Study of destruxin B and tea saponin, their interaction and synergism activities with *Bacillus thuringiensis kurstaki* against *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)

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Abstract

This study reveals the individual and synergistic effects of destruxin B (DB) (mycotoxin from *Metarhizium anisoplae*), tea saponin (Ts) and *Bacillus thuringiensis* (Bt) against *Spodoptera exigua*. DB, Ts and Bt reduced the growth of neonate larvae by up to 91.30, 89.17 and 77.17%. EC_{50} values of DB against 4th and 5th instars were 0.17 and 0.22 mg ml⁻¹, 0.35 and 0.41 mg ml⁻¹ against 4th and 5th instars for Ts and 0.0031 and 0.0035 mg ml⁻¹ after being treated with Bt. The synergism of DB and Ts with Bt resulted in the increased efficiency of these chemicals as mortality percentages significantly increased up to 94.2% with DB+Ts+Bt followed by DB+Bt with 91.99% and were significantly higher than individual treatments of Bt 65.81% and Ts 76.66%. Nutritional analysis revealed the increased toxicity of DB and Ts in combination with Bt, but DB+Bt showed higher efficiency with minimal relative consumption rate (RCR) 2.69 mg/mg/day, relative growth rate (RGR) 1.33 mg/mg/day and efficiency of conversion of ingested food (ECI) 35.77%, respectively. Changes in antioxidant enzymes, superoxidase dismutase (SOD) and catalases (CAT), were noticed to some extent over different exposure times in all treatments. The highest SOD activity was observed in individual DB treatment, while individual Bt treatment caused the highest variation in CAT values. In all treatments, SOD and CAT values fell sharply on the final day except for individual Bt treatment, with which the values were relatively stable on the final day.

Key words: *Spodoptera exigua*; destruxin B; Tea saponin; *Bacillus thuringiensis*

INTRODUCTION

The use of conventional insecticides over a long period of time has caused an imbalance in the ecosystem by effecting natural enemies and the environment, and has become a high risk to human health. This exhaustive use of insecticides has also resulted in increased resistance in many important pest species. *Spodoptera exigua* is a species that has developed resistance against most pesticides groups (Wu et al., 1995; Moulton et al., 2000; Wang et al., 2001).

S. exigua caterpillars are destructive crop pests responsible for considerable annual economic losses (Jacques et al., 2008). Since 1986, *S. exigua* emerged as a serious pest throughout eastern and

central provinces, especially in Huanghuai and Jianghuai districts, in China. *S. exigua* damaged more than 30,000 km² only in Henan and Shandong provinces during 1999 (Luo et al., 2000). This insect is not only the major agricultural pest, particularly in the southern USA and Asia, but its management is confounded by its migratory dispersal and rapidly evolving resistance to conventional pesticides (Adamczyk et al., 2003; Shimada et al., 2005).

During the last 20 years, much attention has been devoted to natural pest control agents (Ujvary, 2002). In these natural agents, the role of destruxins (mycotoxin from entomophagous fungi *Metarhizium anisoplae*) has been very important. Destruxins are cyclodepsipeptidic mycotoxins iso-

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lated from the extracellular culture medium of *Metarhizium anisoplae* (Metch.) and their insecticidal activity has been proved by many authors against different insect species and has also been found to have synergistic activity with other natural agents, such as *Bacillus thuringiensis* (Bt), against some insects (Brousseau et al., 1998). Similarly, plants produce a variety of compounds that provide certain protection against insect attack (Felton and Gatehouse, 1996). Glycosylated sterols or triterpenoids, known as saponins, have been identified in 80 plant families and many have been found to interfere with insect growth and development (Adel et al., 2000). Bt has been successfully tested against more than 137 insect species of Lepidoptera, Hymenoptera, Diptera and Coleoptera (Gill et al., 1992), but in the recent years there have been many reports on the development of resistance to Bt (Tabashnik and Carriere, 2004). The combinations of biopesticides and Bt can be helpful to ease the increasing problem of resistance (Senthil Nathan et al., 2006).

The toxic effects of biochemicals on insects can be manifested in several manners, including toxicity, mortality, antifeedent growth inhibition and efficiency against defense system of pests. Antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), form an important part of the defense system (Joannis and Storey, 1996), protecting cells against reactive oxygen species. Oxidative stress after exposure to pesticides and other xenobiotics has been widely documented within the animal kingdom (Harris, 1992; Adamski and Ziemnicki, 2004; Suzuki et al., 2004; Yang et al., 2004) and could contribute to developing resistance in insects.

Biochemicals can be degraded more easily than conventional insecticides so these chemicals must be the main focus of pest management in this era; therefore, the present investigation was undertaken to study the insecticidal activities of destruxin B (DB, a mycotoxin from *Metarhizium anisoplae*) and tea saponin (Ts, glycoside compound extracted from *Camellia* seeds) individually, in combination, and with Bt against *S. exigua* (Hübner) to determine their interactive and synergistic impact on bio-efficacy, nutritional efficiency and antioxidative defense system (SOD, CAT in the fat bodies of insects).

MATERIALS AND METHODS

Insects. Larvae of *S. exigua* Hübner (Lepidoptera: Noctuidae) were reared on a semi-synthetic diet (David et al., 1975) and kept in growth chambers at $26 \pm 2^\circ\text{C}$ with a 16L8D photoperiod.

Chemicals. Destruxin B (DB) was isolated from strain MaQ10 of *Metarhizium anisoplae* (Metch.) according to the references (Pais et al., 1981; Liu et al., 2004; Hu et al., 2006). Destruxin was identified by high-performance liquid chromatography (HPLC), mass spectrometry, and quantified with standards by HPLC. DB was mixed with acetone (5 mg in 1 ml) as a stock solution and diluted with distilled water to make different concentrations.

Tea saponin (Ts) is commercially available and was purchased from Chenxi County Hanqing Bio-Technology Co., Ltd. (Hunan, China). It was dissolved in 5% methanol as a stock solution and different concentrations were prepared with distilled water.

B. thuringiensis (Bt) was purchased from Yangzhou lu-yuan Biochemical Ltd. (Yangzhou, China) at a concentration of 16,000 IU/mg. Different concentrations of Bt were prepared with distilled water to study individual and synergistic effects with DB and Ts.

Evaluation of bio-efficacy.

EC₅₀, LC₅₀ of DB, Ts, Bt and effect on larval growth. In this experiment DB, Ts and Bt were used to determine their individual effects on the growth of early instars of *S. exigua*. For this purpose, 1-day-old neonate larvae were placed on 1 g fresh artificial diet. Insects were placed individually in separate cups (Koul et al., 1990). Artificial diet was treated with six concentrations of DB [DB1 (90 µg/ml), DB2 (75 µg/ml), DB3 (60 µg/ml), DB4 (45 µg/ml), DB5 (30 µg/ml), DB6 (15 µg/ml)], Ts [Ts1 (90 µg/ml), Ts2 (75 µg/ml), Ts3 (60 µg/ml), Ts4 (45 µg/ml), Ts5 (30 µg/ml), Ts6 (15 µg/ml)] and Bt [Bt1 (1.9 µg/ml), Bt2 (1.6 µg/ml), Bt3 (1.30 µg/ml), Bt4 (1.0 µg/ml), Bt5 (0.70 µg/ml), Bt6 (0.40 µg/ml)] and carrier solvents were allowed to evaporate while control diet was prepared in the same way but chemical application was omitted. Larval growth was assessed as a percentage of the control after seven days of feeding and data were analyzed by analysis of variance (ANOVA) using SAS 8.01, 2000 and the means were compared by Tukey's multiple range test.

To determine EC_{50} and LC_{50} values, 4th and 5th instar larvae were separately subjected to six different concentrations of DB, Ts (0.6, 0.5, 0.4, 0.3, 0.2, 0.1 $mg\ ml^{-1}$) and Bt (2, 4, 6, 8, 10, 12 $\mu g\ ml^{-1}$ or 0.002, 0.004, 0.006, 0.008, 0.010, 0.012 $mg\ ml^{-1}$). Mean effective concentrations (EC_{50}) were determined by regression analysis while lethal concentrations causing 50% mortality (LC_{50}) were determined using probit-log analysis.

EC_{50} values of these bio chemicals were further used in nutritional and enzyme analysis either individually or in combination with Bt and each other.

Mortality bioassay. Mortality bioassay was carried out using three concentrations of each DB [DB1 (0.6 $mg\ ml^{-1}$), DB2 (0.5 $mg\ ml^{-1}$), DB3 (0.4 $mg\ ml^{-1}$)], Ts [Ts1 (0.6 $mg\ ml^{-1}$), Ts2 (0.5 $mg\ ml^{-1}$), Ts3 (0.4 $mg\ ml^{-1}$)] and Bt [Bt1 (12 $\mu g\ ml^{-1}$), Bt2 (10 $\mu g\ ml^{-1}$), Bt3 (8 $\mu g\ ml^{-1}$)] individually. In order to study the combined effects, combinations were made as DB1+Ts1, DB2+Ts2, DB3+Ts3, DB1+Bt1, DB2+Bt2, DB3+Bt3, Ts1+Bt1, Ts2+Bt2, Ts3+Bt3, DB1+Ts1+Bt1, DB2+Ts2+Bt2, DB3+Ts3+Bt3. Newly molted 4th instar larvae were starved for 4 h before being exposed to treated diet. Larvae were left to feed on the treated diet until their death or pupation (normal or abnormal). Larval mortality was assessed on a daily basis and mortality percentage was calculated for each concentration at different time intervals to determine the time taken by larvae up to mortality against different chemicals and their combinations. Thirty larvae were used for each concentration with five replications. The experiment was repeated three times. Data were subjected to ANOVA using SAS 8.01, 2000 and the means were compared by Tukey's multiple range test.

Nutrition analysis. In order to clarify behavioral changes from the changes caused by the toxicity of chemicals, nutritional analysis was performed. The experiment was carried out using newly molted fourth instar larvae. Thirty larvae per concentration with five replications were provided with DB, Ts and Bt at concentrations 0.17, 0.35 and 0.0031 $mg\ ml^{-1}$ individually (based on EC_{50} values determined against this larval stage) and in combination (DB+Ts, DB+Bt, Ts+Bt and DB+Ts+Bt). Relative growth per unit weight (RGR) of the insect, relative consumption rate (RCR) and efficiency of conversion of ingested food (ECI) were calculated based on dry weight after 3 days of feeding (Koul

et al., 1997). Data was subjected to ANOVA and the means were compared by Tukey's multiple range test (SAS, 2000).

Enzyme analysis. Newly molted fourth instar larvae were fed on artificial diet treated with DB, Ts, Bt, DB+Ts, DB+Bt, Ts+Bt, DB+Ts+Bt (0.17, 0.35 and 0.0031 $mg\ ml^{-1}$ based on EC_{50} values either individually or in combination against fourth instar larvae) and used for enzyme analysis. Larvae were alternatively provided with treated and untreated diet every 24 h to avoid high mortality rates and to observe the long-term effects on the antioxidant enzyme system in the fat bodies of larvae. Samples from fat bodies were collected randomly at 24-h intervals to observe depletion relevant to the control and were calculated as a percentage of the control. For enzyme analysis, 10 insects were randomly selected with an interval of 48 h up to 8 days. Insects were dissected to obtain fat bodies, which were homogenized in 0.05 $mol\ L^{-1}$ phosphate buffer with pH 7.0 at 0 to 4°C. A Thomas glass homogenizer with a Teflon pestle was used to homogenize the samples. The homogenates obtained (10% w/v) were centrifuged at $6,000\times g$ for 10 min at 4°C and the supernatant was used to measure enzyme activities.

Total SOD activity was measured using the method of Beauchamp and Fridovich (1971). This method is based on superoxide anion generation by reduced flavins under the influence of light, at room temperature. SOD catalyzes the dismutation of radicals and inhibits the formation of formazans.

Total CAT activity was measured by homogenizing fat bodies in 66 mM phosphate buffer (pH 7.0). The reaction mixture was composed of 66 mM phosphate buffer (pH 7.0), sample extract and 3% hydrogen peroxide. CAT activity was measured as the amount of H_2O_2 reduced per minute per milligram of protein (Aebi, 1984).

Protein concentrations in the supernatants were determined by the procedure of Bradford (1976), using bovine serum albumin (Sigma-Aldrich).

The enzyme assays were repeated 5 times. Data are presented as a percent increase or decrease over control at a specific time. Results were analyzed by Student's *t* test using SAS 8.01, 2000 software and $p\leq 0.05$ was significant.

RESULTS

Efficacy of bio-chemicals

Effect of DB and Ts on growth, EC_{50} and LC_{50}

DB, Ts and Bt retarded the growth of early larval instars when incorporated into the artificial diet. A significant decrease in growth was observed with the increase in concentration relative to the control. The highest growth reduction was observed at DB1 (91.30%), which was significantly different from the growth reduction at T1 (84.17%) and Bt1 (77.13%) ($df=17$, $F=155$, $p<0.05$) as shown in Fig. 1.

EC_{50} values of DB, Ts and Bt were 0.17 mg ml^{-1} (95% confidence interval 0.10–0.25) and 0.35 mg ml^{-1} (0.25–0.54) and $0.0031 \text{ mg ml}^{-1}$ (0.0023–0.0049), respectively, against 4th instar larvae while the values remained 0.22 mg ml^{-1} (0.11–0.29), 0.41 mg ml^{-1} (0.30–0.58) and $0.0039 \text{ mg ml}^{-1}$ (0.0031–0.0061) against 5th instar larvae (Table 1). Lethal concentrations causing 50% mor-

tality (LC_{50}) were 0.30 mg ml^{-1} (0.25–0.38), 0.52 mg ml^{-1} (0.41–0.75) and $0.0068 \text{ mg ml}^{-1}$ (0.0053–0.0079) of DB, Ts and Bt against 4th instar larvae whereas the values fell to 0.43 mg ml^{-1} (0.34–0.53), 0.63 mg ml^{-1} (0.49–0.95) and $0.0071 \text{ mg ml}^{-1}$ (0.0060–0.0085) against 5th instar larvae of *S. exigua* (Table 2).

Effect of DB, Ts and Bt on mortality

Dose-dependent mortality was observed with individual treatments of DB, Ts and Bt. The highest mortality in individual treatments reached 90, 76.67 and 65.81% against DB, Ts and Bt on the 8th day. In combinations, the mortality percentage significantly increased from individual treatments with shorter exposure time. The highest mortality percentage (94.2%) was noted on the 6th day with DB1+Ts1+Bt1. The combination of DB and Ts resulted in decreased efficiency of DB as the highest mortality noted was 78.20%, as compared to individual DB treatments with 90% mortality on the 8th day. Ts produced better results with Bt with

Fig. 1. Growth inhibition caused by different treatments of DB, Ts and Bt relative to control in early insect instars. Each point expresses the mean $\pm$ standard error of five replicates. Means followed by the same letter above bars have no significance difference ($p \leq 0.05$) according to Tukey's test. DB1, destruxin B ($90 \mu\text{g/ml}$); DB2, destruxin B ($75 \mu\text{g/ml}$); DB3, destruxin B ($60 \mu\text{g/ml}$); DB4, destruxin B ($45 \mu\text{g/ml}$); DB5, destruxin B ($30 \mu\text{g/ml}$); DB6, destruxin B ($15 \mu\text{g/ml}$); Ts1, tea saponin ($90 \mu\text{g/ml}$); Ts2, tea saponin ($75 \mu\text{g/ml}$); Ts3, tea saponin ($60 \mu\text{g/ml}$); Ts4, tea saponin ($45 \mu\text{g/ml}$); Ts5, tea saponin ($30 \mu\text{g/ml}$); Ts6, tea saponin ($15 \mu\text{g/ml}$); Bt1, *B. thuringiensis* ($1.90 \mu\text{g/ml}$); Bt2, *B. thuringiensis* ($1.60 \mu\text{g/ml}$); Bt3, *B. thuringiensis* ($1.30 \mu\text{g/ml}$); Bt4, *B. thuringiensis* ($1.0 \mu\text{g/ml}$); Bt5, *B. thuringiensis* ($0.70 \mu\text{g/ml}$); Bt6, *B. thuringiensis* ($0.40 \mu\text{g/ml}$).

Table 1. EC_{50} and EC_{95} values (mg ml^{-1}) of DB, Ts and Bt to 4th and 5th instar larvae of *S. exigua*

Compounds	EC_{50}	CI 95%	EC_{95}	CI 95%	Slope	χ^2
DB (4th instar)	0.17 ± 0.05	0.10–0.25	1.10 ± 0.12	0.83–3.10	1.54 ± 0.20	5.10
DB (5th instar)	0.22 ± 0.04	0.11–0.29	2.01 ± 0.10	0.91–4.12	1.89 ± 0.23	5.99
Ts (4th instar)	0.35 ± 0.06	0.25–0.54	1.90 ± 0.14	1.09–5.77	1.98 ± 0.22	6.35
Ts (5th instar)	0.41 ± 0.07	0.30–0.58	2.33 ± 0.11	1.21–5.99	2.15 ± 0.21	4.03
Bt (4th instar)	0.0031 ± 0.0004	0.0023–0.0049	0.00873 ± 0.0007	0.0082–0.0091	2.43 ± 0.45	1.73
Bt (5th instar)	0.0039 ± 0.0006	0.0031–0.0061	0.0098 ± 0.0004	0.0089–0.011	2.37 ± 0.41	3.04

DB, destruxin B; Ts, tea saponin; Bt, *B. thuringiensis kurstaki*.

Table 2. LC_{50} and LC_{95} values ($mg\ ml^{-1}$) of DB, Ts and Bt to 4th and 5th instar larvae of *S. exigua*

Compounds	LC_{50}	CI 95%	LC_{95}	CI 95%	Slope	χ^2
DB (4th instar)	0.30 ± 0.02	0.25–0.38	1.46 ± 0.14	0.84–2.01	2.41 ± 0.27	5.71
DB (5th instar)	0.43 ± 0.11	0.34–0.53	1.97 ± 0.16	1.12–2.35	2.49 ± 0.26	4.22
Ts (4th instar)	0.52 ± 0.05	0.41–0.75	2.24 ± 0.10	1.39–4.77	2.71 ± 0.83	3.66
Ts (5th instar)	0.63 ± 0.14	0.49–0.95	2.78 ± 0.13	1.98–6.87	2.98 ± 0.87	7.46
Bt (4th instar)	0.0068 ± 0.0005	0.0053–0.0079	0.013 ± 0.0007	0.012–0.014	2.57 ± 0.35	4.87
Bt (5th instar)	0.0071 ± 0.0005	0.0060–0.0085	0.020 ± 0.0009	0.0177–0.024	1.62 ± 0.45	5.29

DB, destruxin B; Ts, tea saponin; Bt, *B. thuringiensis kurstaki*.

80.67% mortality, particularly when the three were mixed at different concentrations. The combination of DB and Bt resulted in high increased efficiency of both with 91.99% mortality, especially in comparison to individual Bt treatment, which resulted in 65.81% mortality (Table 3).

Nutritional efficiency

Nutritional analysis after feeding larvae on treated diet showed that these bio-chemicals proved toxic to some extent when ingested by larvae. EC_{50} (0.17, 0.35 and $0.0031\ mg\ ml^{-1}$ of DB, Ts and Bt, respectively) values were used either individually or in combination for this experiment. There was a decrease in RCR, RGR and ECI as compared to the control. In individual treatments, RCR and RGR values of larvae fed on DB, Ts and Bt were 2.84, 3.92, 3.07 $mg/mg/day$ and 1.45, 2.20 and 1.73 $mg/mg/day$, respectively. The lowest RCR, RGR values were found with DB+Bt, 2.69 $mg/mg/day$ and 1.33 $mg/mg/day$ and 35.33% of the control, respectively. Ts also showed better results, with Bt having significant lower values of RCR and RGR than individual treatment. The combination of DB+Ts improved Ts efficiency and the results of RCR; RGR was significantly different from individual Ts treatment (Table 4).

Enzyme analysis

Fat body weight of insects changed over time up to the final day (8th day) in comparison to the control. The maximum reduction in fat body weight (48%) was obtained on the 8th day after being treated with DB, whereas the minimum reduction was found after one day of treatment with Ts+Bt (11.67%) relative to the control (Fig. 2). A general trend of increased reduction on the final day, in relation to the control, was found, except with Ts and DB+Ts+Bt treatments where, after a certain in-

crease, it decreased on the final day (Fig. 2).

When fourth instar larvae were exposed to artificial diet treated with DB, Ts and Bt individually (at EC_{50} values) or in combination there was a certain change in SOD and CAT activity of treated larvae in comparison to the control (Tables 5 and 6) at different exposure times. With SOD, the highest variation in activity compared to the control was found on the 6th day after DB treatment (+29.21%) and the lowest activity was noted on the 8th day (−5.30%) after treatment with DB+Bt (Table 5). The maximum CAT activity was found on the 6th day (+24.64%) after treatment with Bt whereas the lowest value was noted on the 8th day (−9.51%) after DB treatment (Table 6). All chemicals changed SOD and CAT activity to some extent but with a common trend of decreased values on the final days, but the decrease in values with Bt was not as sharp as with other treatments (Tables 5 and 6).

DISCUSSION

Synthetic insecticides have proven toxic to beneficial insects, such as natural parasitoids and predators (Abudulai et al., 2001). The best alternative may be biochemicals obtained from natural origins, including plants, fungi or bacteria. In this study, three biochemicals were tested, either individually or in combination, and exhibited some toxicity against *S. exigua*, although some combinations exhibited higher efficiency than others. These chemicals significantly affected larval growth, nutrition efficiency and had important poisonous effects on *S. exigua* larvae, and their efficiency was increased when used in combination. This toxicity appeared to be related with decreased larval weight and inhibition of development. Such disruptions were often

Table 3. Mortality percentages at different exposure times, treated individually or in combination with DB, Ts and Bt

Compounds	Days							
	1	2	3	4	5	6	7	8
DB1	0 ^f	6.67±2.7 ^{ef}	26.67±2.0 ^{efghi}	43.33±3.3 ^{defg}	53.33±4.1 ^{cde}	73.33±2.3 ^{bcd}	80.00±3.8 ^a	90.00±2.6 ^a
DB2	0 ^f	0.00 ^f	6.67±2.2 ^k	20.00±3.5 ^{hi}	33.33±3.4 ^{fghij}	50.00±3.5 ^{efgh}	63.33±3.1 ^{bc}	73.00±1.9 ^{bc}
DB3	0 ^f	3.33±1.1 ^f	10.00±3.1 ^k	20.00±1.7 ^{hi}	30.00±2.0 ^{ghij}	36.67±2.9 ^{hij}	50.00±3.3 ^{cde}	60.00±2.0 ^{cde}
Ts1	0 ^f	0.00 ^f	13.33±1.85 ^{ijk}	30.00±3.4 ^{gh}	43.33±1.7 ^{defgh}	60.00±3.4 ^{cdef}	66.67±2.2 ^{abc}	76.67±2.7 ^{abc}
Ts2	0 ^f	0.00 ^f	3.33±1.2 ^k	10.00±1.3 ^j	23.33±3.8 ^{ij}	30.00±1.5 ^j	43.33±1.4 ^{def}	53.33±1.8 ^{de}
Ts3	0 ^f	3.33±0.8 ^f	6.67±1.6 ^k	10.00±1.2 ^j	16.67±1.5 ^{jk}	23.33±1.1 ^j	30.00±2.9 ^f	36.67±3.5 ^f
Bt1	13.2±1.4 ^{bcd}	24.26±3.0 ^{bcd}	34.23±1.7 ^{cdef}	39.56±3.2 ^{efg}	47.23±3.5 ^{defg}	55.09±3.7 ^{defg}	57.89±2.8 ^{cd}	65.81±3.7 ^{cd}
Bt2	15.36±2.0 ^{bcd}	27.26±2.0 ^{bcd}	31.21±2.4 ^{defg}	34.23±2.8 ^{fgh}	38.12±3.7 ^{efghi}	43.67±3.4 ^{fghi}	45.80±2.2 ^{def}	47.87±2.7 ^{ef}
Bt3	3.15±0.7 ^{ef}	12.30±1.4 ^{def}	17.23±2.1 ^{ghijk}	21.36±2.0 ^{hi}	28.69±2.2 ^{hij}	31.89±3.0 ^{ij}	34.23±3.5 ^{ef}	35.25±2.5 ^f
DB1+Ts1	15.05±1.0 ^{bcd}	30.91±2.3 ^{bc}	49.93±2.9 ^b	58.20±3.5 ^{bcd}	69.45±3.7 ^{abc}	72.16±2.5 ^{bcd}	78.20±2.9 ^{ab}	0.00 ^g
DB2+Ts2	21.12±1.5 ^{ab}	33.01±2.2 ^b	47.31±2.4 ^{bc}	58.00±3.3 ^{bcd}	59.09±2.7 ^{bcd}	61.78±4.0 ^{bcd}	0.00 ^g	0.00 ^g
DB3+Ts3	13.15±1.7 ^{bcd}	24.16±2.7 ^{bcd}	37.36±4.2 ^{bcd}	47.27±3.6 ^{cdef}	51.24±3.5 ^{de}	52.10±3.9 ^{efgh}	53.99±3.0 ^{cd}	0.00 ^g
DB1+Bt1	32.12±2.7 ^a	69.01±4.6 ^a	77.31±3.8 ^a	81.00±2.5 ^a	85.00±3.1 ^a	91.99±2.5 ^a	0.00 ^g	0.00 ^g
DB2+Bt2	21.35±1.5 ^{ab}	35.00±3.0 ^b	47.12±2.4 ^{bc}	61.32±4.0 ^{bc}	72.78±2.5 ^{ab}	79.08±3.6 ^{ab}	81.68±2.7 ^a	0.00 ^g
DB3+Bt3	23.15±2.6 ^b	34.16±2.5 ^b	42.36±1.5 ^{bcd}	52.27±2.0 ^{cde}	58.70±3.5 ^{bcd}	60.46±3.2 ^{def}	64.78±3.4 ^{abc}	66.89±3.0 ^{cd}
Ts1+Bt1	8.15±1.1 ^{cdef}	16.00±1.5 ^{cdef}	21.00±1.8 ^{fghij}	39.34±3.2 ^{efg}	51.36±3.0 ^{de}	67.96±3.0 ^{bcd}	80.67±1.9 ^a	0.00 ^g
Ts2+Bt2	6.39±1.1 ^{cdef}	20.69±1.8 ^{bcd}	29.36±2.0 ^{defgh}	41.26±2.7 ^{efg}	57.23±3.8 ^{bcd}	65.12±2.5 ^{bcd}	66.42±2.1 ^{abc}	67.34±2.4 ^{cd}
Ts3+Bt3	0 ^f	7.27±1.3 ^{ef}	11.32±1.7 ^{ijk}	19.00±1.4 ⁱ	21.36±1.5 ^{ij}	36.21±2.7 ^{ij}	41.10±2.8 ^{def}	46.23±1.2 ^{ef}
DB1+Ts1+Bt1	16.32±1.5 ^{bc}	24.26±1.7 ^{bcd}	48.65±3.0 ^{bc}	69.41±4.7 ^{ab}	77.23±3.7 ^a	94.20±1.3 ^a	0.00 ^g	0.00 ^g
DB2+Ts2+Bt2	20.3±1.7 ^b	35.20±2.4 ^b	46.32±3.3 ^{bc}	57.26±2.5 ^{bcd}	72.95±3.6 ^{ab}	78.26±2.7 ^{abc}	81.36±1.9 ^a	85.23±3.0 ^{ab}
DB3+Ts3+Bt3	4.28±0.5 ^{def}	12.32±1.3 ^{def}	15.30±1.7 ^{hijk}	39.24±3.3 ^{efg}	49.7±82.3 ^{def}	66.20±2.5 ^{bcd}	75.31±2.4 ^{ab}	77.25±3.9 ^{abc}
Control	0 ^f	0.00 ^f	3.00±0.3 ^j	3.00±0.3 ^j	6.33±1.1 ^k	6.33±1.1 ^k	9.67±1.4 ^g	13.00±1.9 ^{fg}
	df=21, F=22.20, p<0.0001	df=21, F=32.87, p<0.0001	df=21, F=53.97, p<0.0001	df=21, F=56.42, p<0.0001	df=21, F=41.32, p<0.0001	df=21, F=43.84, p<0.0001	df=21, F=82.50, p<0.0001	df=21, F=118.64, p<0.0001

Mortality percentages by DB, Ts and Bt either individually or in combination, at different time intervals (from day 1 to day 8). Each point expresses the means ± standard errors of five replicates. Values with the same letter in the column are not significantly different from each other according to Tukey's test at $p<0.0001$.

DB1, destruxin B (0.6 mg/ml); DB2, destruxin B (0.5 mg/ml); DB3, destruxin B (0.4 mg/ml); Ts1, tea saponin (0.6 mg/ml); Ts2, tea saponin (0.5 mg/ml); Ts3, tea saponin (0.4 mg/ml); Bt1, *B. thuringiensis* (12 µg/ml); Bt2, *B. thuringiensis* (10 µg/ml); Bt3, *B. thuringiensis* (8 µg/ml).

described in insects reared on extracts from natural resources or on pure allelochemicals with insecticidal activity (Rharrabe et al., 2007).

Table 4. Feeding, growth and efficiency of conversion of ingested food by 4th instar larvae of *S. exigua*

Compounds	RCR (mg/mg/day)	RGR (mg/mg/day)	ECI (%)
DB	2.84±0.13 ^{cde}	1.45±0.14 ^c	37.33±2.90 ^{ab}
Ts	3.92±0.12 ^b	2.20±0.10 ^b	46.10±1.53 ^{ab}
Bt	3.07±0.10 ^{bc}	1.73±0.17 ^{bc}	43.19±3.29 ^{ab}
DB+Ts	3.39±0.13 ^{bc}	1.55±0.15 ^{bc}	45.33±5.61 ^{ab}
DB+Bt	2.69±0.16 ^{de}	1.33±0.09 ^c	35.77±2.85 ^b
Ts+Bt	2.99±0.16 ^{cd}	1.77±0.11 ^{bc}	44.21±3.05 ^{ab}
DB+Ts+Bt	3.37±0.08 ^{bc}	2.14±0.13 ^{bc}	44.98±3.75 ^{ab}
Control	6.05±0.15 ^a	3.11±0.19 ^a	52.23±3.26 ^a

Mean values within a column with different letters are significantly different at $p \leq 0.05$ based on Tukey's test.

RCR, relative consumption rate; RGR, relative growth rate; ECI, efficiency of conversion of ingested food.

DB, destruxin B; Ts, tea saponin; Bt, *B. thuringiensis kurstaki*.

The mortality caused by individual and different combined treatments of biochemicals was found to be concentration and time (exposure period) dependent. The combination of DB1+Ts1+Bt1 was found to be the most lethal with highest mortality (94.2%). The combination of Ts+Bt at different concentrations exhibited the significantly increased efficiency of both as compared to individual performance and the highest mortality was caused by Ts1+Bt1 (80.67%), which was much higher than the highest mortality caused by individual Ts (76.66%) and Bt1 (65.81%). Lower mortality in individual Bt treatment could be because of stable SOD and CAT values in the final days (Tables 5 and 6). These results show the working compatibility of DB and Ts with Bt against *S. exigua*. Similar results were found by Gould et al. (1991) as they observed increased mortality with the addition of Bt to plant extracts. The combination of DB+Ts resulted in decreased mortality as compared to the individual performance of DB.

Some scientists believe that insecticidal activi-

Fig. 2. Effect of biochemicals (DB, Ts, Bt) individually or in combination with each other (based on their respective EC_{50} values), on the fat body weight of *S. exigua* larvae. Each point expresses the mean±standard error of five replicates. Means followed by the same letter above bars have no significance difference ($p \leq 0.05$) according to Tukey's test. DB, destruxin B; Ts, tea saponin; Bt, *B. thuringiensis kurstaki*.

Table 5. Effect of biochemicals on total superoxide dismutase (SOD) activity in fat bodies

Time of exposure (days)	SOD (% over control)						
	DB	Ts	Bt	DB+Ts	DB+Bt	Ts+Bt	DB+Ts+Bt
2	13.54+*	9.02+*	11.43+*	5.12+ns	13.15+ns	9.54+*	5.04+ns
4	27.40+*	15.94+ns	16.69+*	13.69+ns	23.51+*	18.01+ns	14.40+ns
6	29.21+*	21.87+ns	21.27+ns	25.43+ns	18.25+*	16.48+ns	19.97+*
8	3.24−ns	2.02+*	18.95+*	7.48+*	5.30−ns	8.47+*	6.80+ns

* Significantly different from the control ($\alpha=0.05$). ns, not significant.

+, % increase over control; −, % decrease over control.

DB, destruxin B; Ts, tea saponin; Bt, *B. thuringiensis kurstaki*.

Table 6. Effect of biochemicals on total catalase (CAT) activity in fat bodies

Time of exposure (days)	CAT (% over control)						
	DB	Ts	Bt	DB+Ts	DB+Bt	Ts+Bt	DB+Ts+Bt
2	7.50+ns	12.30+ns	9.63+*	4.12+ns	10.47+ns	8.73+ns	13.67+ns
4	19.21+*	16.69+*	18.09+ns	8.44+ns	14.58+*	14.89+*	14.97+*
6	15.89+*	23.57+*	24.64+*	11.98+*	16.20+*	15.23+*	11.91+ns
8	9.51-*	1.68-ns	13.42+*	1.79+ns	7.07+ns	1.51+ns	4.48+ns

*Significantly different from the control ($\alpha=0.05$). ns, not significant.

+, % increase over control; -, % decrease over control.

DB, destruxin B; Ts, tea saponin; Bt, *B. thuringiensis kurstaki*.

ties of destruxins are due to the combination of starvation and toxicity effects (Amiri et al., 1999). This phenomenon was confirmed in our experiment, as the RCR value against DB was considerably lower than Ts and Bt in individual treatments. The lowest RCR value noted with DB+Bt exhibited the higher interactive ability of the two, as there was a considerable decrease in comparison to individual treatments. The same results were found with DB+Bt with the lowest RGR values. The combination of Ts and Bt resulted in significantly lower RCR and RGR values than individual treatments of Ts without effecting the ECI value. Significantly lower ECI values against DB+Bt confirmed the multidimensional impact on the insect as reduced ECI can result from the decreased efficiency of *S. exigua* to change the consumed food into growth, perhaps by diverting energy from biomass production into detoxification. Strong antifeedant activity of DB has also been documented by many researchers, such as Hu et al. (2007), who identified antifeedant activity of DB against *Spodoptera litura*. On the other side, fourth instar *S. exigua* larvae were relatively insusceptible to individual Ts treatment as it caused less reduction in growth and ECI, as noticed by Adel et al. (2000), who have reported that treated *S. littoralis* gained almost the same weight as the control; however, in our experiment, increased toxicity of Ts was observed when mixed with Bt with lower RGR, RCR and ECI values as compared to individual treatment of Ts.

Fat bodies of insects had been the focus of study by many scientists and were reported to be a source of high metabolic rates in herbivorous insects (Ahmad et al., 1991). In our experiment, all biochemicals, individually or in combination, not only

caused the depletion of fat body weight as compared to the control but increased activities of SOD and CAT enzymes in fat bodies were also noted. Similarly Adamski et al. (2003) and Yu (2004) found fluctuations in the activities of different enzymes in fat bodies after the exposure of insects to different chemicals. Fat bodies are supposed to be sensitive to toxicity and play a vital role in the detoxification of insecticides with increased enzyme activities, as Kafel et al. (2003) reported that the increase in enzyme activities in fat bodies is dependent on exposure time.

Antioxidant enzymes SOD and CAT play a vital role in detoxifying destructive oxidant species by catalyzing the detoxification of $O_2^{\cdot-}$ to O_2 and H_2O_2 , SOD blocks $O_2^{\cdot-}$ -driven cell damage (McCord and Fridovich, 1969). CAT can break down H_2O_2 to H_2O and O_2 . Biochemicals can effect many enzymatic activities in different tissues where cells are producing enzymes (Timmins and Reynolds, 1992). Enhanced activity of antioxidant enzymes play an important role in the insect immune system and can be a cause of resistance. In this study, we found significantly higher values of these enzymes at different intervals when compared to the control, which confirms the presence of an antioxidant defense system in the insect body. The maximum SOD value was observed with individual DB treatment after 6 days of treatment. The highest CAT value was observed on the 6th day of individual Bt treatment. The combination of all biochemicals (DB+Ts+Bt) resulted in suppressed SOD values but did not differ significantly from the control except on day 6 and after 4 days of treatment with CAT. The values of these enzymes may fluctuate as documented by researchers (Casano et al., 1997; Cakmak, 2000) that high lev-

els of H_2O_2 inhibit SOD through the formation of excess hydroxyl radicals, whereas the CAT enzyme is sensitive to $O_2^{\cdot-}$ and can be inactivated by its increased levels. The activity of these enzymes fell with the increased exposure time, except with Bt, indicating that the detoxification mechanism was unable to match the stress produced by DB and Ts (Sowjanya and Padmaja, 2008). The lower enzyme activities on the final days could have been the cause of the lower survival (Adamczyk et al., 2003).

To summarize, this study not only establishes the bioinsecticidal effects of DB and Ts on the growth, mortality and nutritional aspects of *S. exigua* but also provides a comparison with Bt and their compatibility in combination, especially with Bt. This information can be helpful to increase the efficacy of not only these biochemicals but this phenomenon can also be used with other biochemicals from natural sources against different insect species. This study also provides information about the activity of antioxidant enzymes against these biochemicals, as antioxidant enzymes are supposed to be involved in the resistance mechanism of insects, so these investigations should be helpful to establish the relationship between biochemicals from natural resources and the defense system of insects, which can further enhance their activities and the better control of insects.

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