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Combined Effects of Some Microbial Control Agents Mixed with Botanical Extracts on Some Stored Product Insects

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Abstract: The effect of two microbial entomopathogens (*Bacillus thuringiensis and Beauveria bassiana*) and three botanical extracts; methanolic extract of *Taxodium distichum* leaves, volatile oil (V.O) of *Taxodium distichum* leaves and (V.O) *Boswella carterii* (gum resin) were studied on three stored product insects, *Plodia interpunctella*, *Ephestia cautella* and *Ephestia küehniella*. At 5% concentration level to the botanical extract, *T. distichum* leaves (methanolic extract), scored the highest potential effects against the target insects (88-90% mortalities) . The other botanical extracts at this concentration caused also high effects (75-81 mortalities).The different stages of the target insects were affected by a lower concentration at 0.5%. The treatments, beside inducing reasonable larval mortality, caused increase in the malformed pupae and deformed adults. Botanical extracts tested when combined with *B. thuringiensis* caused a significant enhancement to the pathogens and increased the mortality in almost all cases. When the extract in combination with *B. bassiana* caused a less than 50% reduction in the LC50 especially *Taxodium distichum* (V.O) .

Key wards: *Bacillus thuringiensis, Beauveria bassiana, Taxodium distichum* leaves, volatile oil (V.O), *Boswella carterii* (V.O), *Plodia interpunctella, Ephestia küehniella, E. cautella*

Introduction

Indian meal moth, *Plodia interpunctella*; almond moth, *Ephestia cautella* and Mediterranean flour moth, *Ephestia küehniella* are the major pests of stored grains in Egypt. Most of the damage is done when larvae interfere with flour production during spinning webbing that clogs Machiney and by biting holes resulting in accumulation of static flour. Using chemical control for these pests is undesirable, hence safe and yet effective control methods are being sought. Microbial control agents are considered as good replacement due to the absence of vertebrate toxicity or pathogenicity compatibility with organisms and biodegradability (Goettel, 1991). Several authors studied the toxic effects of different commercial bacterial formulations containing *Bacillus thuringiensis* and *Beauveria bassiana* against some insects (Kinsinger *et al.*, 1980; Salama *et al.*, 1991; Kayaa, *et al.*, 1991; El-Mandarawy, 1992).

Plant extracts were used as toxicants, oviposition repellents, growth regulators or antifeedants for many insects (Ismail, 1994; Nassar *et al.*, 1997).

The present work was conducted to evaluate the toxicity of two microbial control agents (*B. thuringiensis* and *B. bassiana*) and methanolic extract of *T. distichum* leaves, volatile oil and *B. carterii,* against three stored product pests, *P. interpunctella*, *E. cautella* and *E. küehniella.*

Materials and Methods

Rearing the target insects on semi-artificial diet: The target insects were reared under laboratory conditions on semi artificial diet (fine wheat with some adherent endosperm), with 20% glycerin and 5% yeast powder. Populations were held at 26±2°C and 70-80% R.H, for the diet of Burgus and Hurst (1977).

Groups of 100 one-day old eggs were placed, each in 12 cm petridishes comprising a thin layer of diet.

The preparation of the plant extracts

Plant materials

***Taxodium distichum* leaves:** The fresh leaves of *Taxodium distichum* were obtained from Moshtohor, Kalubia farm.

Method of extraction

Volatile oil extraction of *T. distichum* (V.O) : The volatile oil of *T. distichum* leaves was prepared by hydro distillation method using Clevenger apparatus (Egyptian Pharmacopoeia, 1984).

Methanolic extraction: *T. distichum* leaves were defatted with methanol (v/v) at 40-60°C and subjected to percolation, the extract was concentrated under vacuum to give reddish brown material of *T. distichum* leaves extract.

Volatile oil extraction of *Boswella carterii* (V.O): *Boswella carterii* (gum resin exudates) were obtained from the Haraz market. Volatile oil of *B. carterii* was extracted by hydro distillation method as described in Egyptian Pharmacopoeia (1984) using Clevenger apparatus, the oil was collected and dried over anhydrous sodium sulphate.

Methanolic extraction of *B. carterii:* About 200 g of the defatted resin was subjected to percolation with methanol (v/v) at 24°C for 48 h. The extract was dried under vacuum to give brown colour materials. For each extract 5 g was dissolved in 100 ml distilled water to obtain 5% of the plant extracts. Further dilutions were made to obtain 0.5 and 0.05% of the plant extract in the diet.

Assessments of botanical extracts effects: Egg masses from each species were collected from the corresponding culture and kept till hatching under laboratory conditions of 26±2°C and 70-80 RH. Fifty hatched larvae were fed on the corresponding semi-artificial diet. Treated diets were prepared by mixing (10 ml) of the prescribed concentrations of the botanical extract with 100 g diet to obtain 5% , 0.5 and 0.05% extract in the diet, then placed in a glass jar (15x20 cm^2). The percent mortalities were calculated after 7 days. Each experiment was replicated four times. Control larvae were fed on uncontaminated diet.

Effect of the botanical extract on certain developmental stages of the target insects: Fifty-fourth instar larvae were reared on a diet containing 0.5% of the plant extract. The percentage of larval mortality, pupation, malformed pupae, deformed adults and percentage of hatched eggs were calculated.

Bioassay of *B. thuringiensis* against the target insect: Dipel 2X (*Bacillus thuringiensis* var *Kurstaki* 32.000 IU/mg) was used. The assay procedure proposed by Dulmage *et al.* (1971) was adopted. The suspensions of *B. thuringiensis* were added to the semi-artificial diets used for each species @ 500, 250, 125, 63, 32 and 16 µg Bt/ml diet. The control larvae were reared on untreated semi-artificial diet. Mortalities were counted after 7 days. The experiments were replicated four times at 26±2°C and 60-70% RH. Mortalities were corrected according to Abbott (1925), while LC50 were calculated through probit analysis (Finney, 1964).

Isolation of *B. bassiana:* *Beauveria bassiana* was isolated from diseased *P. interpunctella*, *E. cautella* and *E. küehniella* larvae. Isolates were subcultured on nutrient PDA medium. Isolates were identified at N.R.C. Plant Pathology Department. The spores of *B. bassiana* were collected from agar surface fungus cultures in 15 cm diameter Petri-dish. Spore suspension in water 0.1% tween-80 was prepared. The strength of the original culture was $16.5x10^8$ conidia ml^{-1}.

Bioassay of *B. bassiana* against the target insect: The target insects, were fed on an artificial diet containing different concentrations of *B. bassiana* in diet. About $16.5x10^7$, $8.25x10^7$, $4.125x10^7$, $2.06x10^7$, $1.03x10^7$ and $0.51x10^7$ conidia ml^{-1}, were added to the semi artificial diets @ 10 ml of the suspension to 100 g of diet (v/w) used for each species. Mortalities were counted after 7 days.

The interaction between the pathogen and the plant extracts: The methanolic extracts of leaves and V.O of *Taxodium distichum* and *Boswella carterii* (0.05%) were added to the original concentration of each entomopathogen (*B. thuringiensis* or *B. bassiana*) (v/v). Then 1-2 fold dilutions were made and 10 ml from each concentration were added to 100 g of the diet. Ten fourth instar larvae for each insect species were kept in a glass jar (15x5 cm^2) fed on a diet containing the prescribed treatment.

All the above experiments were carried out under laboratory conditions at 26±2°C and 60-70% RH. The experiments were replicated 4 times. Mortalities were counted after 7 days. The control insects were fed on uncontaminated diet. The corrected mortality was calculated according to Abbott (1925).

Reduction in LC50 calculated according to the following formula =

$$\frac{\text{(LC50 of pathogen alone – LC50 of pathogen after added the extract)}}{\text{LC50 of the pathogen alone}} \times 100$$

Results and Discussion

Effect of botanical extract on larvae of tested insects: The methanolic extract of *Taxodium distichum* leaves at 5% concentration caused the highest mortality of 89, 90 and 88%. This is compared with 0, 1 and 2% in the control for *P. interpunctella, E. cautella* and *E. küehniella*, respectively (Table 1, 2). When the concentrations decreased to 0.5%. the percentage of mortalities ranged between 14.24 and 32.13% for the tested species.

When the target insect species were treated with *T. distichum* (V.O) at 5% concentration, the mortalities

Table 1: Effect of tested botanical extracts against fourth larval stage on the three target insects

		% of larval mortality of		
Treatments	Concentrations%	*P. interpunctella*	*E. cautella*	*E. kiiehniella*
T. distichum leaves	5	89.00	90.00	88.00
	0.5	14.24	28.19	32.13
	0.05	0.00	1.00	4.00
T. distichum (V.O)	5	80.00	75.00	81.00
	0.5	15.20	16.14	23.30
	0.05	1.00	2.00	2.00
B. carterii (V.O)	5	79.00	76.00	78.30
	0.5	41.10	31.2	51.20
	0.05	5.00	4.00	6.00
Control (untreated)	0	0.00	1.00	2.00

Table 2: Effect of 3 botanical extracts at 0.5% on some biological aspects of target insects

Insect pest	Treatments	% of larval mortality	% of pupation	% of malformed pupae	% of adult deformed	% of egg hatched /female
P. interpunctella	T. distichum leaves	14.24	60	50	30.0	78.0
P. interpunctella	*T. distichum* oil	15.2	50	56	5.0	60.0
P. interpunctella	*B. carterii*	41.1	25	51	6.0	79.0
Control (untreated)	Control (untreated)	0	97	1	0.0	100.0
F value=661 LSD 1%= 1.73	F=3096 LSD=2.09	F=2772	LSD=1.48	F=1118 LDS=1.48	F=37 LDS=9.75	
E.cautella	*T. distichum leaves*	28.19	57	70	28.3	80.0
E.cautella	*T. distichum oil*	16.14	41	80	14.0	69.0
E.cautella	*B. carterii*	31.2	32	53	18.0	60.0
Control (untreated)	Control (untreated)	1	99	0	0.0	100.0
F value=261 LDS 1%= 2.26	F= 488 LDS=5.34	F=696	LSD=3.65	F=187 LSD=5.48	F=49 LSD=11.34	
E. kiehniella	*T. distichum* leaves	32.13	40	88	303.0	71.0
E. kiehniella	*T. distichum* oil	23.3	31	70	13.0	62.0
E. kiehniella	*B. carterii*	51.2	33	63	10.0	63.0
Control (untreated)	Control (untreated)	2	100	0	0.0	98.0
F value=390 LSD 1%=1.10	F=57.9 LSD=13.09	F=177	LSD=4.69	F=363 LSD=2.1	F=39 LSD=12.06	

Table 3: Effect of *B. thuringiensis* and *B. bassiana* on the fourth larval instars of the target insects

Pathogen	Insect pest	LC50	Slope	Variance	95% confidence limits
B. thuringiensis	*P. interpunctella*	152	1.5	0.07	131-163
	E. cautella	142	1.3	0.09	128-160
	E. kiehniella	131	1.4	0.09	118-144
B. bassiana	*P. interpunctella*	$73x10^7$	1.4	0.08	$50\text{-}84x10^7$
	E. cautella	$61x10^7$	1.0	0.09	$31\text{-}81x10^7$
	E. kiehniella	$50x10^7$	1.0	0.09	$28\text{-}77x10^7$

Table 4: Combined effect of pathogen with 0.05% of *T. distichum* leaves on the target insects.

T. distichum leaves+	Insect pests	LC50	% of reduction	Slope	Variance	95% confidence limits
B.thuringiiensis	*P. interpunctella*	44	71	1.4	0.1	21-68
	E. cautella	51	63	2.1	0.2	29-77
	E. kiehniella	40	69	1.3	0.08	23-67
B. bassiana	*P. interpunctella*	$40x10^7$	45	2.3	0.09	$13\text{-}63x10^7$
	E. cautella	$40x10^7$	35	1.5	0.5	$22\text{-}66x10^7$
	E. kiehniella	$40x10^7$	20	1.6	0.08	$26\text{-}69x10^7$

Table 5: Combined effect of the pathogen with 0.05% of *T. distichum* (v.o) on the target insects

T.distichum (V.O)+	Insect pests	LC50	% of reduction	Slope	Variance	95% confidence limits
B.thuringiensis	*P. interpunctella*	49	67	2.3	0.1	31-71
	E. cautella	51	63	1.5	0.2	35-78
	E. kiehniella	33	74	1.4	0.06	21-55
B. bassiana	*P. interpunctella*	$41X10^7$	43	2.2	0.08	$22\text{-}65X10^7$
	E. cautella	$35X10^7$	43	2.5	0.09	$23\text{-}66X10^7$
	E. kiehniella	$33X10^7$	34	1.3	0.1	$20\text{-}65X10^7$

Table 6: Combined effect of pathogen with 0.05% of *B. carterii* on the target insects

B. carterri (V.O)+	Insect pests	LC50	% of reduction	Slope	variance	95% confidence limits
B.thuringiensis	*P. interpunctella*	50	67	1.7	0.07	19-69
	E. cautella	39	72	1.4	1.01	22-71
	E. kiehniella	27	79	1.5	0.08	14-56
B. bassiana	*P. interpunctella*	$51x10^7$	30	2.4	0.09	$33\text{-}69x10^7$
	E. cautella	$33x10^7$	46	1.4	0.09	$21\text{-}70x10^7$
	E. kiehniella	$31x10^7$	38	1.8	1.00	$11\text{-}37x10^7$

reached 80, 75 and 81%, for, *P. interpunctella, E. cautella* and *E. küehniella*, respectively. The decrease in concentration of the *T. distichum* (V.O) to 0.5% led to a decrease in the mortality of the tested species to 15.20, 16.14 and 23.30%.

The *Boswella carterii* (V.O) caused mortality rates of 79, 76 and 78% in *P. interpunctella, E. cautella* and *E. küehniella*, respectively when used at 5%, however the mortality decreased to a range between 31.20 - 51.20% in the three species.

However, 0.05% concentration level for all the tested botanical extracts had no effects on all the target insects.

Many authors recommended the traditional use of a number of plant materials as protectants of stored products (Mohiuddin *et al*., 1987). In this concern, Nasseh (1981) reported that extract of *Allium sativum* caused a strong anti-feedent effect on *Epilachna varivestis*. Jacobson *et al*. (1987) also reviewed protection of stored product with phyto-chemicals. Nassar *et al.* (1997) reported that *Parasarcophaga argyrostonna* can be controlled by *Nerium oleander* and *Sorghum bicolor* extracts. El-Din (2001) found that the oil of 3 umbellifrous plants caused a higher toxicity against *Tribolium confusum, Sitophilus oryza* and *Rhizopetha dominance*. Nassar *et al.* (1999) reported that the botanical extracts of jochia, curcuma, zygophylum and sorghum reduced the infestation of potato tuber moth. Salama and Ahmed (1997) found that the chinaberry *Melia azedrach* at 100 ppm added to the diet could act as a stomach poison for cotton leafworm. Dandage *et al.* (1998) found that several Zingiberaceae *Curcuma zedoaria* and *Alpini galangal* contain compounds that showed insecticidal effects against *Callosobruchus chinensis.*

Effect of botanical extracts at the level 0.5% on the biology of the target insects: *Ephestia küehniella* larvae were more susceptible to the extract of *T. distichum* leaves than the volatile oil and *B. carterii.* The percent pupation was significantly decreased to 60, 57 and 40%, as compared with 97, 99 and 100% in control for each of *P. interpunctella, E. cautella and E. küehniella,* respectively. Malformation among pupae was significantly increased to 50, 70 and 88% in comparison as with 1, 0 and 0% in control for *P. interpunctella, E. cautella* and *E. küehniella*, respectively. The percentage of adults deformed ranged between 28.3 and 30.3% for the three insect species. The percentage of egg hatching was significantly decreased to 78, 80 and 71% as compared with 100, 100 and 98% for *P. interpunctella, E. cautella* and *E. küehniella*, respectively after treatment with 0.5% concentration of *T. distichum* leaves.

The extracts of *T. distichum* (V.O) caused a significant larval mortality @ 15.20, 16.14 and 23.30%, a decrease among the pupation percentage to 50, 41 and 31%, respectively in the tested insects.

The percentage of malformed pupae ranged between 56 and 70% for the tested insects.

The percentage of adults deformed was significantly increased to 5, 14 and 13% as compared to 0% in the control and that of egg hatching was significantly declined to 60, 69 and 62% when the insects were treated with *T. distichum* (V.O) at 0.5% concentrations.

The *Boswella carterii* (V.O) at 0.5% concentration of caused a significant larval mortality (31.20 and 51.20%) for three insect species. The percentage pupation of the three target insects ranged between 25 to 33%. The percentage of malformed pupae was 51, 53 and 63%, respectively. While the percentage of adults deformed ranged between 6 and 33% in the three-target insects. The percentage of egg hatch was 79, 60 and 63% in *P. interpunctella, E. cautella* and *E. küehniella*, respectively.

Bowry *et al.* (1984) also found that the oil seed cake powders from neem to be the most effective in reducing oviposition of curulionid, *Sitophilus oryzae* under laboratory conditions. Nassar *et al.* (1997) found that *Parasarcophaga argyrostoma* larvae malformed and failed to pupate at high concentrations of *Nerium oleander* and *Sorghum bicolor* extracts.

Salem (1991) recorded that the oil extract of *Azadrachta indica* was effective against the potato tuber moth biological aspects. Salem *et al.* (1995) indicated that *Melia* leaves at higher concentrations had a direct effect on emergence of adults and was sufficient to increase the mortality rate of *Agrotis ipsilon* to 100%. Schmidt *et al.* (1997) noticed that the larvae of *S. littoralis* and *Agrotis ipsilon* failed to pupate at higher concentrations of *Melia azedarach* extract.

The effect of *B. thuringensis* and B. bassiana on the target insects: The LC50, with *B. thuringensis* recorded was 152, 141 and 131 μg ml^{-1} for *P. interpunctella, E. cautella* and *E. küehniella* with *B.thuringensis*, respectively. While the LC50 with B.b were 73, 61 and 50 x 10 conidia ml^{-1} for the same three species (Table 3).

The effect of different treatments with the combination of plant extracts tested: The LC50 of *P. interpunctella, E. cautella* and *E. küehniella* was reduced (71, 63 and 69%, respectively) with combination of *T. distichum* leaves + *B.t.* The extract affected significantly as indicated by non overlapping the corresponding limits, according to (Finney, 1964).

The LC50 reduced (by 45, 35 and 20%, respectively) after treatment of *P. interpunctella, E. cautella and E. küehniella* with B.b combined with 0.05% of *T. distichum* leaves (Table 4).

On the other hand the addition of *T. distichum* (V.O) caused an enhancement of *B.t* activity and significantly

reduced the LC50 of *P. interpunctella, E. cautella* and *E. küehniella* by 67, 63 and 74% (Table 5). The addition of *T. distichum* (V.O) to the fungus B.b, caused a reduction in LC50 of the target insects by 43, 43 and 34, respectively (Table 5). The combination *B. carterii* and *B.t* caused a significant reduction in LC50 by 67, 72 and 79% for *P. interpunctella, E. cautella* and *E. küehniella,* respectively.

When *B. carterii* combined with B.b, the LC50 of *P. interpunctella, E. cautella* and *E. küehniella* decreased to 51, 33 and 31x10 conidia ml^{-1}, respectively (Table 6). The three tested botanical extracts at 0.05% concentration caused a significant enhancement in *B. thuringiensis* but after adding the plant extracts to *B. bassiana* the LC50 values were decreased non significantly due to the overlapping in the corresponding 95% confidence limits according to Finney (1964).

Similar results were also obtained by Nassar *et al.* (1999), who reported that the treatments of potato tuber moth with *B. thuringiensis* and botanical extracts can destroy the epithelial membrane of the mid gut and caused higher larval mortality. The addition of all plant extracts tested to *B. thuringiensis* caused a significant decrease in LC50 levels and caused reduction more than 50% in LC50 value, while the addition of these to the fungus (*B. bassiana*) caused a non significant decrease in LC50 values and less than 50% reduction.

In this connection, Sabbour and Ismail (2001) reported that the combination of *B. thuringiensis* or *B. bassiana* with botanical extracts of *Solanum nigrum, Atropa belladonna* and *Hyoscyamus* showed a synergistic effect against *Pthorimarea operculella.* Similarly, Ismail and Sabbour (2002) found that the combination of *B. bassiana* with Comphene, a-pipene and citronella caused a reduction of *Earias insulana, Pectinophora gossypiella* and *Heliothis armigera* in laboratory experiments and the combination with certain terpenes and bioinsecticied increase the yield in the field experiments.

Novan *et al.* (1992) recorded a knowledge about the interaction of microbial insecticides and plant extracts on lepidopterous insects, who reported that the allelochemicals antagonized the insecticide activity of the microbes by means of feeding reduction. On the other hand, microbial activity was enhanced either by causing insect toxicity or by potentiation of the crystal protein. Ludlum *et al.* (1991) found that the alkylation possibly improved the solubilization and proteolysis of the pathogen crystals protein of microbe formulation to enhance or synergistic the activity of bacterial or fungal insecticides.

The combination between the plant extracts (*Taxodium distichum* (leaves, V.O) and *Boswella carterii* cause an enhancement to the potency of *Beauveria bassiana* and *Bacillus thuringiensis.*

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