

INTERACTION OF TWO BOTANICAL COMPOUNDS WITH *Bacillus thuringiensis* KURSTAKI AGAINST *Spodoptera littoralis*

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ABSTRACT

The toxicity of *B.t.* (Dipel 2X 6.4% and Protecto 9.4%) alone and combinations of *B.t.* and garlic and hot pepper soaks in water and mineral oil (Alphaz.) was examined on the first instar larvae (neonate) of *Spodoptera littoralis* (Boisd). Pepper and garlic soaks in water or in oil increased the efficiency of Dipel 2x and protecto against the neonates of the cotton leaf worm. The toxicity of Dipel 2x was increased by 2.82, 14.55, 8.41 and 13.61 folds when it used in mixture with pepper soak in water, pepper soak in oil, garlic soak in water and garlic soak in oil respectively .

The results also indicate that the addition of garlic or hot pepper soaks in water or oil to protecto gave the highest values of relative toxicity, whereas 5.15, 9.44, 6.39 and 14.06 folds also obtained with the same mixtures, respectively.

On the other hand, twenty-two compounds were identified in the water extract of hot pepper. The dominant compound was 4H-pyran-4-one, 2-3 dihydro -3,5-dihydroxy 6-methyl which appeared at Rt 7.61 min with 29.64% area. While twenty-one compounds were detected and identified in water garlic extract. 2- Furan carboxaldehyde, 5 hydroxy methyl was the dominant compound represented 66.31% of the total area of this extract .

INTRODUCTION

For several decades since its discovery, formulations of *Bacillus thuringiensis* have been seen as the ideal means of controlling Lepidopteran pests in agriculture because of the many attributes that differentiate these microbial insecticides from the synthetic chemical formulations. No toxicity to mammals, environmental friendliness, and good integration with other pest control methods, all made *B.t.* the much needed tool for IPM programmes in developing countries.

It is known that *B.t.* has a very short effective residual life. Previous studies have shown that the incorporation of certain toxic and nontoxic additives such as emulsifying agents, protein solubilizing agent, mineral oil, nitrogenous and aromatic compounds, inorganic salts and allelochemicals in insect diet along with *B.t.* ssp *kurstaki* can potentiate the action of the pathogen, resulting in higher larval mortality and lower feeding activity in noctuid larvae (Salaam *et al.*, 1984, 1989, Morris *et al.*, 1995 and Besheli, 2007)

The purpose of the present study is to investigate the interaction of some botanical compounds extracts with *Bacillus thuringiensis* ssp. *kurstaki* against the neonate of the cotton leaf worm *Spodoptera littoralis* Boisd and identification of the active components of crude test extracts by using GC/MS.

MATERIALS AND METHODS

Compounds used:

A. Bio-insecticides:

1-Dipel 2X: it is a wettable powder formulation based on B.t. Supsp *Kurstaki*. It contains lepidopteran active toxin 6.4% and 93.6% inert material, produced by Abbot Laboratories. North Chicago USA.

2- Protecto: it is a wettable powder formulation based on B.t. Supsp *Kurstaki*. It contains lepidopteran active toxin 9.4% and 90.6% inert material, produced by Plant Protection Research Institute, ARC, Dokki, Cairo, Egypt.

B. Experimental oil:

Alpha Z-oil 96.4% EC: it is a highly purified paraffinic oil supplied by Cooperative Organization of Petroleum. Its physical properties and structural composition were determined according to ASTM method (Anonymous, 1980).

C. Garlic and hot pepper soaks:

Garlic *Allium sativum* and hot pepper *Capsicum* sp. used in this experiment were selected free from diseases hundred grams of chopped garlic or hot pepper were added to 100 ml of distilled water and the same weight of garlic or hot pepper was added to 100 ml of Alpha Z-oil EC 96.4%. The mixtures were allowed to stand for 24 hours. The extracts were filtered through gauze and kept in a dark brown glass bottle until use.

Test insect:

The first instar larvae (neonate) of lab. strain of *Spodoptera littoralis* were reared on the semi synthetic diet Ahmed (1996), which consists of the following components :

Dry powdered lima beans	150gm
Dry yeast	15 gm
Ascorbic acid	3gm
Nipagin (methyl L- P- hydroxyl benzoate)	3gm
Agar	10 gm
Distilled water	600ml

Agar was dissolved in 200 ml of boiling water. The other ingredients were blended in 400ml water. They were mixed after cooling below 60°C and the diet was poured into a number of plastic cups (3×4 cm, diameter x depth).

Testing and evaluation:

There were six experimental treatments:

- 1- Dipel 2x or protecto alone
- 2- Dipel 2x or protecto mixed with garlic soak in water at 1:1.
- 3- Dipel 2x or protecto mixed with garlic soak in tested mineral oil at 1:1.
- 4- Dipel 2x or protecto mixed with hot pepper soak in water at 1:1.
- 5- Dipel 2x or protecto mixed with hot pepper soak in oil at 1:1.
- 6- Mineral oil alone.

400µl from every concentration of tested *B.t.* alone or its mixture with plant soaks or oil alone was applied onto the insect diet in the plastic cups using micropipette.

Each concentration was replicated three times, the cups used as control was treated with plant soaks in water and replicated also three times.

Twenty of neonate larvae of *S. littoralis* were placed on the surface of treated cups after it dried. Percentage mortality was calculated after 72 hrs and corrected by Abbott's formula (1925). The corrected percent mortalities were statistically computed according to Finney (1971).

GC /MS. Identification

Ten ml of each water extract filtrate were passed through 13mmGD/Xdisposable syringe filter of 0.45 μm pore size (Whatman Inc., Clifton, NJ, USA) for further purification.

Separation of resulting *A. sativum* and *Capsicums sp* water crude extract, fractions was accomplished on Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary Colum HP-5MS (30m \times 0.32 mm i.d. \times 0.25 μm film thickness). Samples were injected under the following condition:

Helium was used as carrier gas at approximately 1.0 ml /min, pulsed split less mode. The solvent delay was 4 min. and the injection size was 1.0 μl . The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 e.v. scanning from m/z 50 to 700. The ion source temperature was 230 $^{\circ}\text{C}$ and the quadruple temperature 150 $^{\circ}\text{C}$. The electron multiplier voltage (EM voltage) was maintained 1050 v above auto tune. The instrument was manually tuned using perfluorotributyl amino (PFTBA). The GC temperature program was started at 80 $^{\circ}\text{C}$ held on three min., then elevated to 260 $^{\circ}\text{C}$ at rate of 8 $^{\circ}\text{C}/\text{min}$. The detector and injector temperature were set at 280 and 250 $^{\circ}\text{C}$, respectively.

RESULTS AND DISCUSSION

It always seems convenient to measure the efficiency or toxicity of different toxic materials by comparing them with a stander compound. To achieve this, the toxicity index was obtained by comparing the efficiency of the tested compounds, at a fixed level LC₅₀ or LC₉₀ to their most effective compound.

The toxicity index was calculated by the following equation (Barakat *et al.*, 2006)

$$\text{Toxicity index} = \frac{\text{LC}_{50} \text{ or LC}_{90} \text{ of the most effective compound}}{\text{LC}_{50} \text{ or LC}_{90} \text{ of the other tested compound}} \times 100$$

The toxicity index in Table (1) indicated that Dipel 2x with garlic soak in oil, Dipel 2x with garlic soak in water, Dipel 2x with pepper soak in water, Dipel alone and Alpha Z oil were 93.53, 57.80, 19.36, 6.87 and 0.80% as effective as Dipel 2x with pepper soak in oil (100%) at LC₅₀ value. The results in Table 2 revealed that the most effective compound was the protecto with garlic soak in oil. Based on the toxicity index, the tested compound could be arranged discerningly according to their toxicity against

the neonates larvae of *Spodoptera littoralis* as follows : Protecto with garlic soak in oil, protecto with pepper soak in oil, protecto with garlic soak in water, protecto with pepper soak in water, protecto alone and Alpha-Z oil. Their toxicity index values based on LC₅₀ were 100, 67.16, 45.47, 36.60, 7.11 and 0.44, respectively: The role of mineral oil EC in augmenting the effectiveness of insecticide formulation could be attributed by the function of oil as an antifeedant and /or as a penetrate aid (Mustafa and El-Attal, 1985).

The role of pepper and garlic soaks, in water or oil in increasing the toxicity of the bio-compounds Dipel 2X and protecto is shown in (Tables 3 and 4). The relative toxicity (R.T.) was calculated from LC₅₀ values by assigning and arbitrary value of 1.0 for the least effective compound.

Table (1): Efficacy of Dipel 2X alone or in admixture with garlic or pepper soaks in water or in mineral oil against the neonate of *Spodoptera littoralis* under laboratory conditions.

Treatments	LC ₅₀ in ppm	TI %	LC ₅₀ in ppm	TI	Slope
Dipel 2X	67.37 (49.30 – 103.16)	6.87	1789.89 (762.77 – 7020.69)	0.94	0.90
Dipel 2X + pepper soak in water	23.92 (18.53 – 31.03)	19.36	517.79 (282.25 – 1302.38)	3.25	0.96
Dipel 2X + pepper soak in oil	4.63 (2.09 – 6.24)	100	16.87 (12.69 – 39.54)	100	2.28
Dipel 2X + garlic soak in water	8.01 (1.81 – 12.89)	57.80	120.60 (119.07 – 128.21)	13.99	1.09
Dipel 2X + garlic soak in oil	4.95 (3.77 – 6.08)	93.53	25.75 (20.61 – 34.85)	65.51	1.79
Alpha Z-oil	2370.25 (2032.75 – 2759.35)	0.20	12785.62 (9946.26 – 17617.31)	0.13	1.75

T. I.: Toxicity Index

Table (2): Efficacy of Protecto alone or in admixture with garlic or pepper soaks in water or in mineral oil against the neonate of *Spodoptera littoralis* under laboratory conditions.

Treatments	LC ₅₀ in ppm	TI %	LC ₅₀ in ppm	TI %	Slope
Protecto	147.46 (109.68 – 222.26)	7.11	3477.21 (1426.35 – 17320.25)	2.33	0.93
Protecto + pepper soak in water	28.66 (22.36 – 35.53)	36.60	574.82 (337.32 – 1306.20)	14.09	1.19
Protecto + pepper soak in oil	15.62 (11.20 – 20.00)	67.16	169.37 (115.85 – 302.62)	47.79	1.24
Protecto + garlic soak in water	23.07 (16.03 – 30.65)	45.47	339.25 (233.74 – 570.44)	23.86	0.92
Protecto + garlic soak in oil	10.49 (7.32 – 13.57)	100	80.94 (62.40 – 116.23)	100	1.44
Alpha Z-oil	2370.25 (2032.75 – 2759.35)	0.44	12785.62 (9946.26 – 17617.31)	0.63	1.75

T. I.: Toxicity Index

The toxicity of Dipel 2X was increased by 2.82, 14.55, 8.41 and 13.61 folds when it used in admixture with pepper soak in water, pepper soak in oil, garlic soak in water and garlic soak in oil, respectively (Table 3). Also the

toxicity of protecto was increased by 5.15, 9.44, 6.39 and 14.06 folds when it used in admixture with the same previously soaks (Table 4).

Tables (5) and (6) show the chemical name, Rt, % area, molecular weight (M.W) and chemical formula of the identified compounds in water extract of hot pepper and garlic.

Twenty-two compounds were identified in the water extract of hot pepper on the basis of their molecular weight (M.W), molecular formula and scan, after comparing them with library data of the instrument. The quantitative determination was carried out based on peak area integration (Table 5). Data show that 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl (no.13) which appeared at Rt 7.61 min was the dominate compound with 29.64 as % area followed by 1,2-Bis (trimethylsilyl) benzene (no.9) with 12.1% and 4-octanone, 2-methyl-thiopivsllic acid (no .5) with 9.72%.

George *et al* (2007) reported that fruit extracts of *Capicun annuvm* and *C. frutescence* could be useful for managing of cabbage loppers and spider mites, which could reduce reliance on synthetic pesticides.

Table (6) shows that twenty-one compounds were detected and identified in water garlic extract. 2-Furancarboxaldehyde,5-(hydroxymethyl) was the dominate compound represented 66.31 % of the total area of this extract followed by 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl amounting to 13.76%.

Table (3): The role of garlic and pepper soaks in water and mineral oil on the efficiency of Dipel 2X against the neonate of *Spodoptera littoralis*.

Treatments	LC ₅₀ in ppm	R. T.
Dipel 2X	67.37	1.00
Dipel 2X + pepper soak in water	23.92	2.82
Dipel 2X + pepper soak in oil	4.63	14.55
Dipel 2X + garlic soak in water	8.01	8.41
Dipel 2X + garlic soak in oil	4.95	13.61

R.T. =Relative Toxicity

Table (4): The role of garlic and pepper soaks in water and mineral oil on the efficiency of Protecto against the neonate of *Spodoptera littoralis*.

Treatments	LC ₅₀ in ppm	R. T.
Protecto	147.46	1.00
Protecto + pepper soak in water	28.66	5.15
Protecto + pepper soak in oil	15.62	9.44
Protecto + garlic soak in water	23.07	6.39
Protecto + garlic soak in oil	10.49	14.06

R.T. =Relative Toxicity

Table (5): Major compounds detected in the water hot pepper extract after GC / MS identification.

No.	Compounds	Rt min.	% Area	M.W.	Chemical Formula
1.	Dihydropyran	4.32	1.53	84.06	C ₆ H ₈ O
2.	Nanofin 2(3H)-furanone,5methyl	4.38	2.34	113.12	C ₇ H ₁₀ N
3.	11-Trimethylsilyloxy-1-undecene	4.88	2.14	242.21	C ₁₄ H ₃₀ OSi
4.	1,2,3,4-Butanetetrol,[S-(R*,R*)]-N-carbethoxy-N-methoxymethyl-amine]-Butanethiol	5.02	4.21	122.06	C ₈ H ₁₆ O ₄
5.	4-Octanone,2-methyl-Thiopivalic acid	5.23	9.72	142.14	C ₉ H ₁₈ O
6.	Oxirane, Phenyl	5.67	2.30	120.06	C ₇ H ₈ O
7.	2-methyl-3-Furanthiol	5.95	3.15	114.01	C ₇ H ₈ OS
8.	2,5-Dimethyl-4hydroxy-3(2H)-Furanone	6.21	4.92	128.05	C ₈ H ₁₀ O ₃
9.	1,2Bis(trimethylsilyl)benzene	6.32	12.10	222.13	C ₁₂ H ₂₂ Si ₂
10.	1-methyl-2-thioxo-4,5-dihydro-4-oxoimidazole	6.64	1.64	128.00	C ₄ H ₄ N ₂ OS
11.	Hexanal,2-ethyl-3Heptanone	6.80	1.99	128.12	C ₈ H ₁₆ O
12.	2-propanamine,N-methyl-N-nitroso-Ethanamine,N-ethyl-N-nitroso	7.50	1.97	102.08	C ₄ H ₁₀ N ₂ O
13.	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	7.61	29.64	144.04	C ₈ H ₈ O ₄
14.	Quinoline,6-ethyl-2-(Trimethylsilyl)thiazole	7.89	1.14	157.09	C ₁₁ H ₁₃ N
15.	Silanol,trimethyl	8.85	5.17	146.08	C ₈ H ₁₄ O ₂ Si
16.	Cyclohexa-2,5-diene-1,4-dione,2-methyl-5-(4-morpholinyl)	10.39	3.34	207.09	C ₁₁ H ₁₃ NO ₃
17.	2-Hexenoic acid,2-methyl-,methyl ester	11.82	2.04	142.10	C ₈ H ₁₄ O ₂
18.	Tricyclo[4.3.1.1.(3,8)]undecane,1-chloro-Phthalic acid,2-cyclo-hexylethyl 2-ethyl-hexyl ester	12.15	1.07	184.10	C ₁₁ H ₁₇ Cl
19.	1H-Pyrazole,3-ethyl-4,5-dihydro-1,4-dimethyl	14.03	2.93	126.12	C ₇ H ₁₀ N ₂
20.	Quinic acid; Cyclohexane carboxylic acid	15.21	1.46	192.06	C ₇ H ₁₂ O ₆
21.	Phenathrene	17.39	4.98	178.08	C ₁₄ H ₁₀
22.	(5-Ethylcyclopent-1-enyl) methanol	23.80	2.95	126.10	C ₈ H ₁₄ O

Table (6): Major compounds detected in the water garlic extract after GC / MS identification.

No.	Compounds	Rt min.	% Area	M.W.	Chemical Formula
1.	2(5H)-Furanone	4.28	0.26	84.02	C ₄ H ₄ O ₂
2.	Cyclohexanone	4.35	1.22	98.07	C ₆ H ₁₀ O
3.	Benzene[(2,2-dimethoxyethyl)sulfonyl]	4.99	1.22	230.06	C ₁₀ H ₁₄ O ₄ S
4.	4-Octanone,2-methyl-Thiopivalic acid	5.25	1.46	142.14	C ₉ H ₁₈ O
5.	2-Cyclopenten-1-one,2-hydroxy-3-methyl	5.71	1.17	112.05	C ₆ H ₈ O ₂
6.	Pyrimidine, 2-Chloro	6.01	0.81	114.00	C ₄ H ₃ ClN ₂
7.	2,5-Dimethyl-4-hydroxy-3(2H)-Furanone	6.31	3.27	128.05	C ₈ H ₁₀ O ₃
8.	2,5-Furandicarboxaldehyde	6.49	0.17	124.02	C ₆ H ₄ O ₃
9.	2,4(1H,3H)-pyrimidinedione,5-hydroxy	6.63	1.42	128.02	C ₄ H ₄ N ₂ O ₃
10.	1,2-Dibutoxyethane	6.81	0.51	174.16	C ₁₀ H ₂₂ O ₂
11.	3-Methoxybut-1-ene	6.97	0.47	86.07	C ₆ H ₁₀ O
12.	1,3-Butadiene,1-[(1-methylethyl)thio]	7.32	0.58	128.07	C ₇ H ₁₂ S
13.	2-Propanamine,N-methyl-N-nitroso-	7.53	0.80	102.08	C ₄ H ₁₀ N ₂ O
14.	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	7.71	13.76	144.04	C ₈ H ₈ O ₄
15.	4H-Pyran-4-one,3,5-dihydroxy-2-methyl-	8.36	0.53	142.03	C ₈ H ₈ O ₄
16.	5-Formyl-2-Furfuryl-methanoate	9.01	0.47	154.03	C ₈ H ₈ O ₄
17.	2-Furancarboxaldehyde,5-(hydroxymethyl)-	9.38	66.31	126.03	C ₆ H ₆ O ₃
18.	5-Acetoxy-methyl-2-Furaldehyde	10.35	0.26	168.04	C ₈ H ₈ O ₄
19.	D-Mannitol, 1,4-anhydro	13.20	0.68	164.07	C ₆ H ₁₂ O ₅
20.	Butanal, 2-methyl	13.69	5.95	86.07	C ₇ H ₁₀ O
21.	Phenanthrene	17.40	0.58	178.08	C ₁₄ H ₁₀

Mustafizur and Motoyama (2000) identified four major peaks resolved were sulfide compounds produced by the rapid degradation of alliin and cyclic compound produced by dehydration. It remains to be determined whether alliin itself, the degradation products, or the mixture of these are responsible for the repellent effect against two stored pests, the maize weevil and the red flour beetle.

George *et al* (2007), reported that crude extracts from pepper fruits can be explored for developing natural products for use as biodegradable alternatives to synthetic insecticides / acaricides. The significant of these findings with respect the toxicity enhancing effect the treated B.t. compounds are low priced, non toxic to human and animals which add to their feasibilities in application.

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التأثير المشترك بين بعض المستخلصات النباتية والمبيدات البكتيرية ضد دودة ورق القطن

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المعمل المركزي للمبيدات - مركز البحوث الزراعية - الدقى - جيزة - مصر

تم اختبار تأثير المركبات البكتيرية دايليل 2X ٦,٤% والبروتكتو ٩,٤% منفردا ومختلطا مع منقوع الثوم والفلفل الحار في كل من الماء أو الزيت المعدني ضد العمر اليرقى الأول لدودة ورق القطن.

ولقد وجد أن منقوع كل من الثوم والفلفل قد عمل على زيادة فعالية كل من الدليل 2X والبروتكتو ضد العمر اليرقى الأول لدودة ورق القطن.

فقد تمت زيادة فعالية الدايليل بمقدار ٢,٨٢ ، ١٤,٥٥ ، ٨,٤١ ، ١٣,٦١ ضعف عند استخدامه مختلطا بمنقوع الفلفل في الماء ومنقوع الفلفل في الزيت ، ومنقوع الثوم في الماء ومنقوع الثوم في الزيت على التوالي.

وكذلك بينت النتائج أن إضافة كل من منقوع الفلفل والثوم في الماء والزيت قد عملت على زيادة سمية المركب البكتيري بروتكتو حيث زادت نسبة السمية بمقدار ٥,١٥ ، ٩,٤٤ ، ٦,٣٩ ، ١٤,٠٦ ضعف عند خلطة بمنقوع الفلفل في الماء ثم منقوعة في الزيت ثم منقوع الثوم في الماء ثم منقوعه في الزيت على التوالي.

ولقد تم التعرف على ٢٢ مركب ضمن محتويات الفلفل الحار في الماء. وأوضحت النتائج أن مركب 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl والذي كانت قيمة الـ Rt له ٧,٦١ دقيقة كان يوجد بنسبه كبيره وصلت الى ٢٩,٦٤ % .

في حين تم التعرف على ٢١ مركب ضمن محتويات الثوم في الماء ووجد أن المركب 2-Furancarboxaldehyde,5-(hydroxymethyl) يوجد بنسبه كبيرة وصلت الى ٦٦,١٣ % من الكمية الكلية للمستخلص المائي للثوم.