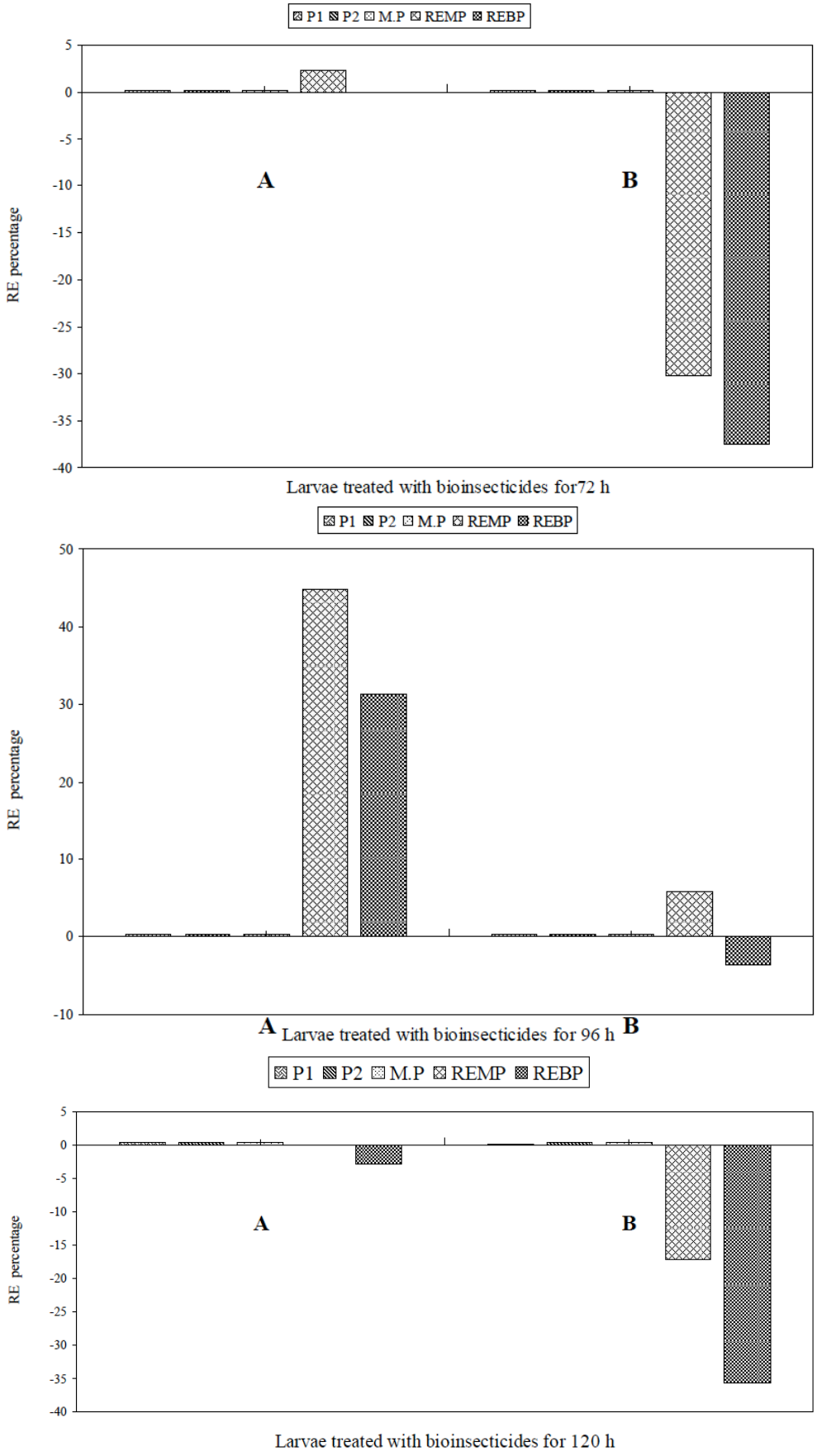


Figure 3: Transconjugant-A.

Figure 3: Continued for transconjugant-A.



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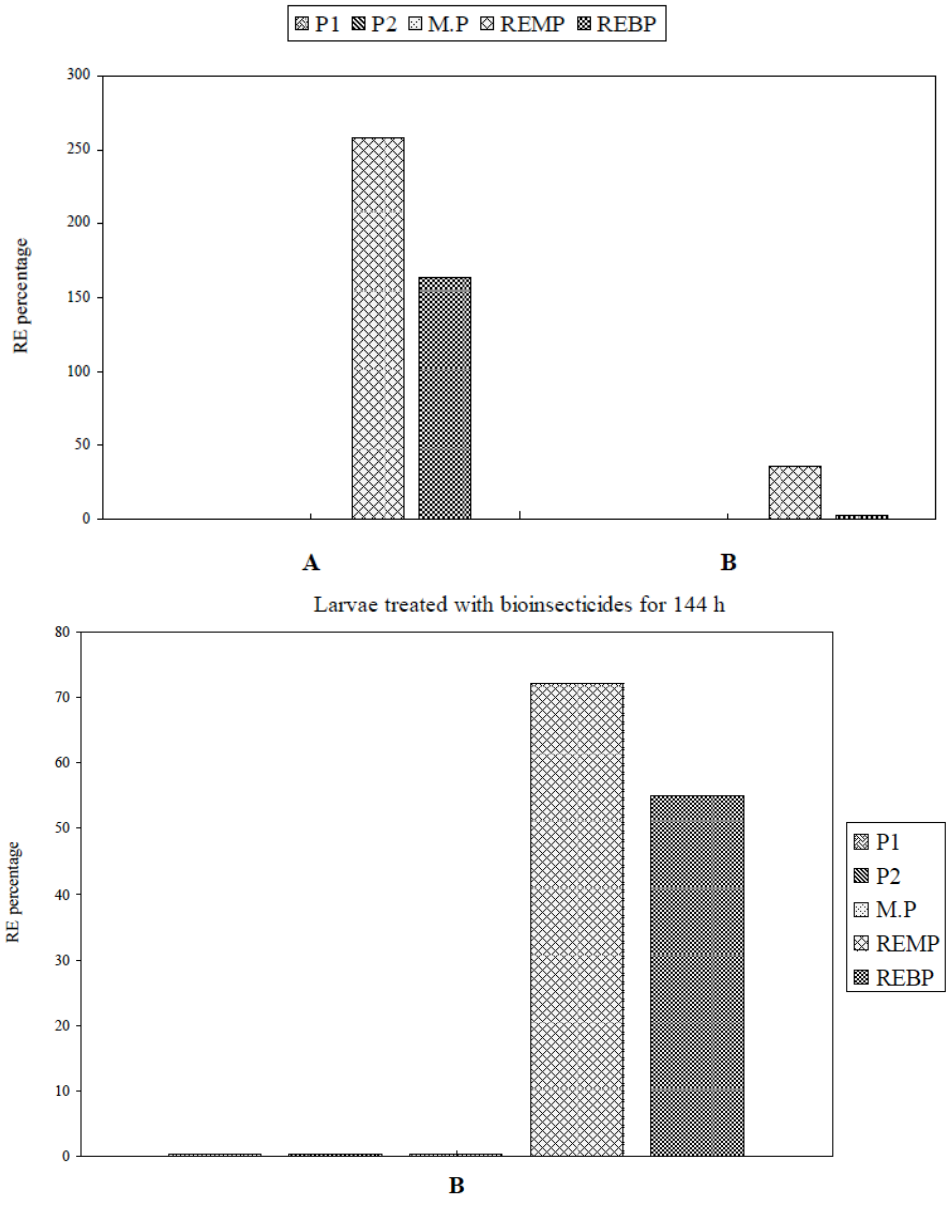


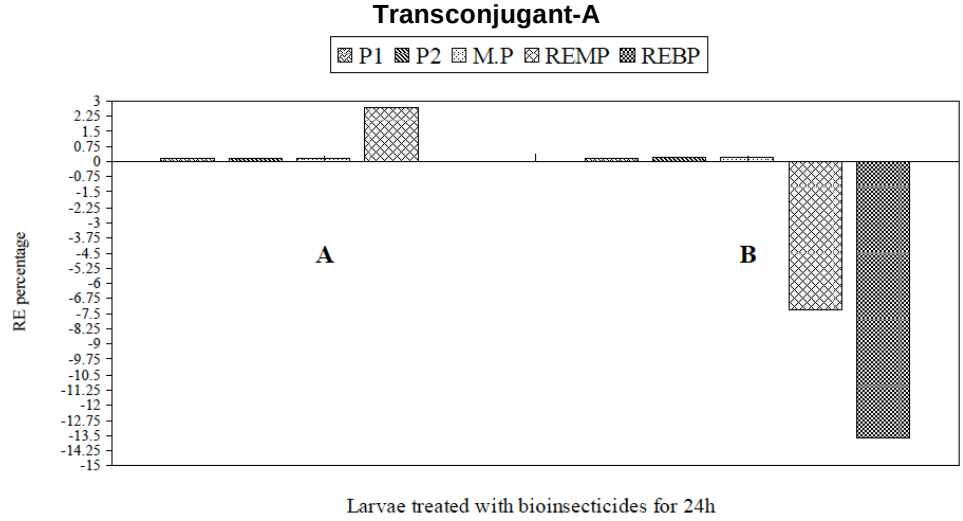
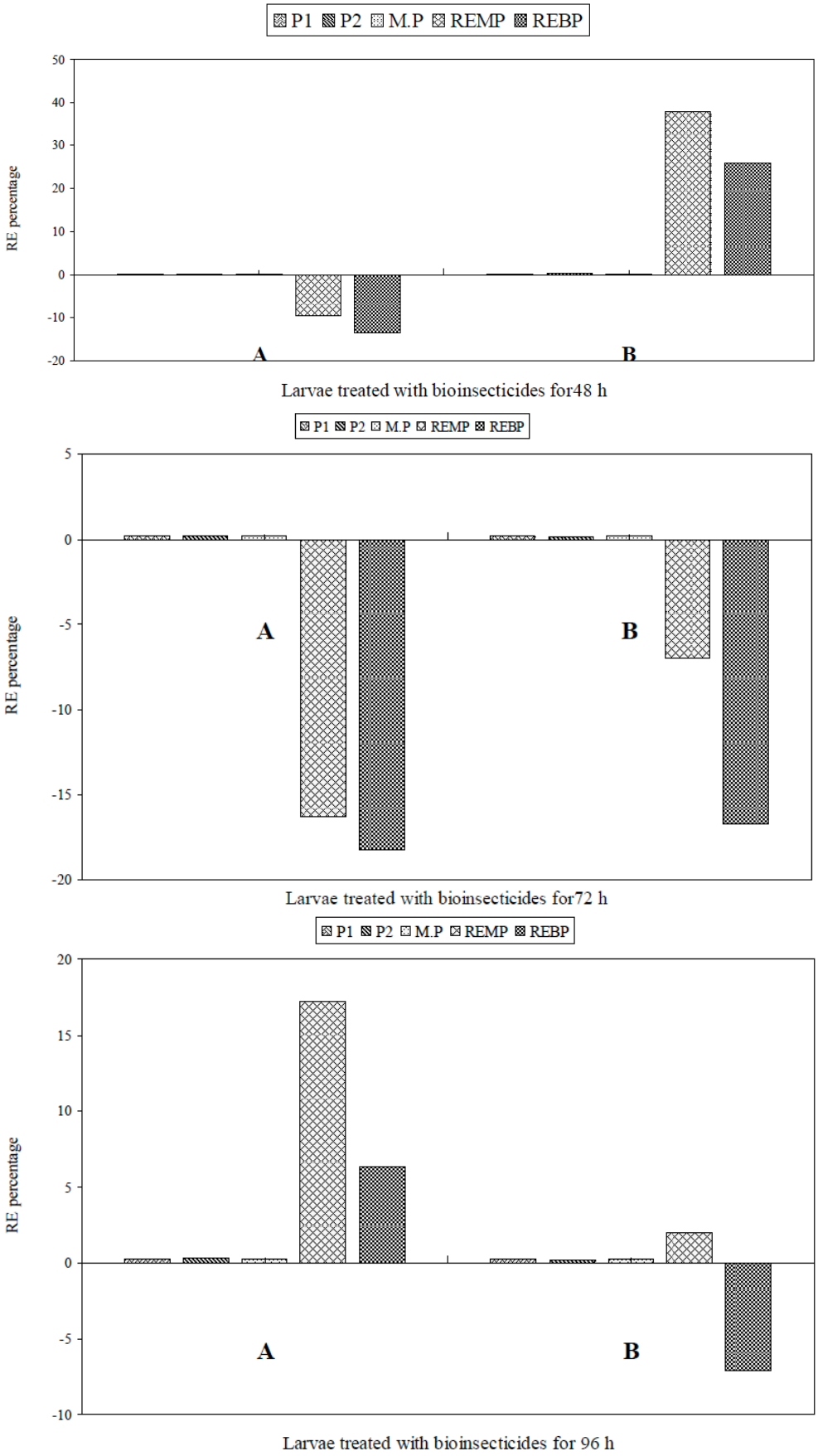


Figure 3: Transconjugant-B.

Figure 3: Continued for transconjugant-B.



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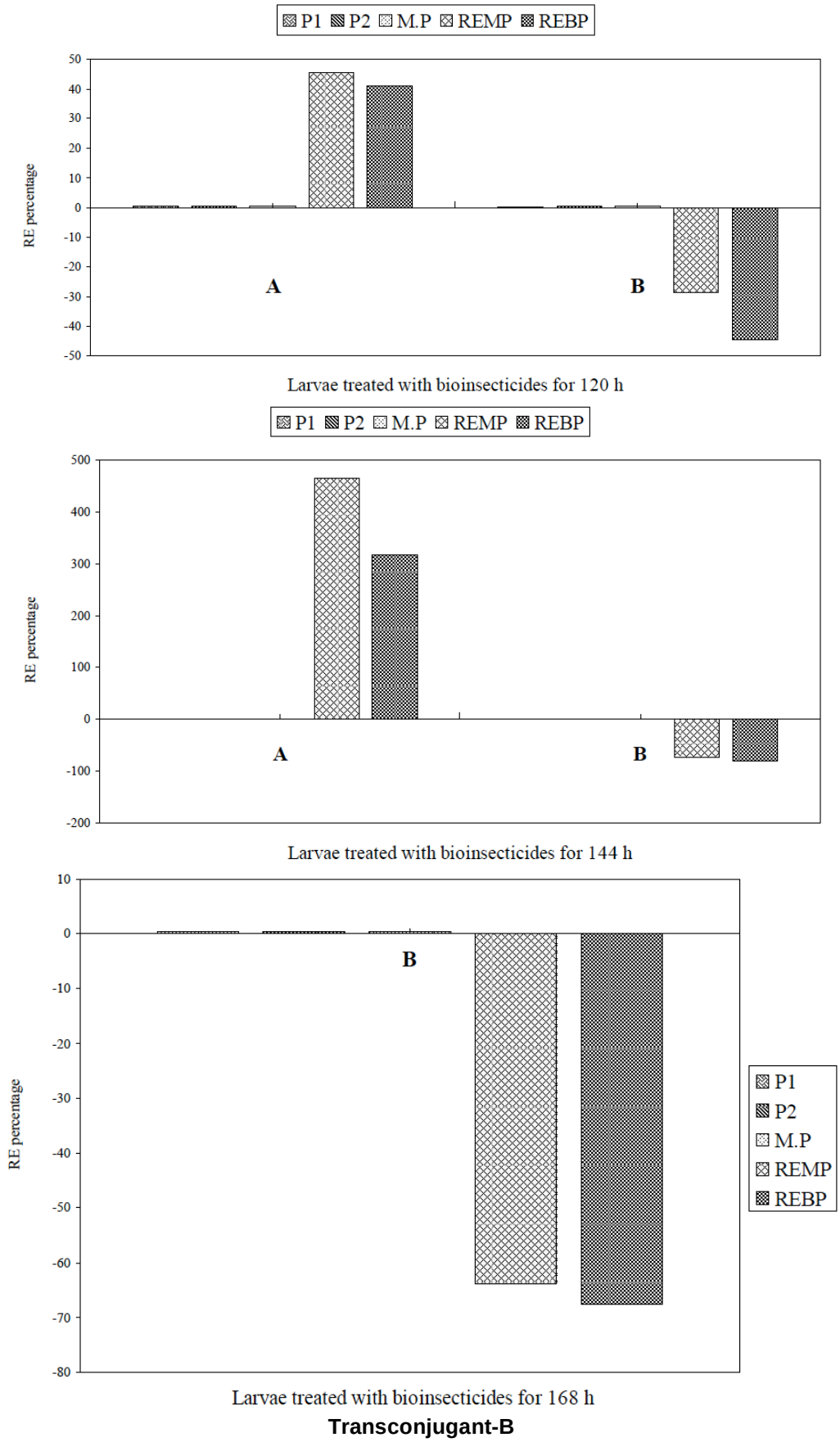
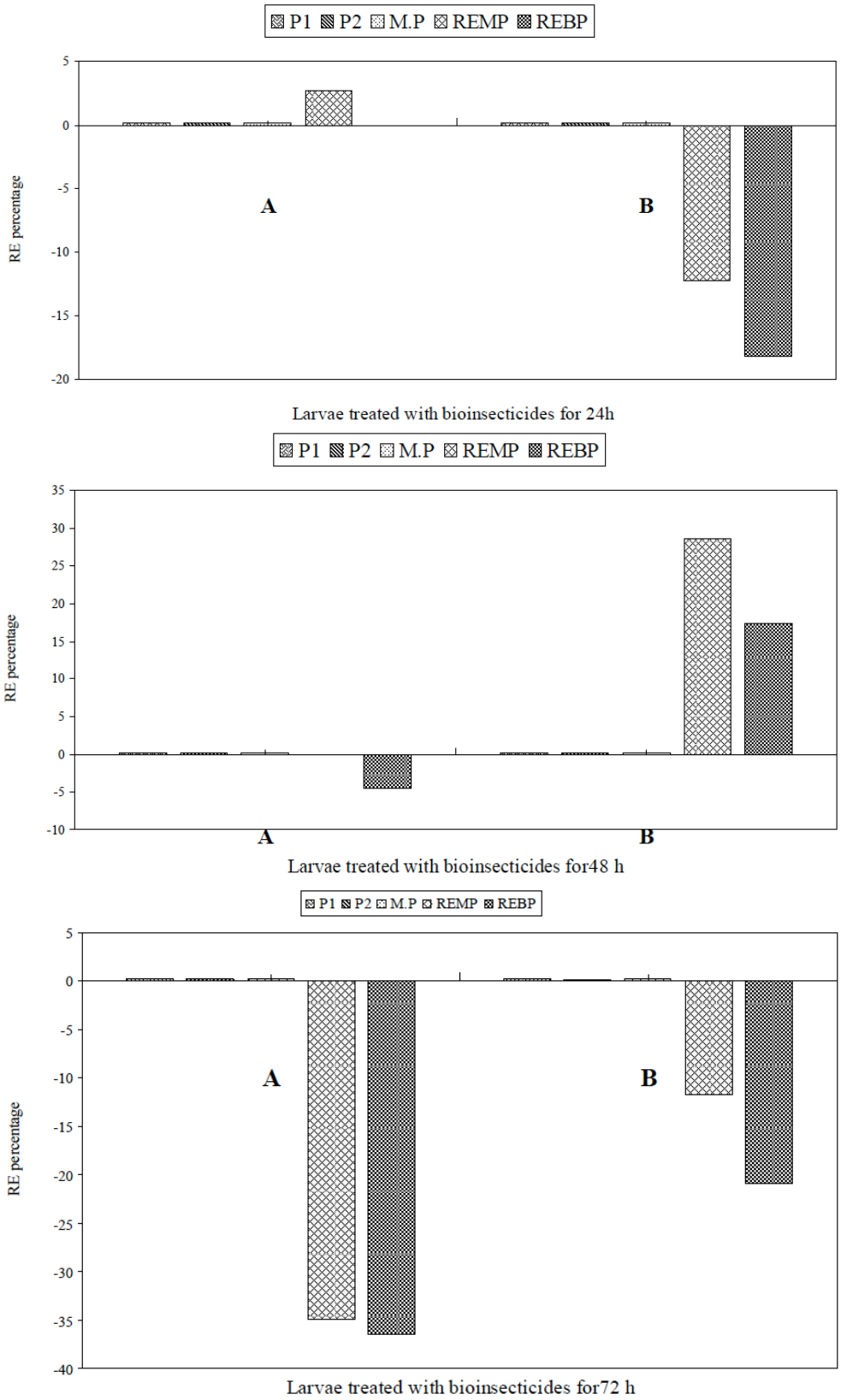


Transconjugant-B

Figure 3: Continued for transconjugant-C.



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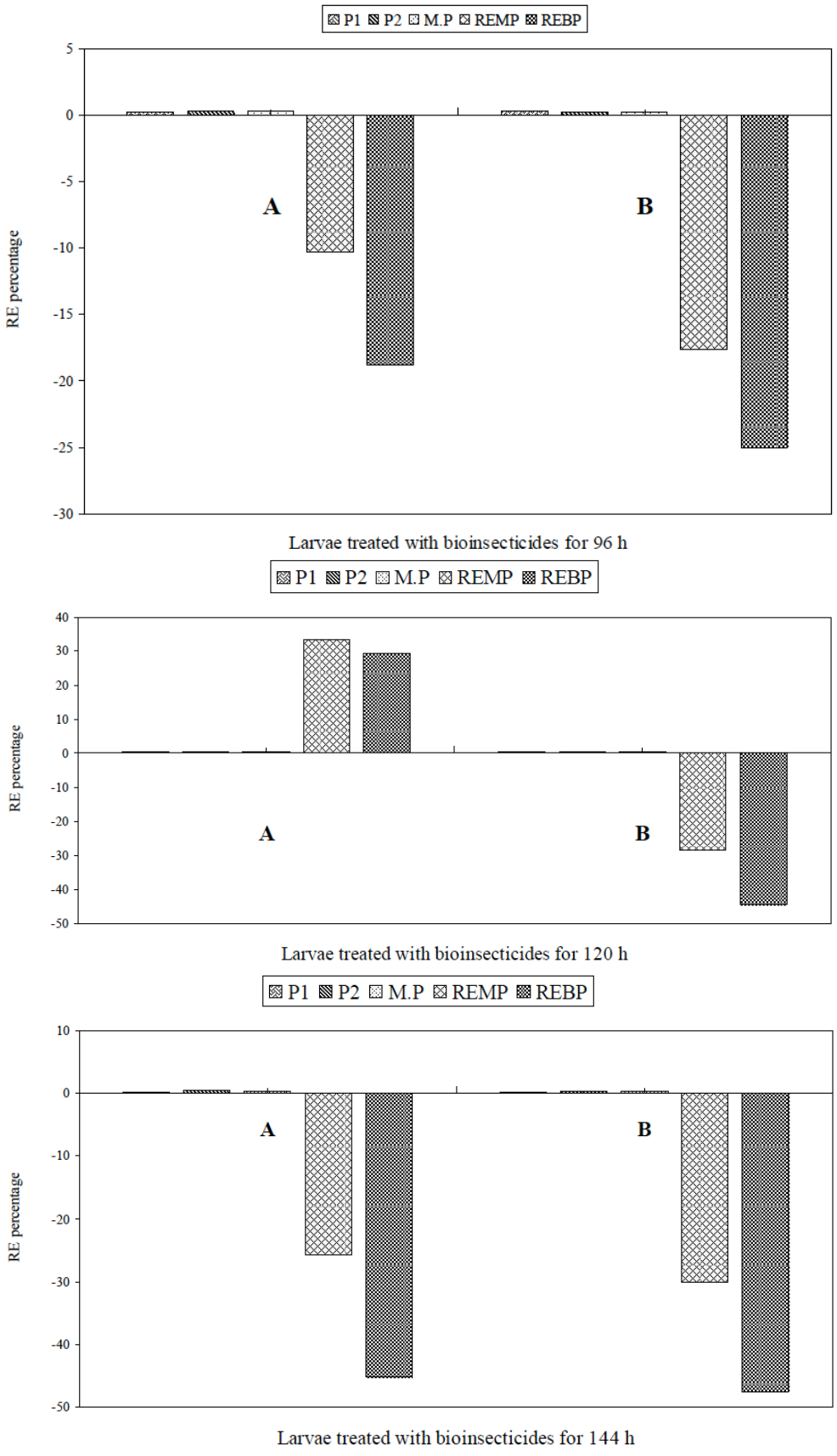

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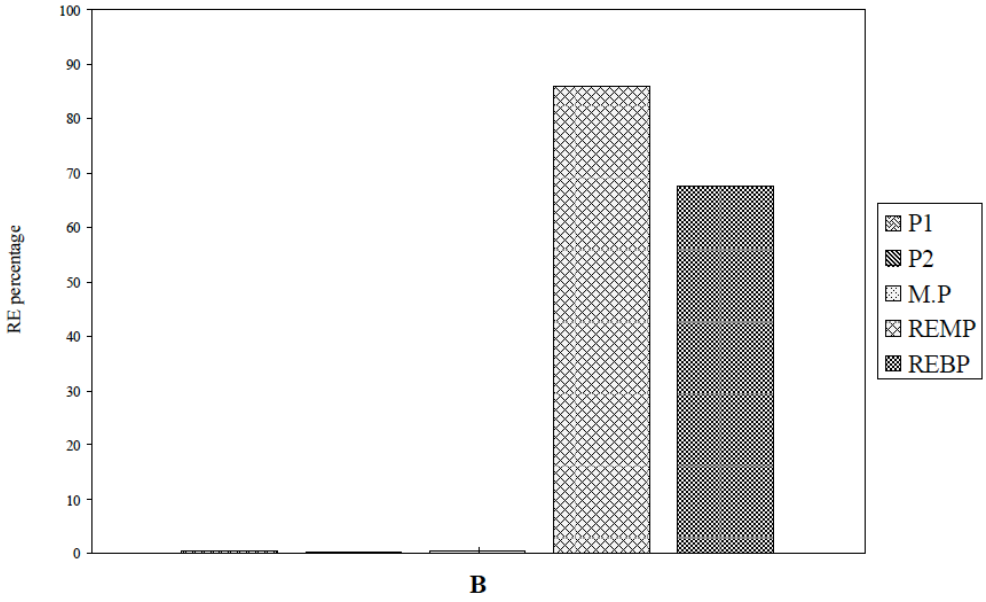
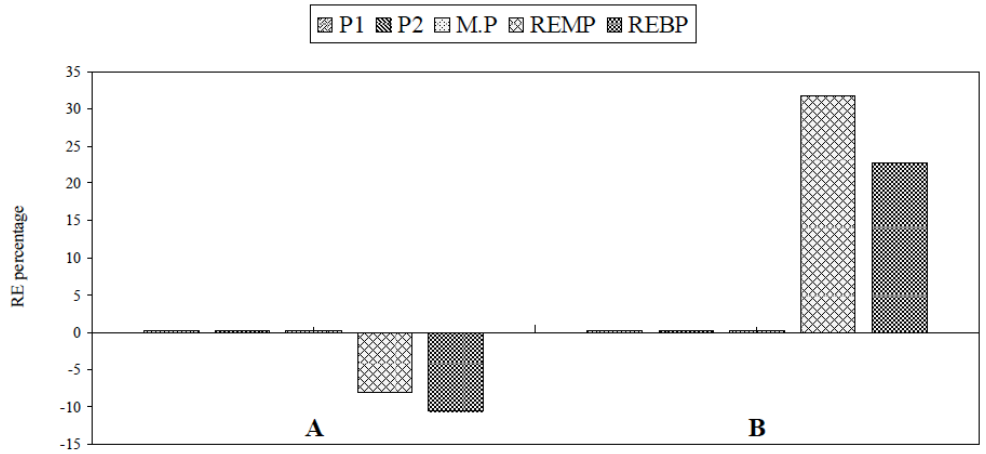


Transconjugant-C



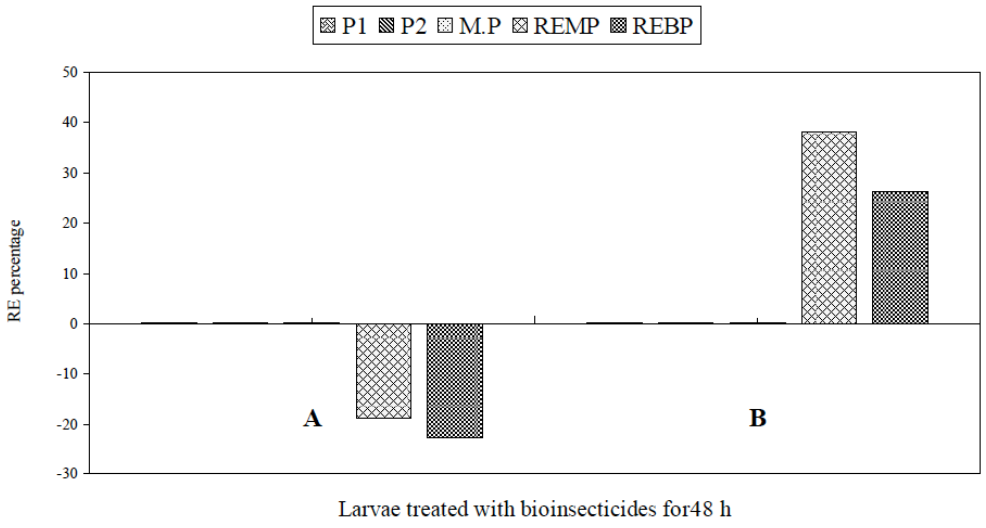
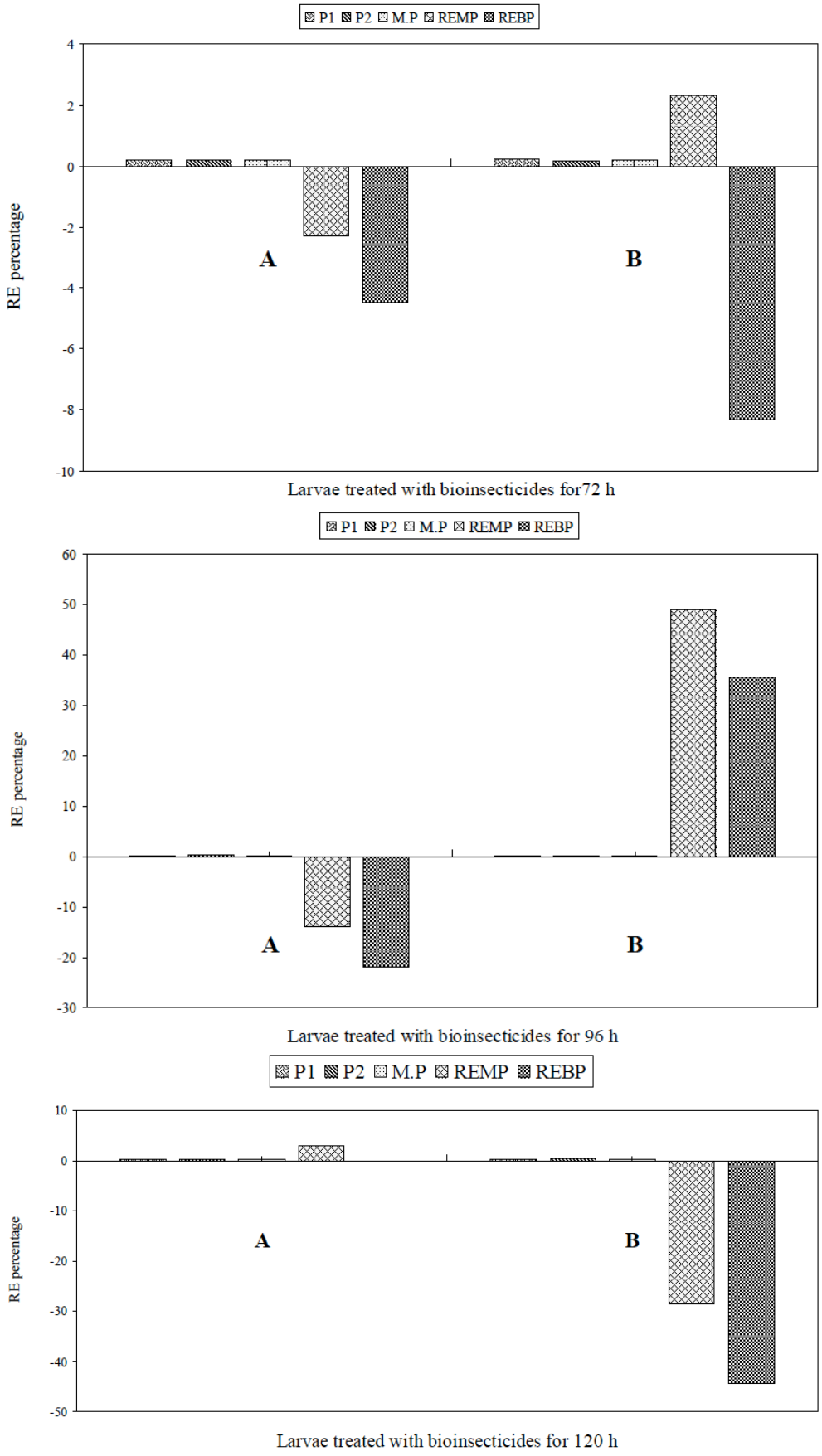


Figure 3: Transconjugant-D.

Figure 3: Continued for transconjugant-D.



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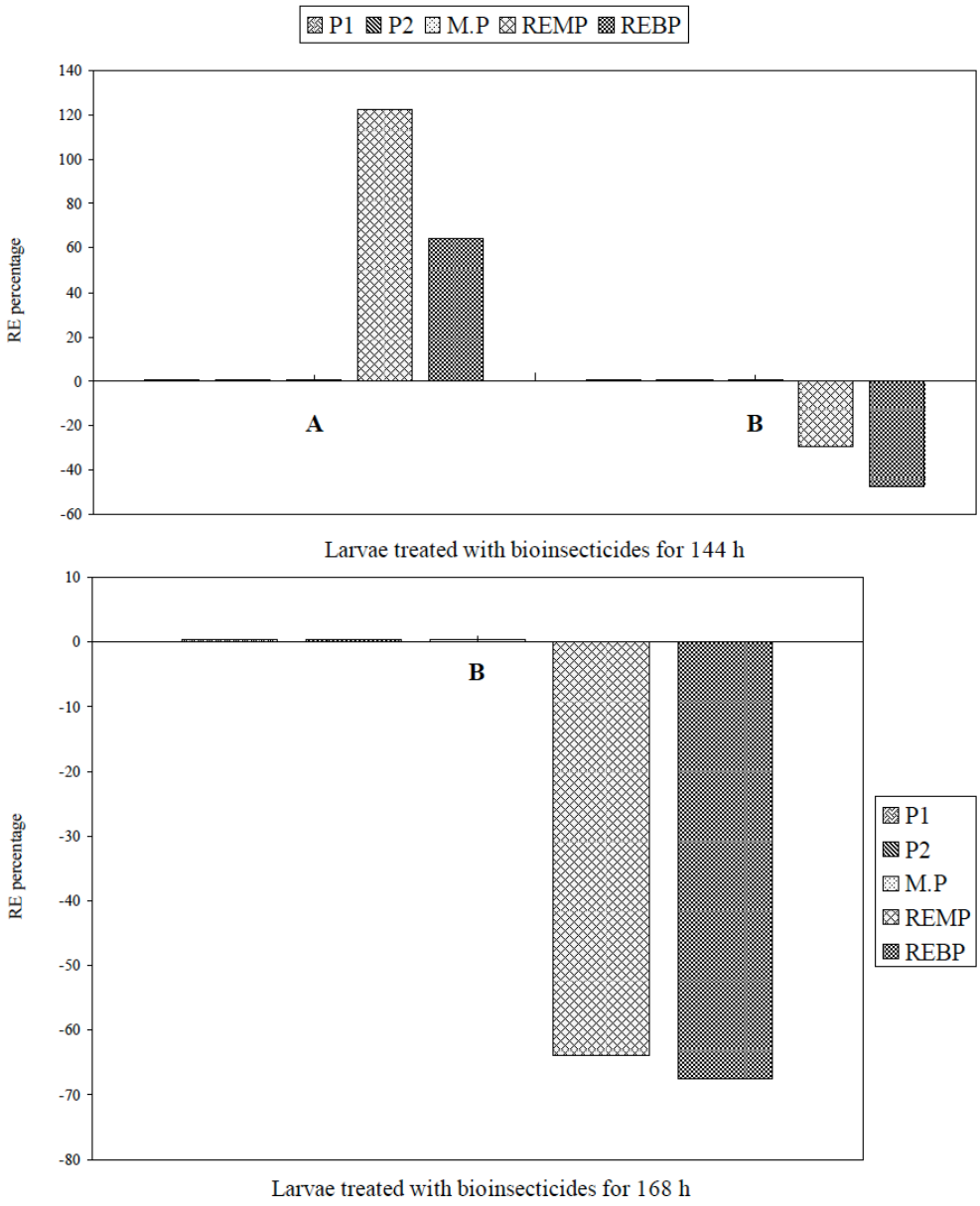


Figure 3: Recombinant efficiency of feeding leaves consumption by larval stage of *Spodoptera littoralis* after treated with bioinsecticides.

Note: A = Crystals, B = Crystals + Endospores, T = Trasconjugant.

Ricinus communis (gram / day) sprayed with bioinsecticides of Bt transconjugants. It is of interest to note that the leaves sprayed with crystals + endospores appeared negative recombinant efficiency in the rate of leaves consumed more than that sprayed with crystals alone as seen at 24 h, 72h, 96h, 120h and 144h. The results indicated that a highly potent recombinant efficiency in reducing leaves consumption was appeared at 72h (crystals), 120h (crystals + endospores) and 144h (crystals + endospors). However, transcongugants B and c was more effective in reducing the rate of consumption at all different times of larval ages. One of the important observations in this study was to select these transconjugants with maximum insecticidal activity to be used in bioinsecticides preparations. The results obtained herein indicated that *Bacillus thuringiensis* transconjugants is the most widely applied as biological insecticide and were used to manage insects that affect forestry and agriculture. This ubiquitous spore-forming bacterium kills insect larvae largely through the action of insecticidal crystal proteins and is commonly

deployed as a direct bacterial spray. Moreover, plants engineered with the *cry* genes encoding the *B. thuringiensis* crystal proteins are the most widely cultivated transgenic crops. For decades, the mechanism of insect killing has been assumed to be toxin-mediated lysis of the gut epithelial cells, which leads to starvation, or *B. thuringiensis* septicemia. This leading to altered the susceptibility of *Spodoptera litura* to *B. thuringiensis* also affected the bacterial community in the larvae's midgut. These results suggest that transcongugants is largely responsible for the septicemia associated with *B. thuringiensis*-induced mortality in *Spodoptera litura* larvae. To cause septicemia, bacteria must be able to enter and multiply in the hemolymph. The present invention indicated that novel *Bacillus thuringiensis* transconjugant strains having improved insecticidal activity.

B. thuringiensis is already a useful alternative or supplement to synthetic chemical pesticide application in commercial agriculture, forest management, and mosquito control. It is also a key source of genes for transgenic expression to provide pest resistance in plants. Most *B. thuringiensis* toxin genes appear to reside on plasmids [22]. The present results agreed with Baum and. Malvar. [23], who reported that developed of *B. thuringiensis* has a fascinating array of molecular mechanisms to produce large amounts of pesticidal toxins during the stationary phase of growth. One can only speculate about the ecological value to the bacterium of using several *cry* gene expression systems. However, coexpression of multiple toxins is likely to increase the host range of a given strain or of a population exchanging toxin genes. One report has suggested plasmid transfer between different *B. thuringiensis* strains during growth within an insect [24].

A common characteristic of the *cry* genes is their expression during the stationary phase. Their products generally accumulate in the mother cell compartment to form a crystal inclusion that can account for 20 to 30% of the dry weight of the sporulated cells. The very high level of crystal protein synthesis in *B. thuringiensis* and its coordination with the stationary phase are controlled by a variety of mechanisms occurring at the transcriptional, posttranscriptional, and posttranslational levels [23].

Effect of Recombinant Bioinsecticides in Reducing the Average Weight of Larvae

As shown from the results presented in Table **3** and diagrammatic in Figure **4** which showed that the efficiency of recombinant bioinsecticides in reducing the average weight of surviving larvae was achieved by transconjugant A at 48h, 96h and 168h in response to spraying feeds with crystals + endospores. However, transconjugant B appeared the same trend at 96h, 120h, 144h and 168h in response to spraying feeds with crystals + endospores. In addition, transconjugant C appeared the same trend at 48h and 96h in response to Cry, Cry + endospores, as well as, transconjugant D at 48h. However, transcongugant D appeared the same trend at 48h, 144h and 168h in response to cry + endospores. The results indicated that feeding the larvae on leaves sprayed with recombinant crystals + endospores caused, in most cases, less weight than that feeding on leaves sprayed with crystals only. The weight of surviving larvae feeding on leaves sprayed with recombinants of Cry, Cry + End. was markedly reduced especially at 48h of larval age. It is of interest because *Spodoptera litura* is one of the more important pests infecting field crops of economic importance in the world. It is resistant to conventional insecticides. The commercially available *B. thuringiensis* HD-1 preparation exhibits poor mortality against this pest [16]. The results obtained here indicated that the effect of microbial product containing crystals + endospores caused recombinant efficiency in a severe weight loss in the surviving larvae than that containing crystals alone.

The results indicated that the domain might be responsible for the formation of lytic pores in the intestinal epithelium of the target organism, one of the proposed mechanisms of Cry toxin activity. The present results agreed with Wang *et al*. [25], who found that a recombinant plasmid pSTK-3A containing *cry3Aa7* gene encoding a coleopteran-specific insecticidal protein was constructed and introduced into wild *Bacillus thuringiensis* subsp. *aizawai* G03, which contained *cry1Aa*, *cry1Ac*, *cry1Ca*, and *cry2Ab* genes and was highly toxic to lepidopteran insect pests. The same authors also found that synthesis of the Cry3Aa7 toxin conferred high and broad toxicity to the recombinant strain against coleopteran order, elm leaf beetle (*Pyrrhalta aenescens)* (LC_{50} 0.35 mg/ml), for which the parental strain was not toxic. Both the parental strain and recombinant strain showed strong insecticidal activity to lepidopteran pests, beet armyworm (*Spodoptera exigua)*, diamondback moth (*Plutella xylostella)*, and cotton bollworm (*Helicoverpa amigera)*, respectively. The lethal concentration 50% (LC_{50}) of G033A against *S*. *exigua*, *P*. *xylostella*, and *H*. *amigera* was 4.26, 0.86, and 1.76 µg/ml, respectively.

Table 3: Recombinant Efficiency Percentage in Reducing (-) the Average Weight of Surviving Larvae (g) of *Spodoptera littoralis* Feeding on Leaves of *Ricinus communis* (gram/day) After Sprayed with *Bacillus thuringiensis* Bioinsecticides.

Bioinsecticides		Treatment time (h)														
		0.0		24		48		72		96		120		144		168
		Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry + End
B. thuringiensis		0.02	0.01	0.04	0.01	0.06	0.03	0.13	0.11	0.22	0.11	0.32	0.15	0.31	0.18	0.22
B. subtilis		0.02	0.01	0.04	0.01	0.08	0.05	0.16	0.11	0.28	0.17	0.36	0.16	0.27	0.19	0.16
Mid Parents		0.02	0.01	0.04	0.01	0.07	0.04	0.14	0.11	0.25	0.14	0.34	0.16	0.29	0.19	0.19
TA	REMP	0.3	0.0	11.4	44.4	18.3	1.3	12.8	26.7	28.9	15.5	9.7	47.0	261.0	7.0	6.8
	REBP	-5.0	-12.5	12.8	18.2	1.2	-13.3	0.0	26.7	15.3	-4.8	3.7	41.1	239.9	6.5	-7.2
TB	REMP	-10.3	-14.3	16.5	55.6	2.8	11.7	6.6	69.5	28.9	-34.3	48.0	-55.9	513.8	-91.4	-89.1
	REBP	-15.0	-25.0	17.9	27.3	-12.0	-4.4	-5.5	69.5	15.3	-45.8	39.9	-57.7	477.9	-91.4	-90.5
TC	REMP	0.3	-14.3	6.3	44.4	7.0	1.3	10.7	78.1	6.5	12.6	17.7	32.3	10.3	29.2	51.6
	REBP	-5.0	-25.0	7.7	18.2	-8.4	-13.3	-1.8	78.1	-4.7	-7.1	11.2	27.0	3.9	28.5	31.7
TD	REMP	-5.0	-14.3	-19.0	22.2	-8.5	-1.3	13.5	108.6	3.7	43.0	39.4	25.9	166.9	-33.5	-16.1
	REBP	-10.0	-25.0	-17.9	0.0	-21.7	-15.6	0.6	108.6	-7.3	17.9	31.7	20.9	151.3	-33.9	-27.1

Cry = Crystals, End = Endospores, T = Transconjugant, REMP = Recombinant efficiency related to mid – parents, REBP = Recombinant efficiency related to better parent.

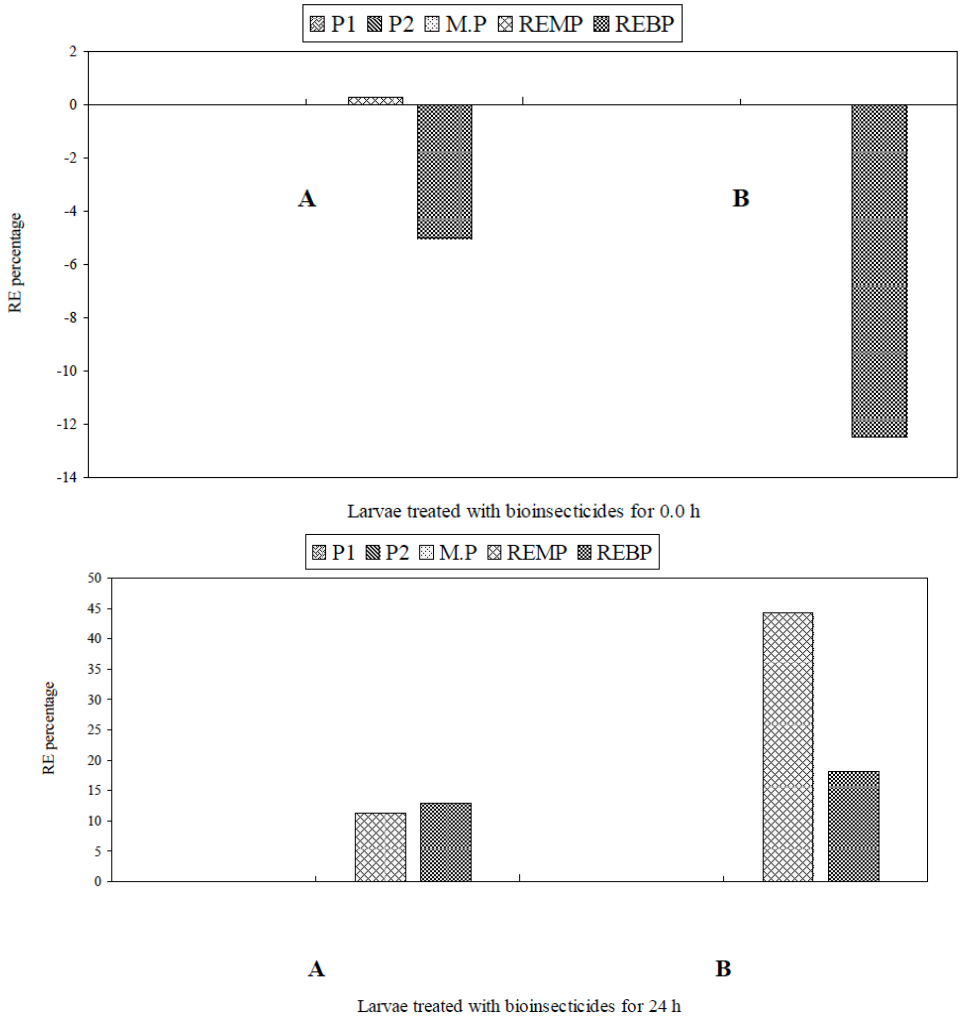
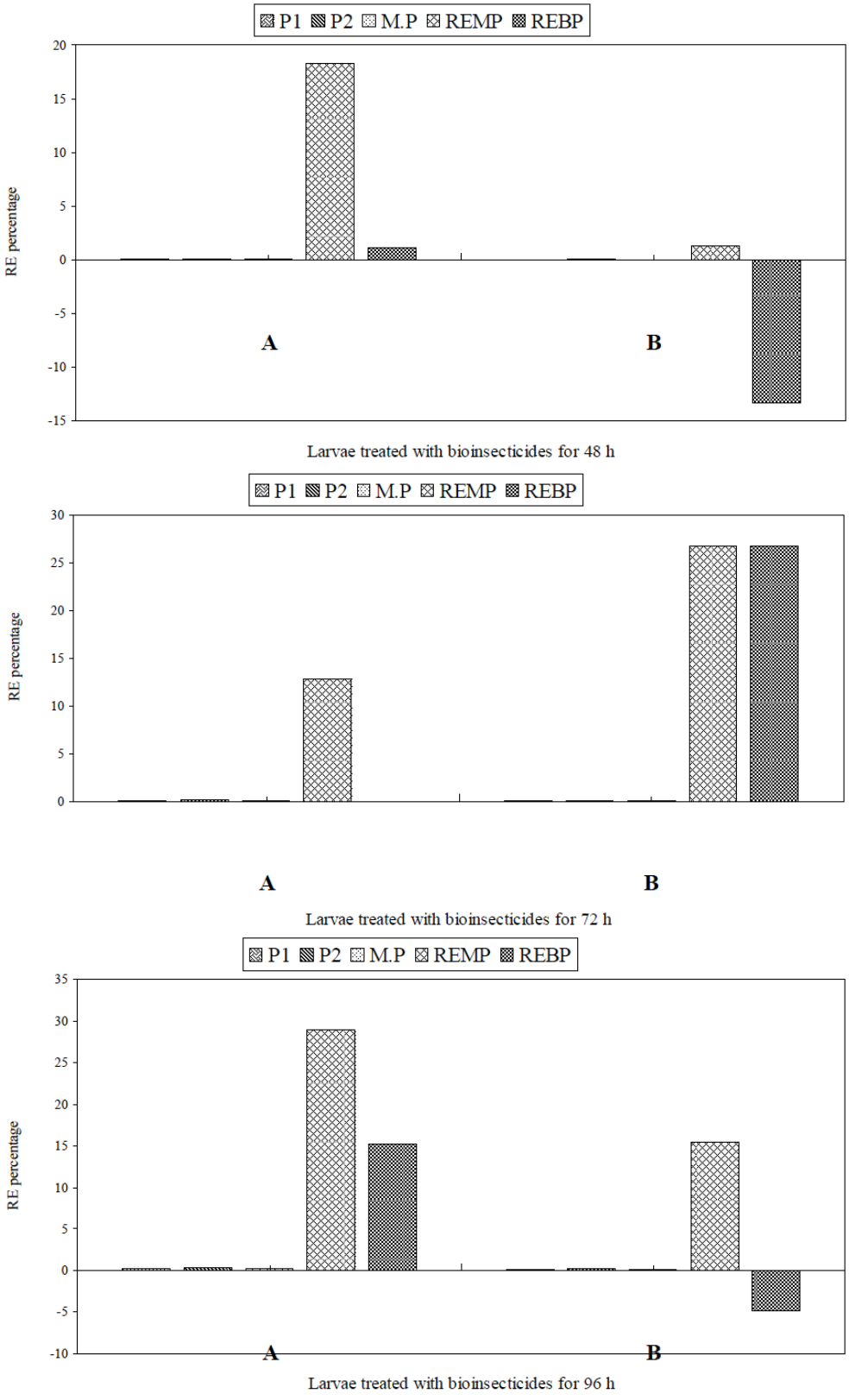


Figure 4: Transconjugant-A.

Figure 4: Continued for transconjugant-A.



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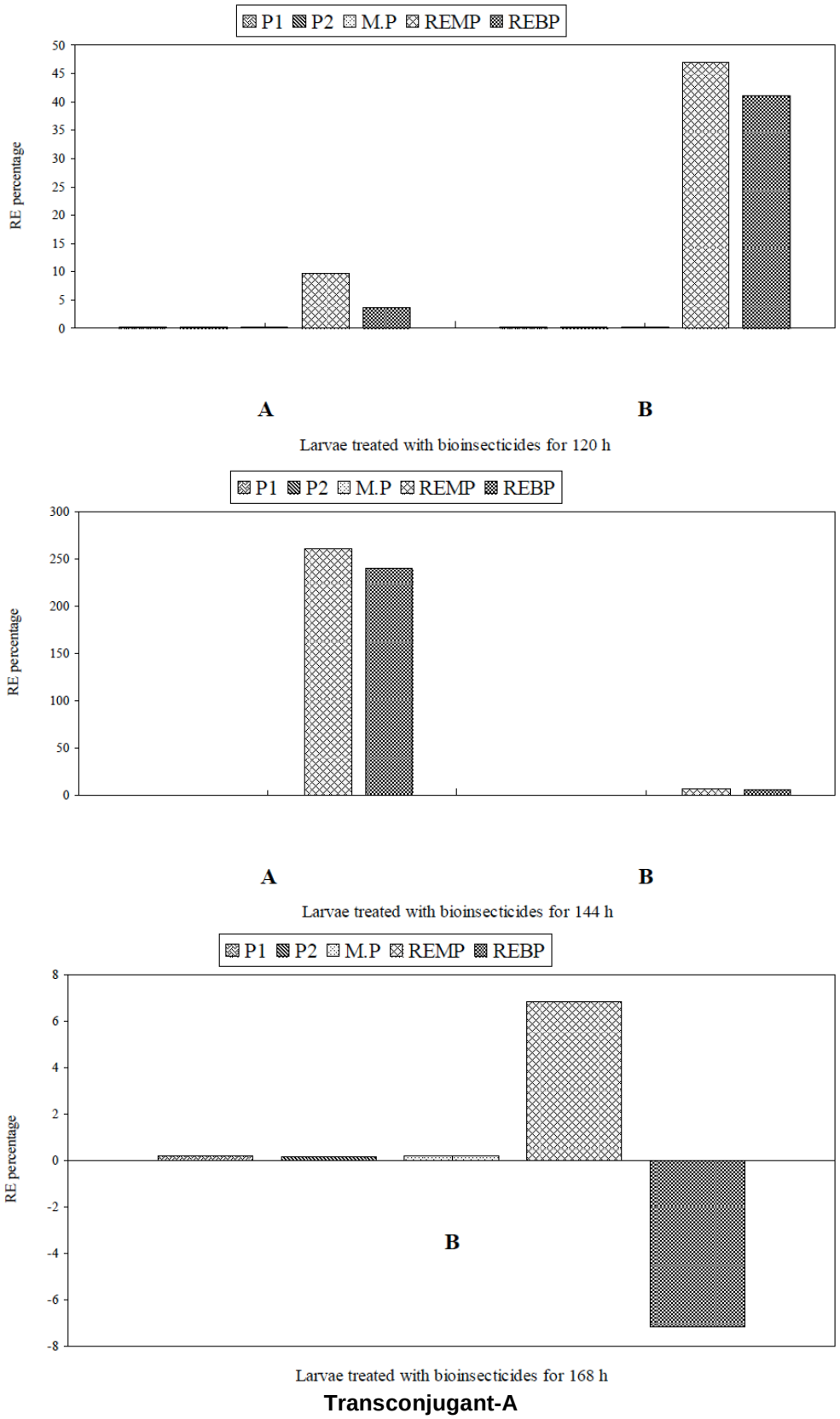
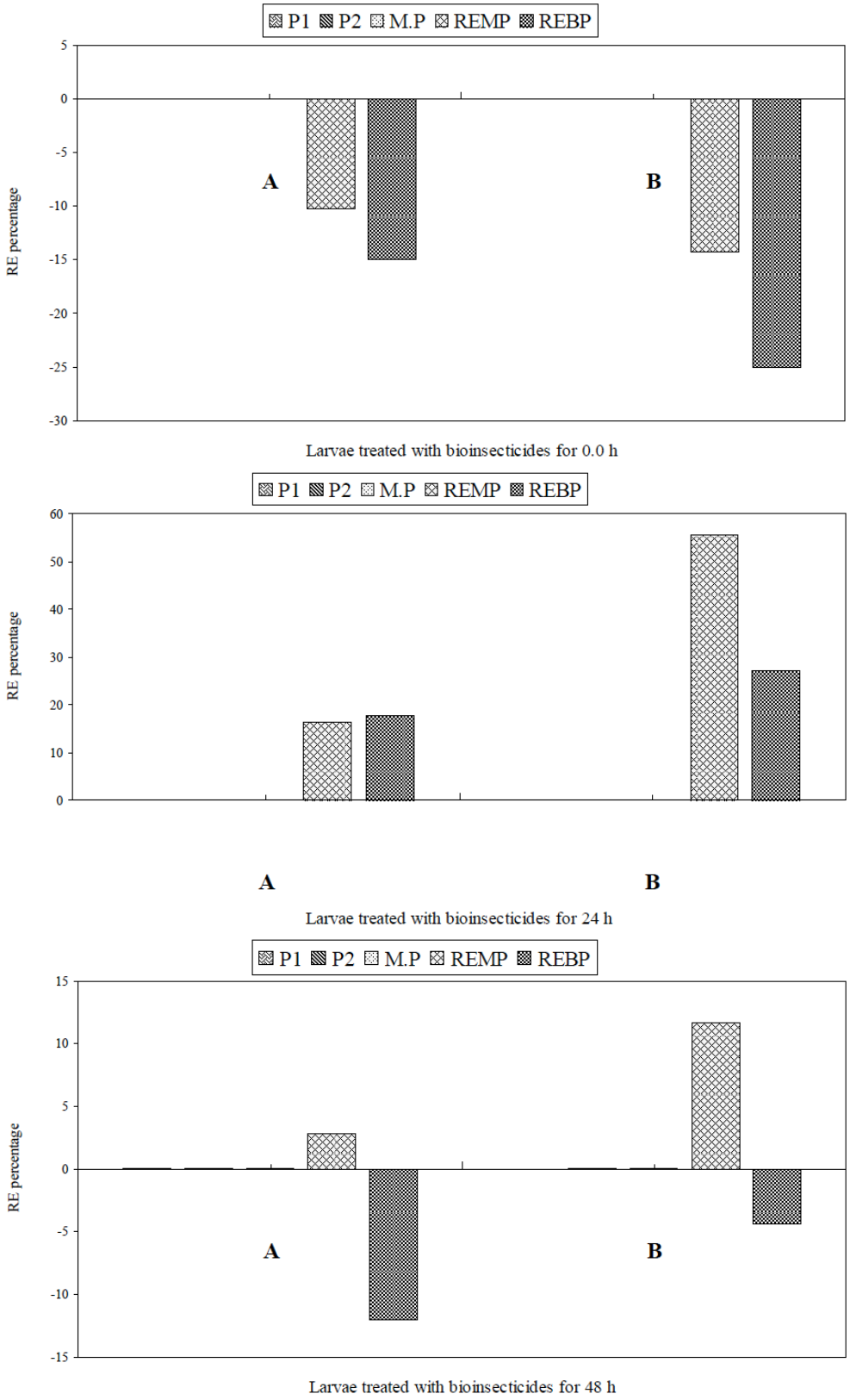


Transconjugant-A

Figure 4: Continued for transconjugant-B.



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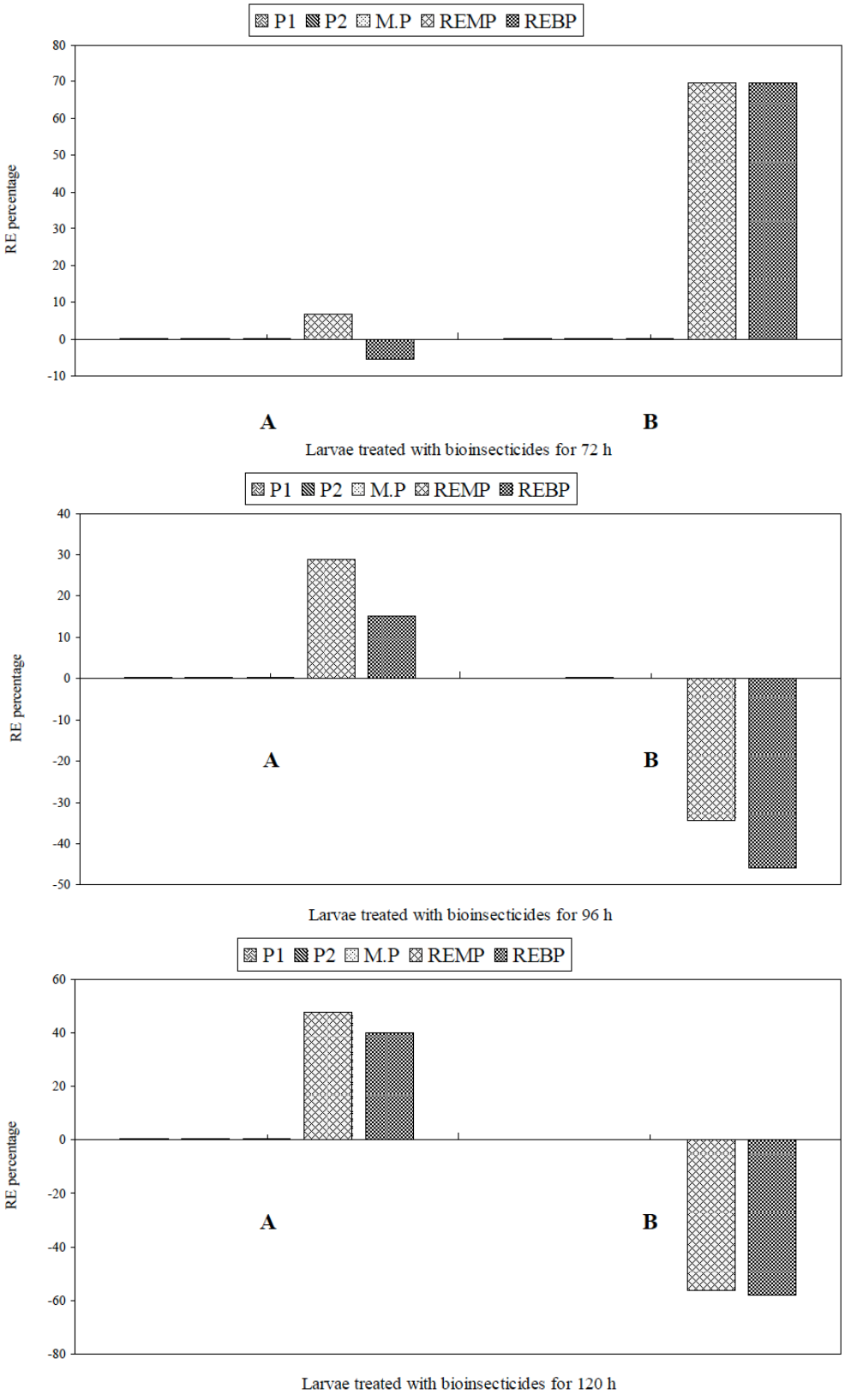

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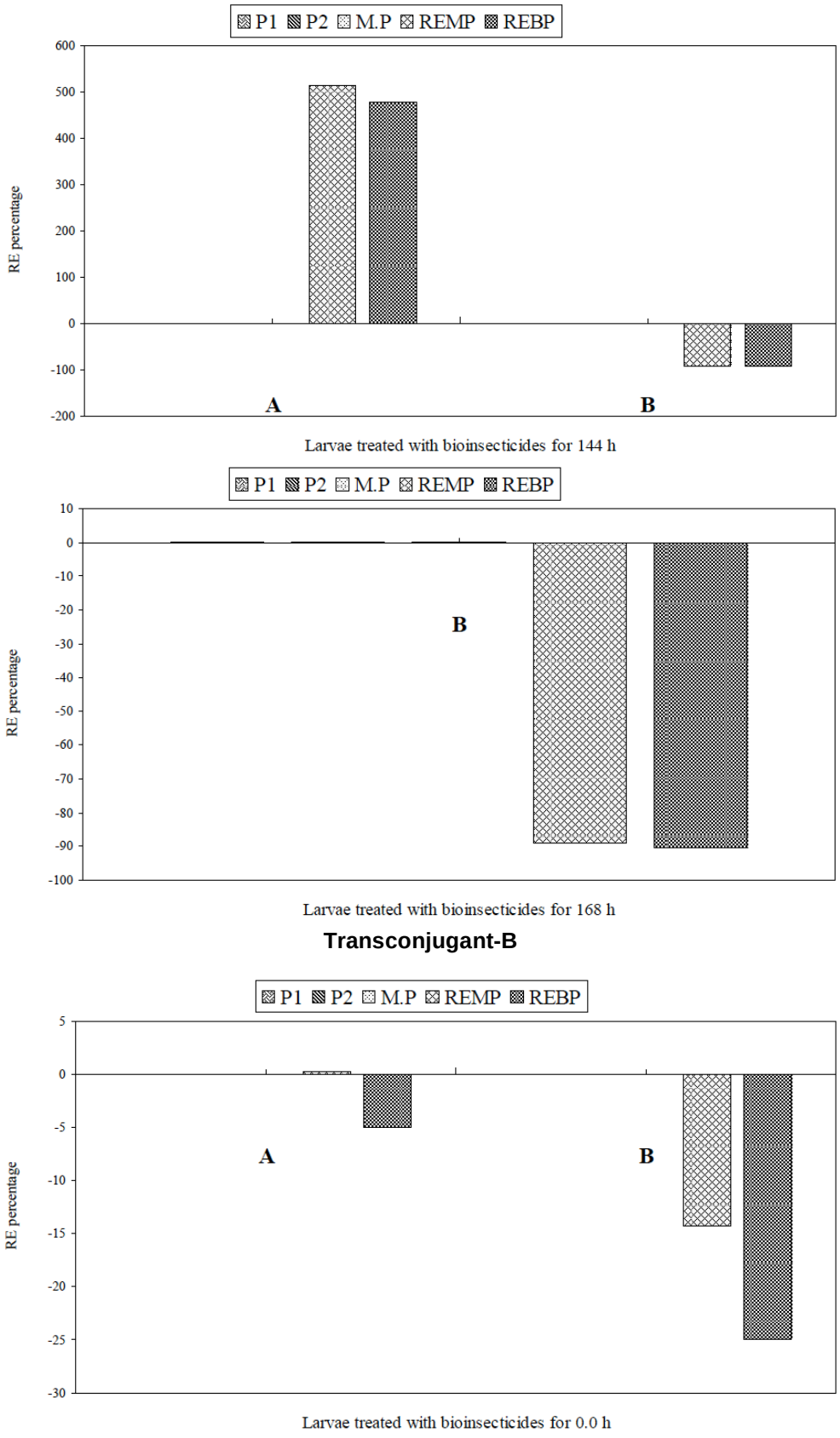
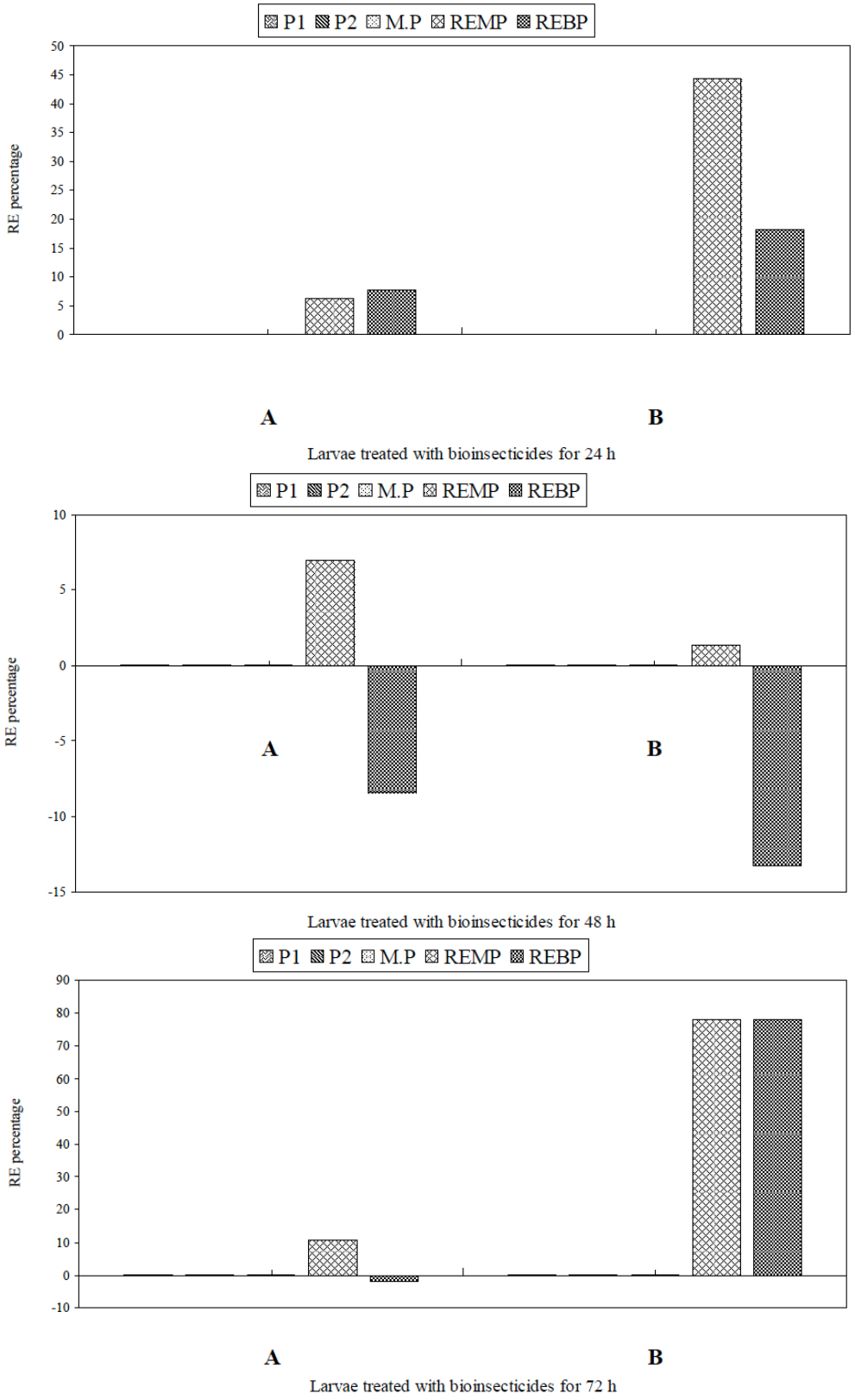


Figure 4: Transconjugant-C.

Figure 4: Continued for transconjugant-C.



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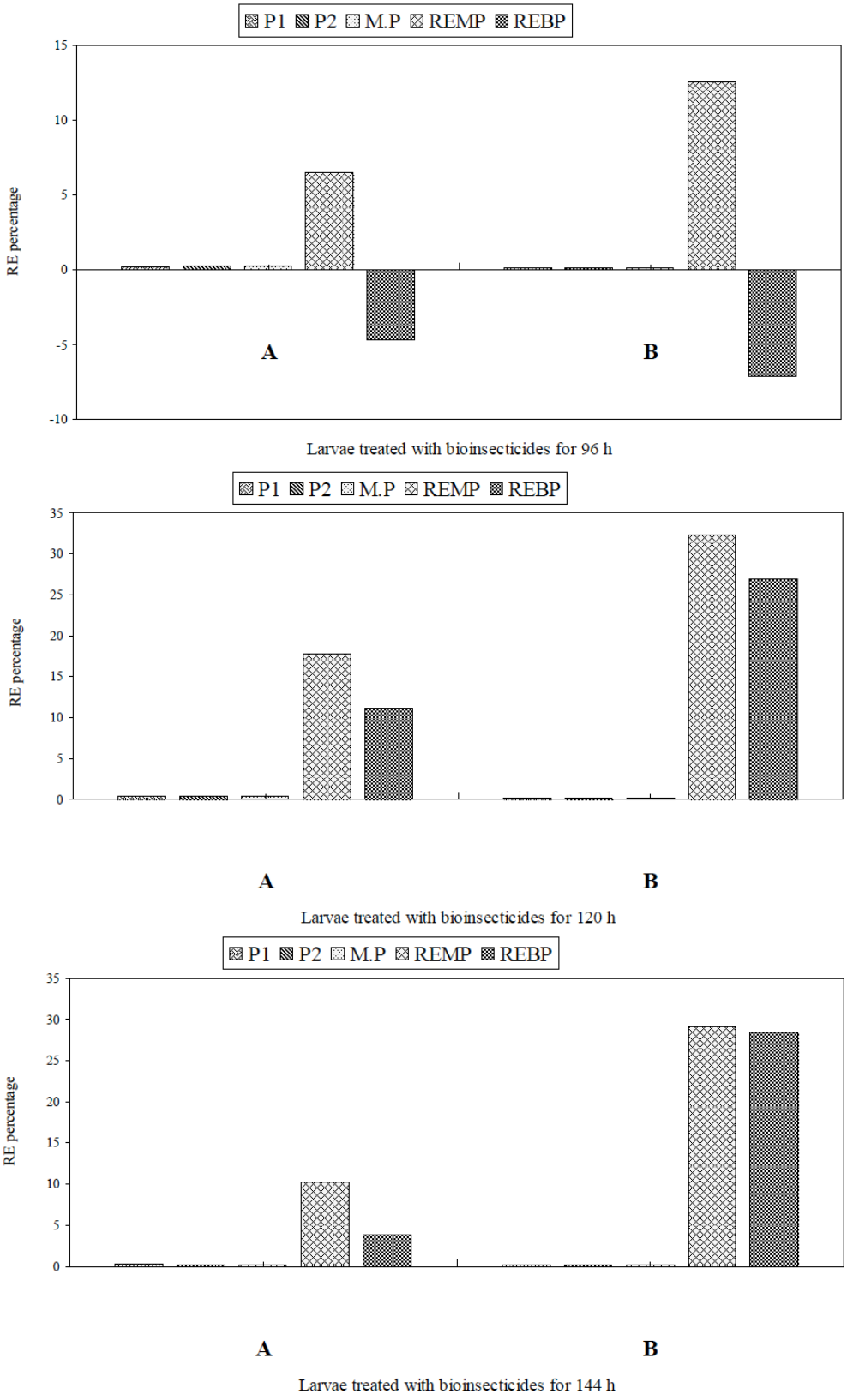

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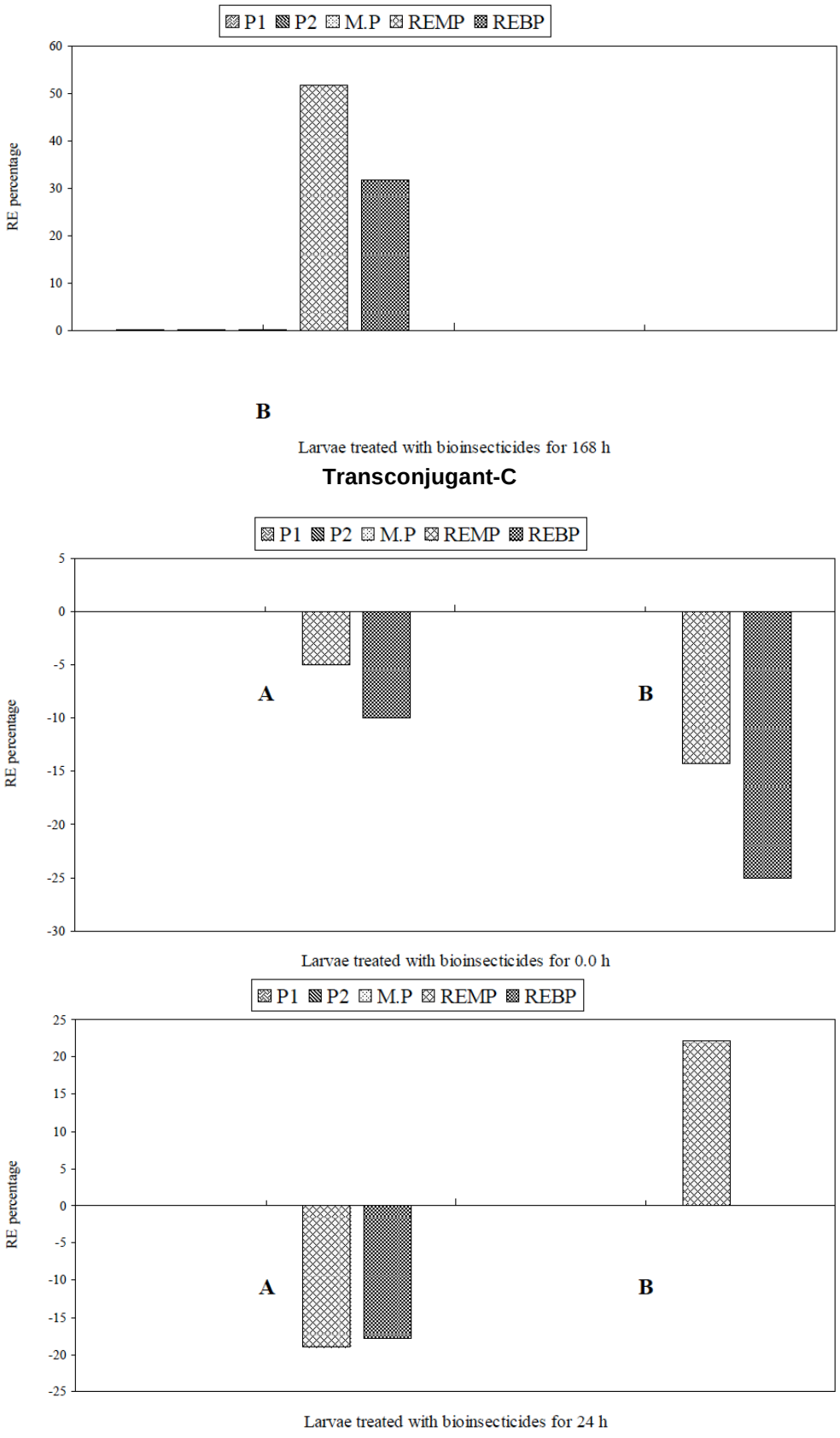
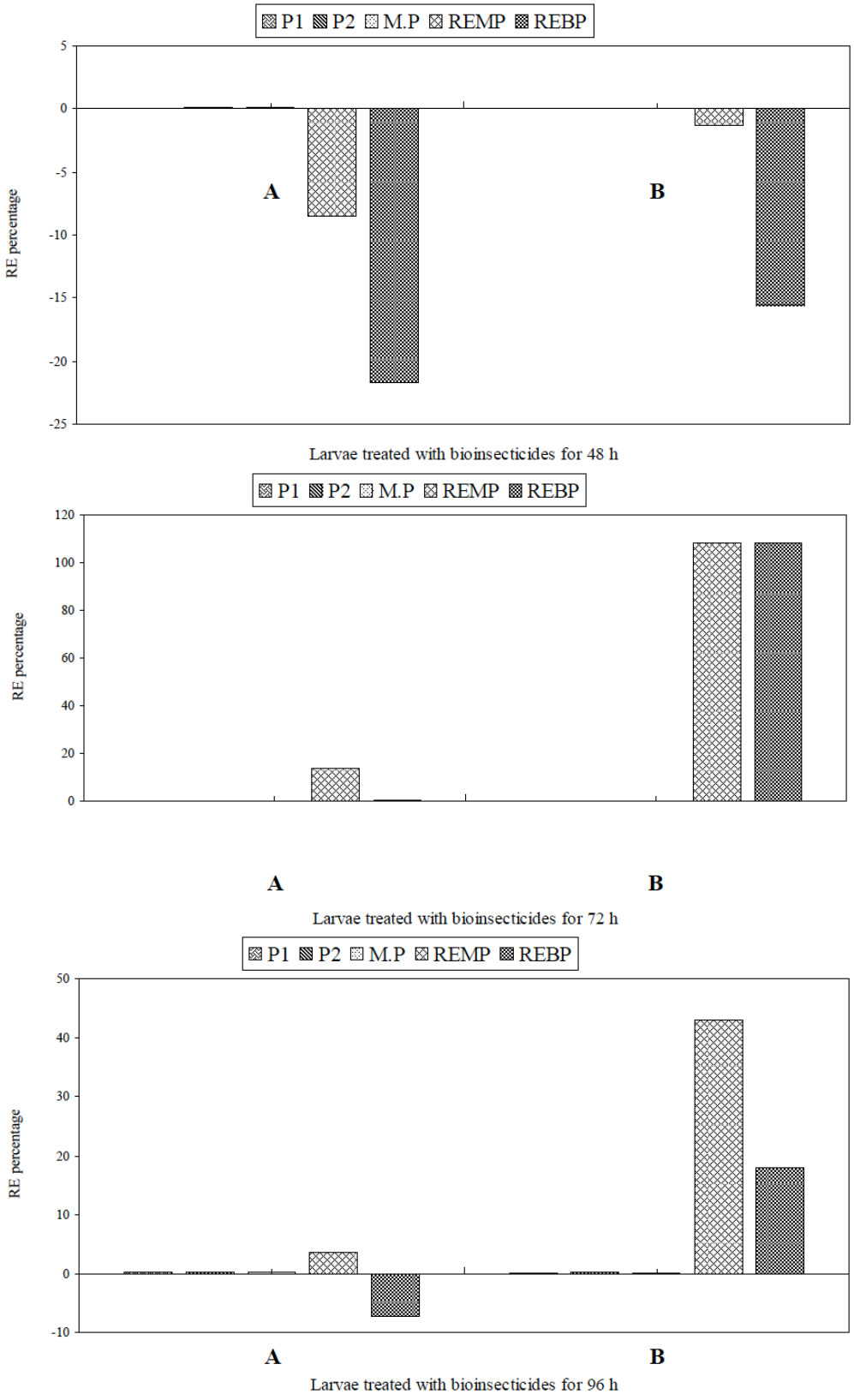


Figure 4: Transconjugant-D.

Figure 4: Continued for transconjugant-D.



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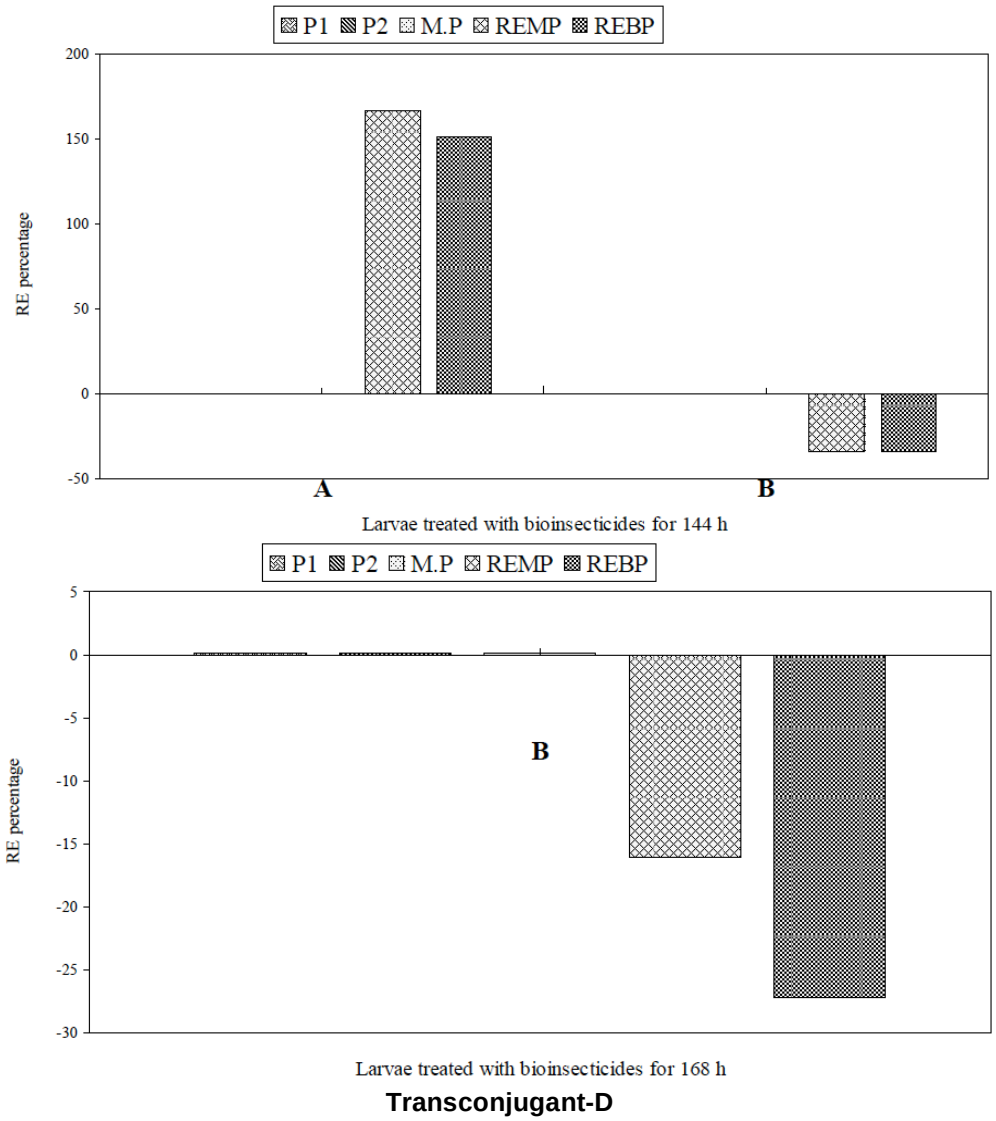


Figure 4: Recombinant efficiency of bioinsecticides affecting on body weight of surviving larvae of *Spodoptera littoralis*.
Note: A = Crystals, B = Crystals + Endospores, T = Transconjugant, RE = Recombinant efficiency.

Recombinant Efficiency in Reducing Survival of *Spodoptera litoralis* Larvae Due to Recombinant Bioinsecticides

Data summarized in Table **4** and diagrammatic in Figure **5** showed that transconjugants A, B and C appeared recombinant efficiency in reduced survival percentage of larvae at 96h, 120h, 144h and 168h in response to recombinants of both cry, cry + endospores. The same trend was also shown by transconjugant C at 48h and 72h in response to both recombinant bioinsecticides. It is of interest to note that recombinant endotoxin preparations containing crystals + endospores was more effective against *Spodoptera litoralis* than that containing crystals only.

The implications of this work encompass both applications and fundamental knowledge. The role of enteric recombinant bacteria in insect mortality by *B. thuringiensis* presents opportunities for designing approaches to manage insect pests by harnessing their indigenous communities or combining *B. thuringiensis* with bacteria that can induce septicemia. This work also raises the possibility that the genes encoding the *B. thuringiensis* toxins could be deployed more effectively in transgenic bacteria by exploiting the role of insect-borne bacteria that enhance insecticidal activity. Additionally, research on both the efficacy and non- target impacts of *B. thuringiensis* has yielded a perplexingly wide range of results with regard to the environmental persistence of activity [26]. Recent

Table 4: Recombinant Efficiency Percentage in Reducing (-) Survival Percentage of *Spodoptera littoralis* Neonate Larvae Fed on Leaves of *Ricinus communis* Sprayed with *Bacillus thuringiensis* Bioinsecticides.

Bioinsecticides		Treatment time (h)												
		24		48		72		96		120		144		168
		Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry + End
B. thuringiensis		100	91.67	95.83	75	95.83	75	95.8	62.5	87.5	58.33	79.17	33.33	29.17
B. subtilis		95.8	83.33	91.67	75	87.5	66.67	79.2	66.67	62.5	41.67	58.33	41.67	37.5
Mid Parents		98	88	94	75	92	71	88	65	75	50	69	38	33
TA	REMP	2.1	4.8	-11.1	5.6	-13.6	11.8	-23.8	-9.7	-16.7	-41.7	-21.2	-44.5	-62.5
	REBP	0.0	0.0	-13.0	5.6	-17.4	5.6	-30.4	-12.5	-28.6	-50.0	-31.6	-50.0	-66.7
TB	REMP	-2.1	-14.3	2.2	-22.2	4.5	-17.7	-14.3	-22.6	-55.6	-33.3	-69.7	-55.5	-62.5
	REBP	-4.2	-18.2	0.0	-22.2	0.0	-22.2	-21.7	-25.0	-61.9	-42.9	-73.7	-60.0	-66.7
TC	REMP	-6.4	-14.3	-2.2	-16.7	0.0	-17.7	4.8	-22.6	11.1	-25.0	15.2	0.0	-25.0
	REBP	-8.3	-18.2	-4.3	-16.7	-4.3	-22.2	-4.3	-25.0	-4.8	-35.7	0.0	-10.0	-33.3
TD	REMP	2.1	-23.8	6.7	-22.2	0.0	-29.4	0.0	-29.0	-33.3	-41.7	-45.5	-33.3	-50.0
	REBP	0.0	-27.3	4.4	-22.2	-4.3	-33.3	-8.7	-31.3	-42.9	-50.0	-52.6	-40.0	-55.5

Cry = Crystals, End = Endospores. T = Transconjugant, REMP = Recombinant efficiency related to mid – parents, REBP = Recombinant efficiency related to better parent.

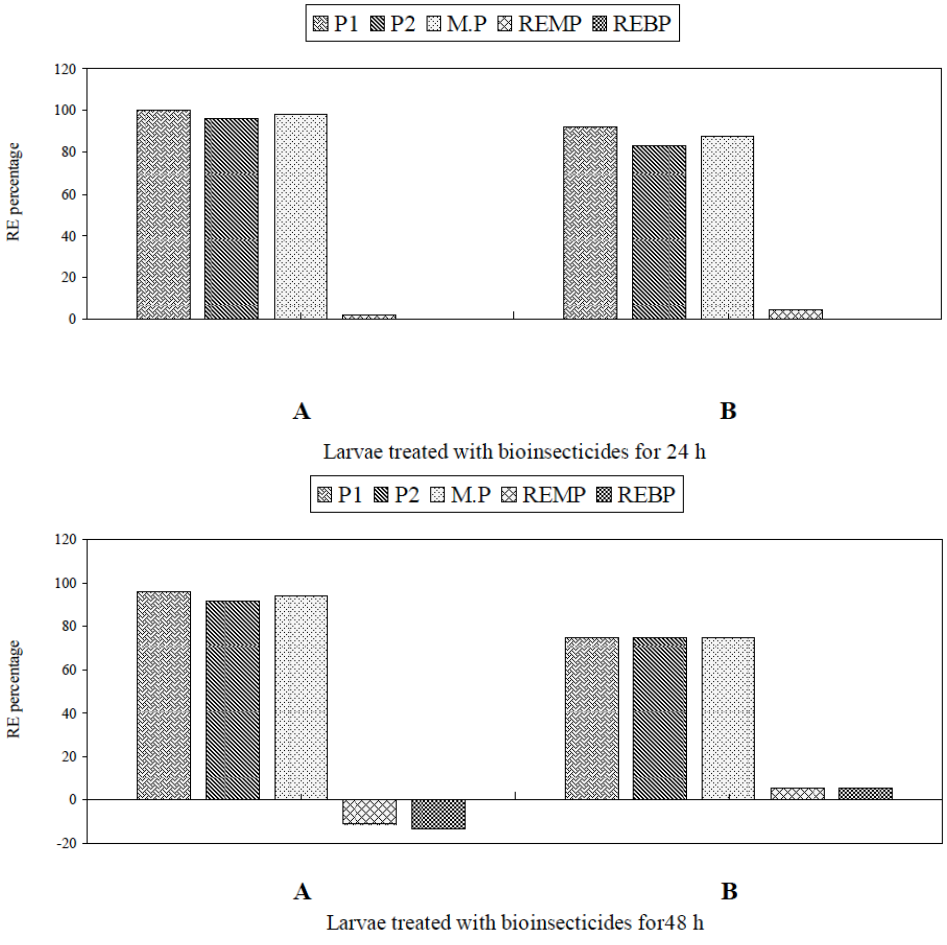
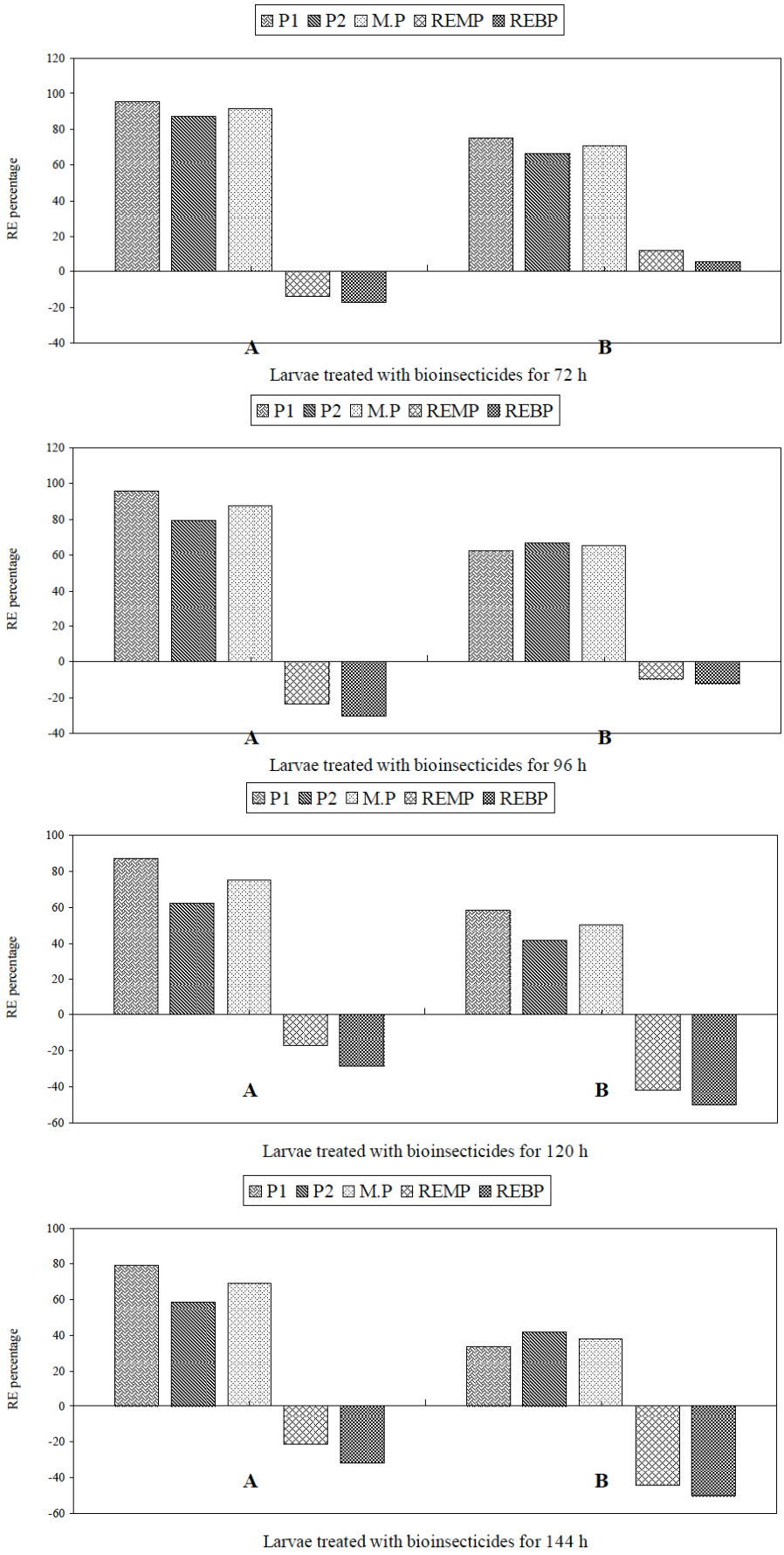


Figure 5: Transconjugant-A.

Figure 5: Continued for transconjugant-A.



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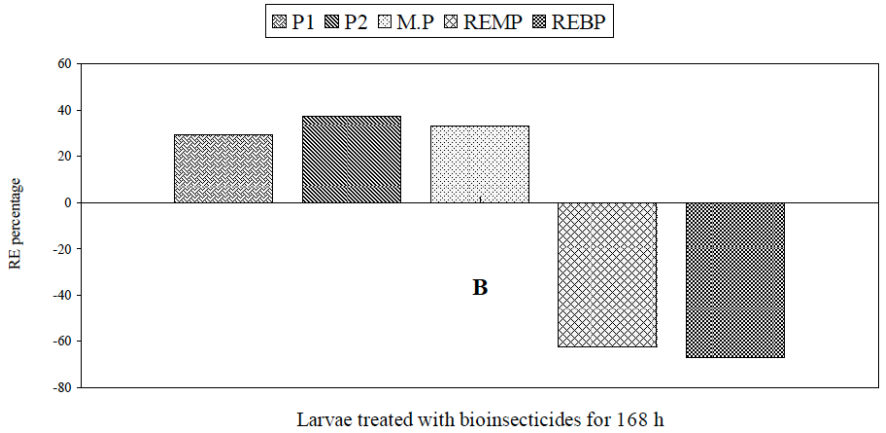
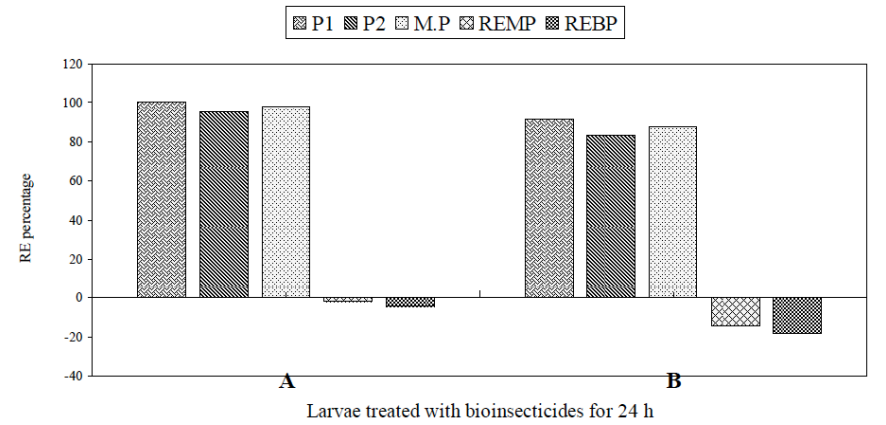
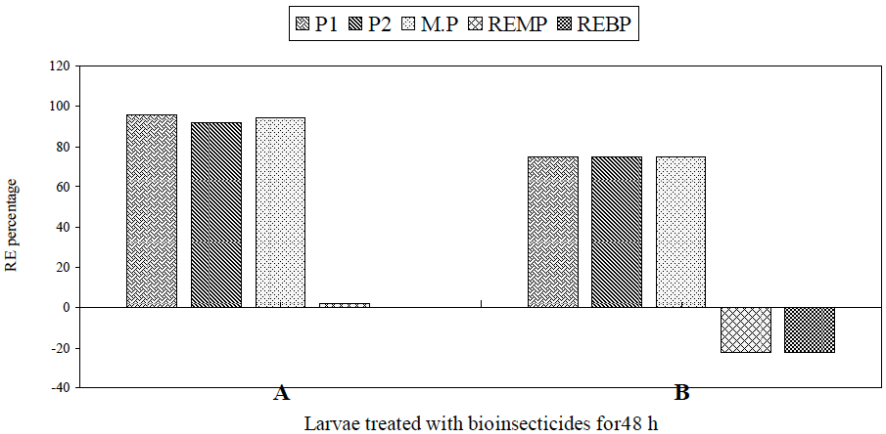


Transconjugant-A





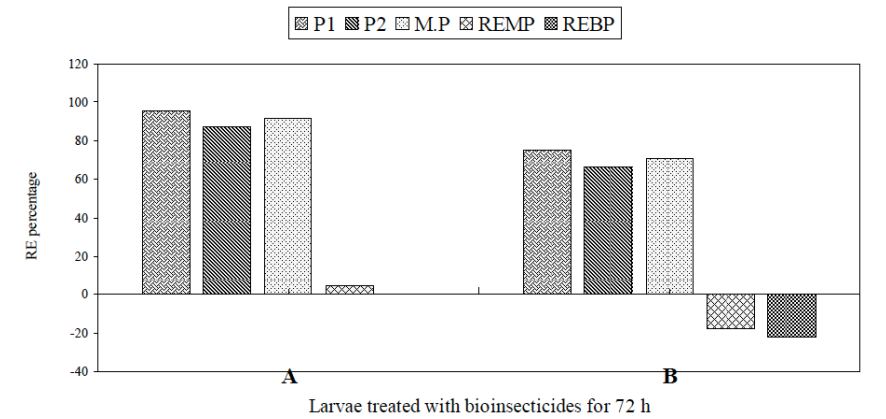
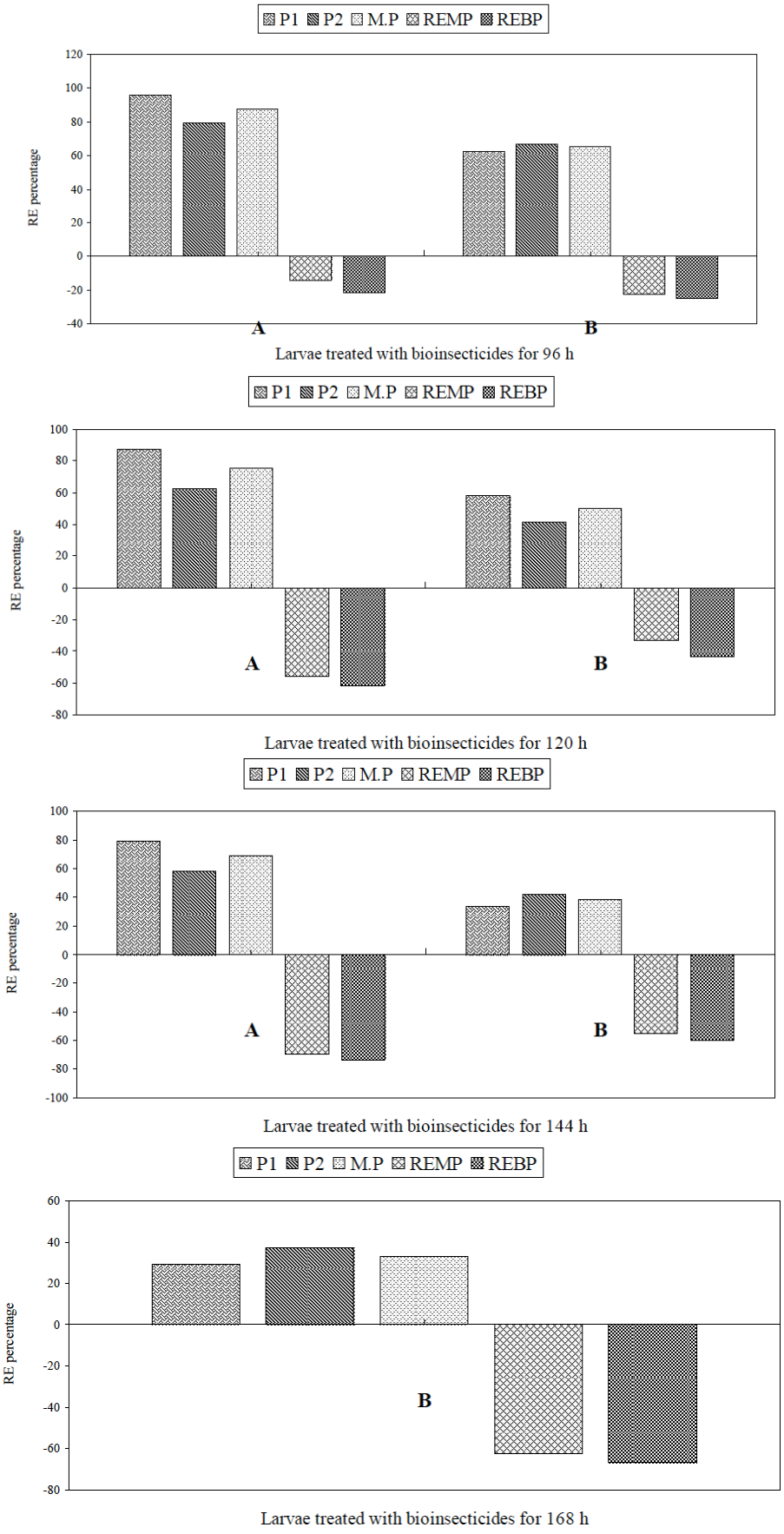


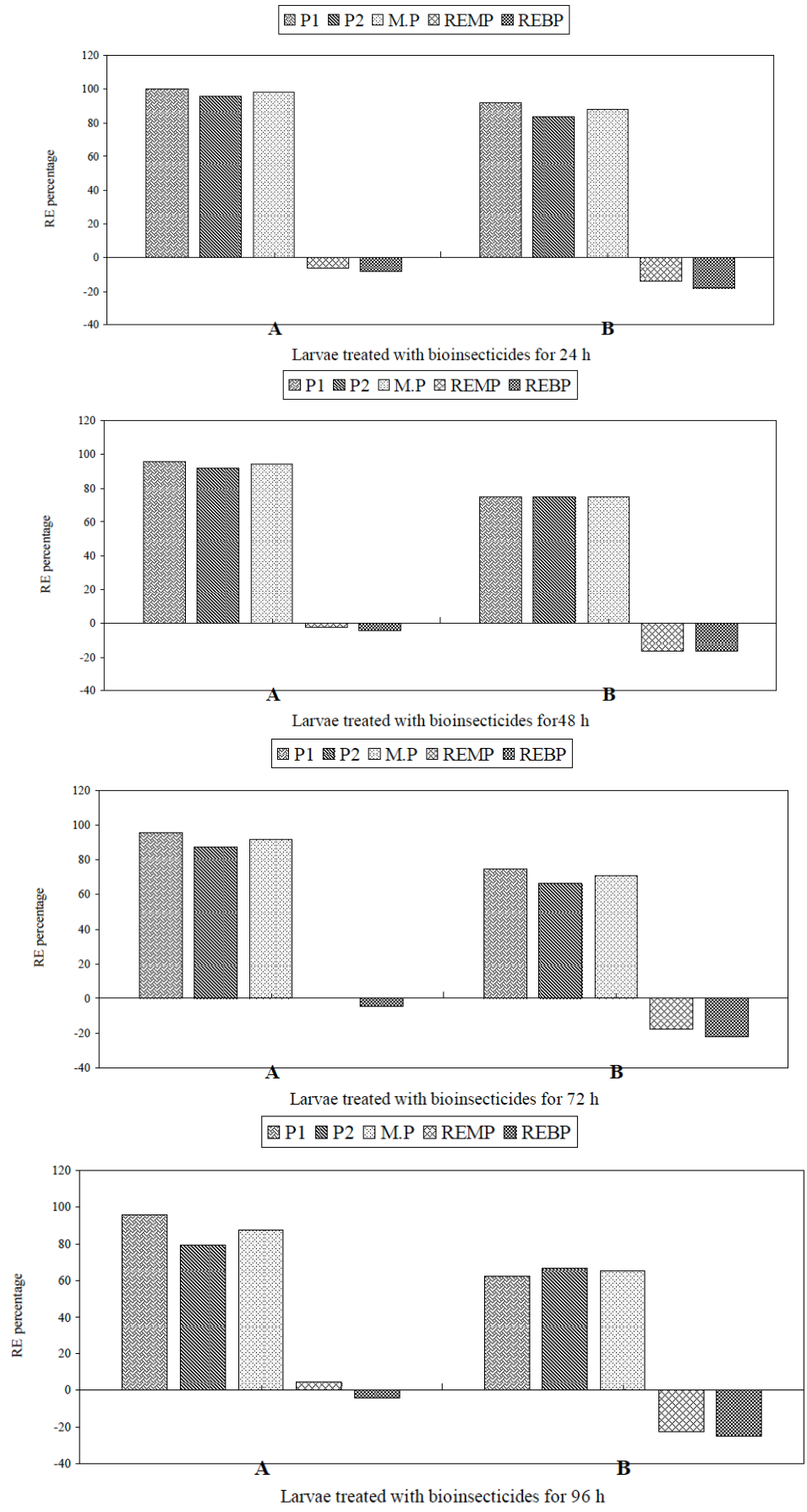
Figure 5: Transconjugant-B.

Figure 5: Continued for transconjugant-B.



Transconjugant-B

Figure 5: Continued for transconjugant-C.



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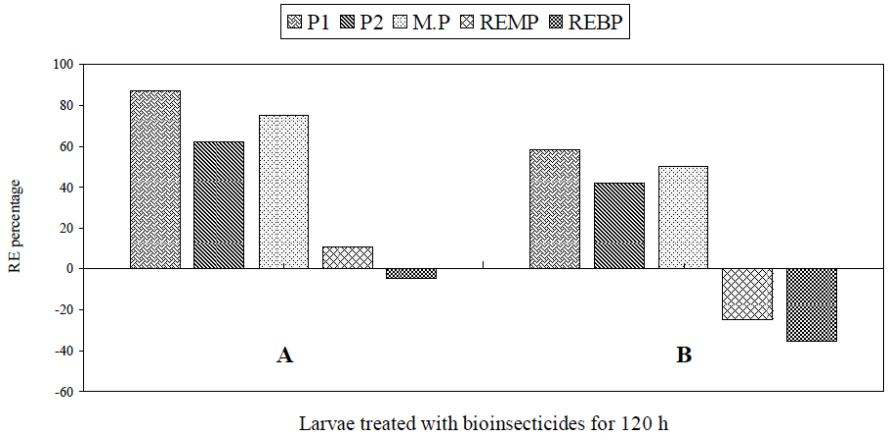
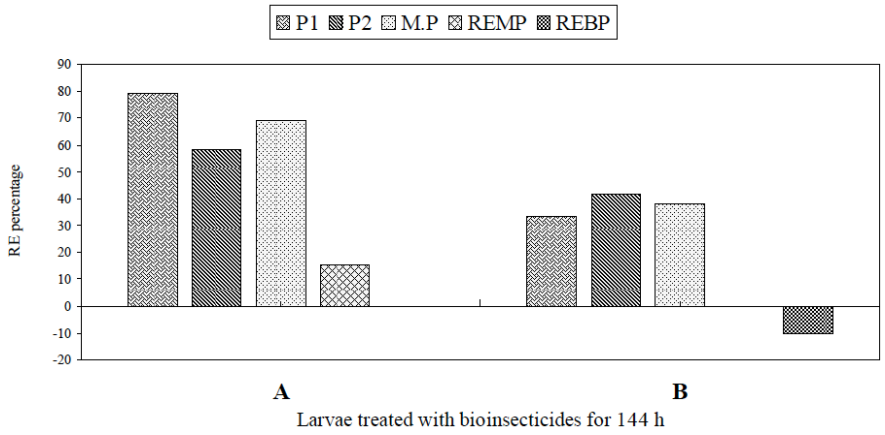
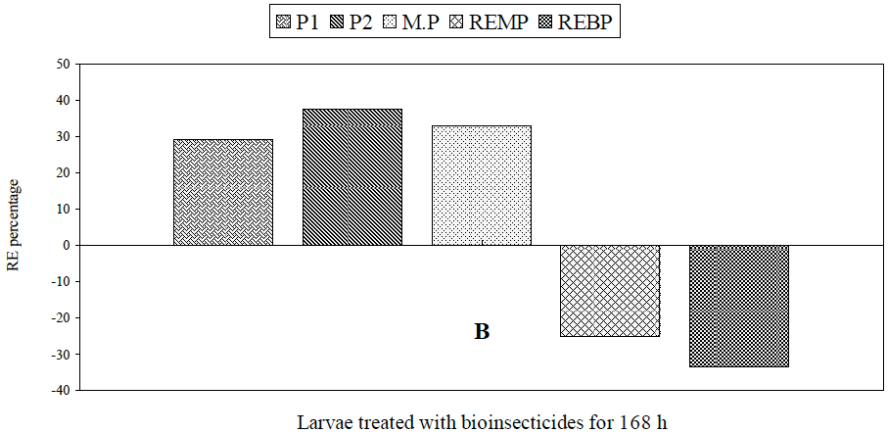






Transconjugant-C

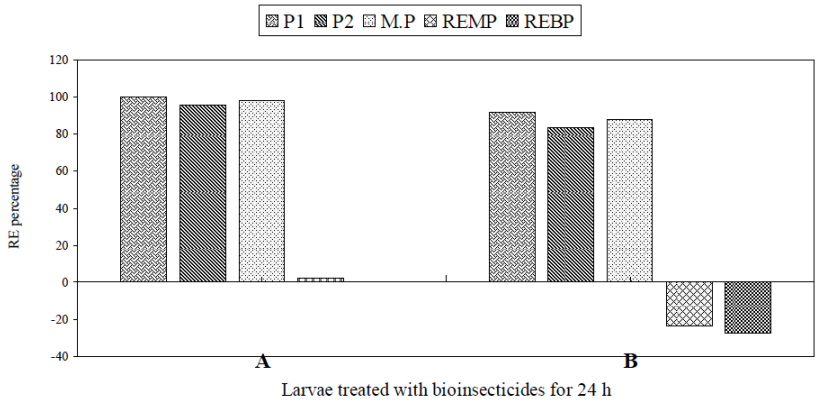
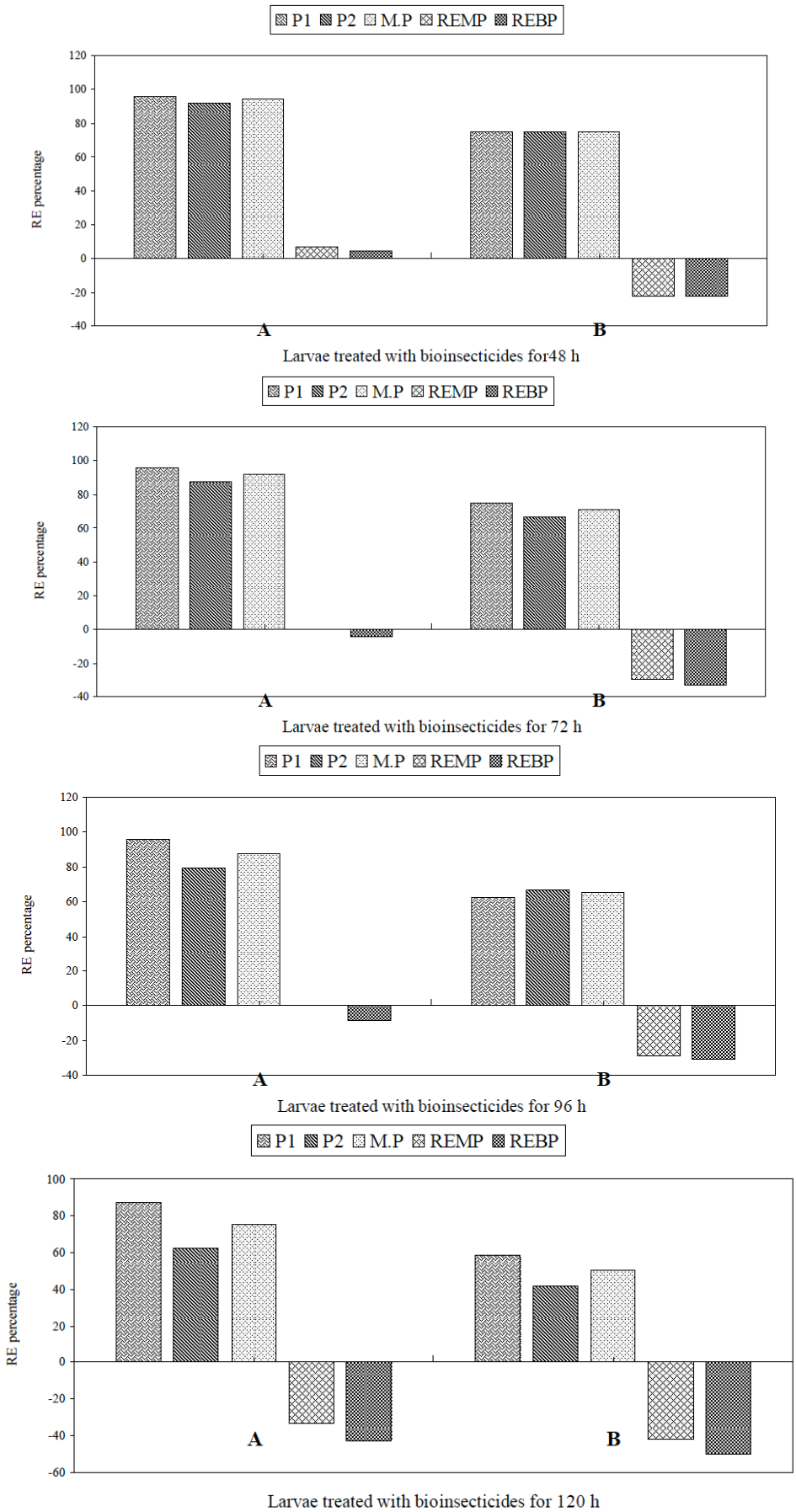


Figure 5: Transconjugant-D.

Figure 5: Continued for transconjugant-D.



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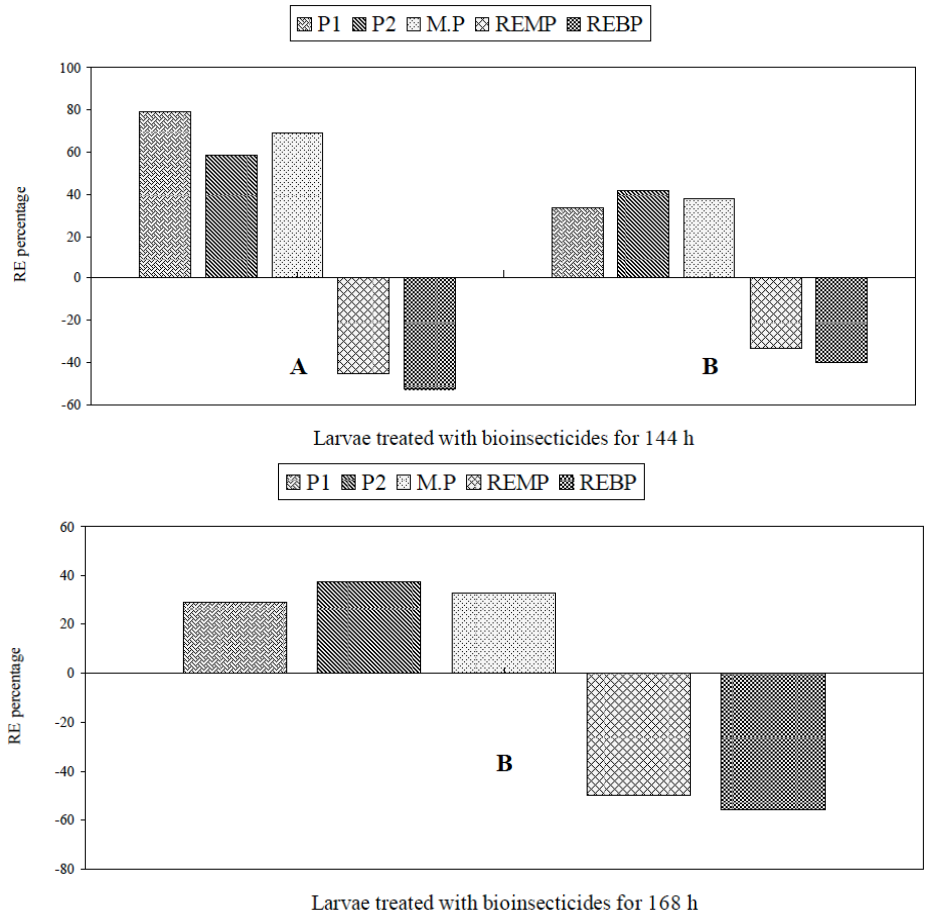

Transconjugant-D

Figure 5: Efficiency of recombinant bioinsecticides affecting on survival of *Spodoptera littoralis* - larval stage.
Note: A = Crystals, B = Crystals + Endospores, T = Transconjugant, TE = Transconjugant efficiency.

studies show that the composition and populations of gut communities can vary widely within insect species [26, 27]; hence, a more thorough understanding of gut communities could help clarify these issues. Recent interest in the role of intestinal bacteria in sepsis invites a parallel between human disease and insect killing by *B. thuringiensis.* This ubiquitous spore-forming bacterium kills insect larvae largely through the action of insecticidal crystal proteins and is commonly deployed as a direct bacterial spray. The results demonstrated that *B. thuringiensis*-induced mortality depends on enteric bacteria. This finding challenges the idea that *B. thuringiensis* induces death by septicemia but is consistent with the model that proposes that septicemia is initiated by the enteric bacteria in the insect gut [28, 29].

In conclusion, new recombinant products of *Bacillus thuringiensis* appeared recombinant efficiency for more effective in controlling *S. littoralis* on crops and vegetables, especially that containing crystals and endospores. Further selection of new strains of *B. thuringiensis* based on potency bioassays and effectiveness studies in the field would be useful to achieve recombinant microbial control of the pests.

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