Insecticidal and biochemical effects of tobacco dust extracts against the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.)

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Abstract: The Egyptian cotton leaf worm, *Spodoptera littoralis* is a key pest infesting several crops, vegetables and ornamentals. The current work was conducted to use tobacco dust as alternative pesticide to avoid pesticides problems such as environmental pollution, resistance development, secondary pest resurgence and the destructive effect on non-target organisms. Biological and biochemical effects of three different tobacco dust extracts (alcohol, petroleum ether and water extracts) on 2nd larval instar of *S. littoralis* were evaluated. Results indicated that alcoholic extract exhibited superior biological activity, followed by petroleum ether then aqueous extraction. In addition, the tested extracts proved latent effects via reduction of pupation, adult emergence rates and decreasing pupal weight. Biochemical analysis revealed that reduction in the total lipids, total protein and total carbohydrate content after treatment with the LC_{50} of each extract. The greatest decrease in these parameters obtained with alcoholic extract followed by petroleum ether and finally water extract. This depletion reflects the action of tobacco dust on the biochemical constituents in treated larvae.

Keywords: Tobacco dust extracts, *Spodoptera littoralis*, biological and biochemical effects.

1.INTRODUCTION

The Egyptian cotton leaf worm *Spodoptera littoralis* (Boisd.) is a dangerous destructive polyphagous insect pest harming about 112 plant species belonging to 44 families. In cotton fields, the pest affects mainly host leaves; moreover it can infest the squares, flowers and green bolls causing serious effect on plant growth and production **(Mokbel *et al.,* 2017; Ghoneim, *et al.,* 2017)**

The pest control accomplishes throughout the application of various pesticides classes. This situation led to negative effects on environmental pollution, non-target organisms (natural enemies and pollinators) and pest resurgence **(Jiang and Ma 2000)**. Therefore, attention was directed to develop an environmental friendly pest management agent such as organic pesticide which exterminate pest with less adverse effects on non-target organism. Plant Extract has a long history of use as biopesticides and in the same time can contribute in insect resistance development suppression **(Rozman *et.al,* 2007).** Economic plants belong to family Solanacae enriched by alkaloids. Many economically important Solanacae plants, e.g., potato, tomato and tobacco, are widely harvested. So, their unused organs can be used as a source of biological insecticides. Tobacco plant has a long and fascinating history as a medicine and an insecticide (**Schmeltz 1971**). Tobacco waste used to control several pests include Carambola friut fly, *Bactrocera carambolae*, Root-Knot-Nematodes (RKN), effectively used as molluscicide to control Golden Apple snail in paddy field, brackish water pond snails (*Cerithidea cingulate* Gmelin) ,mosquito larvae of *Anopheles stephensi* and *culex quinquefasciatus* and against tobacco cutworm, *Spodoptera litura*.. In addition, tobacco waste can be used as organic fertilizer (**Motha *et al.,* 2010; Chaturvedi *et al.,* 2007; Sassanarakkit 2009; Sharma *et al.,* 2011; Prabowo *et al.,* 2016).** Tobacco dust is a byproduct of tobacco industry, available, inexpensive and easily degraded (**Aleem 1988**).

The cvurrent study aim to investigate the insecticidal efficacy of different tobacco dust extracts; explore the effect of the tested treatments on certain biological parameters and their efficacy on certain nutrient component.

2.MATERIAL AND METHODS

2.1.Insects

The cotton leafworm, *S. littoralis* strain was obtained from (Cotton leaf worm Department - Plant Protection Research Institute). This strain was reared on castor bean leaves under constant conditions at 25± 2°C and 65±5% RH according the method described by (El-Defrawi, et al., 1964).

2.2. Preparation of Tobacco dust Extracts

Tobacco dust was brought from El-sharquia for smoke factories, in Giza. Tobacco dust was dried in Oven and pounded in mortar till it became powdery. Hundred and fifty grams (150g) of the powdery tobacco dust was weighed then added to 600 ml of alcohol, petroleum or water separately. They were soaking for 48 hour, shaken properly and filtered with cotton wetted with extracting solvent of each separately. Then the solvent was evaporated. The crude extracts were diluted in water (w/v) according to Turgut *et.al* 2004, and preparing different concentrations that were used on 2nd larval instar of *S. littoralis*.

2.3.Insecticidal and biological activity

Leaf dipping assay was used to evaluate the insecticidal activity of the testes extracts. Serial of concentrations ranged from 5 to 30% were prepared. Freshly molted 2nd instars larvae were used in this experiments. The leaf dipping technique was achieved by dipping freshly castor oil beans leaves in the tested concentration for 30 second, allowed to dry for approximately 15 minutes at room temperature and then used in larval assay. Four replicates contained 10 larvae / jar was used for each treatment. Leaves dipped in the used solvent served as control. The mortality percentages were recorded at three, five and seven days. To investigate the latent effect, the 2nd instars of *S. littoralis* were treated with LC_{50} of the tested extracts. Treated larvae were examined after 48hours then daily until adult emergence to determine mortality, pupation percentage, pupal weight and adult emergence.

2.4.Biochemical studies

Total body tissue samples from late 6-th larval instars treated as 2nd instars with LC_{50} values of the tested three extracts were used. Carbohydrates, lipids and proteins were determined by colorimetric methods and replicated 5 times as follow.

2.4.1.Carbohydrates

Carbohydrate content was determined colorimetrically according to (Morris 1948; Fairbairn 1953) throughout glucose standard method. Known weight of the previous samples (about 10 larvae) was homogenized in 5.0 ml of distilled water and the homogenate diluted to 50.0 ml. Subsequently, 2.0 ml samples of the homogenate were treated with 5.0 ml Dreywood's anthrone reagent.

2.4.2.Lipids

For lipids quantitative determination, known weight from the aforementioned sample was extracted with chloroformmethanol (2:1 v/v) in a Goldfisch fat extractor® (Model 1578, Laboratory Construction Co., Kansas City, USA) for 16 h. (Folch et al. 1951). Then the constant weight of the lipid-free carcasses was determined by drying them at 70±0.5°C for 48 h, and this weight was subtracted from the original weight of the insects. The difference (= lipid content) was expressed as a percentage of body weight. This method was used instead of weighing the exact amount of lipid extracted.

2.4.3.Protein

Protein determination was carried out with Folin -Ciocalteu reagent (Layne 1957). Known weight of 6th larval instars was homogenized in 5.0 ml of distilled water, and the protein was precipitated with 5.0 ml of 20% trichloroacetic acid. The precipitated protein and the crude body tissues (insoluble fraction of proteins, if any) were separated by centrifuging, and then redissolved by boiling for 30 min in 20.0 ml of N-NaOH solution. Thereafter, samples of different sizes from these alkaline protein solutions were analyzed, using bovine albumin as a standard according to the methods of Lowry et al. (1951) for soluble proteins.

2.5. Statistical analysis

Mortality was corrected by Abbott's formula, **Abbott (1925).** Then, data were subjected to probit analysis **Finney, (1971)** to obtain values of LC_{50} by using the software package EPA probit analysis version 1.5. Then, toxicity index was determined according to **Sun (1950).** Means of pupation percent, pupal weight, adult emergence percent and nutrition component were calculated by using SPSS version 19. Significance difference was accepted at $\alpha = 0.05$ in the LSD test.

3.RESULTS

3.1.Toxicity of different tobacco dust Extracts

Table (1) showed the effect of tobacco dust extracts against 2nd larval instars of *S. littoralis***.** Based on values of LC_{25}, $LC_{50}$and $LC_{90,}$ alcohol extract exhibited the highest efficacy followed by petroleum ether and finally water extract. In relation to slope values of the tested treatments, it ranged from 2.59 to 2.67. Slopes indicate the homogeneity of the tested strain in relation to certain substance. Slope values of the tested extracts were clearly similar and proved moderate homogeneity of treated larvae.

$$\text{*Toxicity index} = \frac{LC_{50} \text{ of the most toxic compound}}{LC_{50} \text{ of other compounds}} X100$$

Comparing the values of LC_{50} throughout toxicity index produced the relative toxicity of the treatments. Comparing the toxic action of the three extracts by using toxicity index revealed that: alcoholic extract was the standard followed by petroleum ether extract and finally Water extract with toxicity index values of 100, 83.688, and 69.832 respectively.

3.2.Effect on certain developmental stages

Data presented in **Table (2)**, showed that there was a remarkable decrease in % pupation as they were 36.6, 46.6 and 70 when using alcohol extract, petroleum ether and water extract, respectively comparing to 96.6 of control larvae. There is no significant effect on mean pupal weight of larvae with water extract while there is a slight decrease in mean pupal weight when petroleum ether extract was used. In addition, there was significant effect when alcohol extract was used compared with control larvae. Also, the percentage of adult emergence resulted from the larvae treated as 2nd larval instar with different extracts was presented in **Table (2).**

Data showed that percentage of adult emergence were highly reduced as % decrease recorded 61.58, 46.58 and 33.25% in alcohol extract, petroleum ether and water extract respectively on the cotton leaf worm *S. littoralis* as comparison with control.

3.3.Effect on certain biochemical parameters

Data represented in **Table (3)** showed that total carbohydrates content of *S. littoralis* was significantly decreased in all treatments. The most reduction was occurred by the treatments of alcohol extract the corresponding reduction was (8.3), while petroleum ether and water extract caused slightly decrease which being (11.73

Table1. Efficacy and toxicity index of different crude extracts of tobacco dust on the 2nd larval instars of *S. littoralis* (Boisd.) after 7 days.

Tobacco dust Extracts	Slope ± SE	LC_{25} (95%CL)	LC_{50} (95%CL)	LC_{90} (95%CL)	Toxicity Index*
alcohol extract	2.67 ±0.232	7.27(4.55-8.72)	13.01(9.83-16.19)	39.28(32.73-68.15)	100
petroleum ether	2.61 ±0.237	8.59(4.89-10.17)	15.55(11.39-20.81)	48.04(42.31-108.69)	83.68
Water extract	2.59±0.248	10.25(6.28-12.28)	18.63(14.40- 25.69)	58.01(50.82-142.92)	69.83

CL: Confidence limits
LC values calculated as % of crude extracts

Table2. Latent effect of tobacco dust extracts on the 2^{-end} instar larvae of *S. littoralis* (Boisd.) treated with LC_{50} for each extracts.

parameter	control	alcohol extract	petroleum ether	Water extract	LSD
Pupation %	96.6 ±0.33a	36.6±0.57b	46.6± 0.33b	70± 0.66c	7.45
Mean pupal weight (mg)	307.153±1.52a	274.816±1.20b	293.906± 1.20c	302.706±1.20d	1.83
Adult emergence %	96.58±0.33a	35±0.33b	50±0.33b	63.33±0.33c	0.047

*The mean difference is significant at the 0.05 level
*The same letters means no significant difference and the different letters means a significant difference

and 12.40), respectively. In addition, total protein significantly decreased in in alcohol extract as it was 17.86 while it was slightly decreased in petroleum ether and water extract as they were 20.56 and 21.33, respectively. Also, total lipid was slightly decreased in all extracts which being 5.11, 5.27 and 5.55 in alcohol, petroleum ether and water extract respectively in comparison with control.

4.DISCUSSION

Currently, Botanical insecticides considered ideal alternatives to hazardous synthetic pesticides. Moreover, it is reported that plant derivatives are large specific and non-toxic to non-target organisms **(Senthil Nathan 2006)**.a lot of plant products have been tested as insecticides. One potential indigenous bio pesticide is tobacco dust, a waste product of the tobacco and cigarette industry **(Borlongan *et al.,* 1998)**.Several bio-wastes have shown the potential not only to improve soil organic content but also to use as insecticide/ fungicide. Wastes of *Nicotiana*, *Jatropha*,*Azadirachta*, *Salvadora*, *Maduca* and *Pongamia* represent examples of plants which their commercial exploitation results in biowaste production (**Kulkarni *et al.,* 2007**).Tobacco waste contains mainly the active ingredient nicotine (**Konar, 1970**), and was used to control several pests.

In the present study, tobacco dust extracts exhibited toxic effect against *S. littoralis.* The alcohol extract proved the most efficiency followed by petroleum ether extract and finally water extract with LC_{50} values of 13.016, 15.553 and 18.639 respectively. The activity of tobacco dust extracts was agreed with the results of several previous researches. For instance, aqueous extract of tobacco leaf showed high toxicity against house fly larvae (**Ogbalu *et.al.* 2014**). Similar results were obtained with *Sitophilus zeamais* and *Sitophilus oryzea as* a result to treatment with tobacco leaves extract **(Kuhna *et al.,* 2014). Borlongan *et al.,* 1998** stated that lethal efficiency proportionally with a nicotine content of the tobacco dust. Also, tobacco dust exhibited significant efficacy against various pests includes the currant aphis and the apple red bug. **(Kuhna *et al.,* 2014)** and as molluscicide to combat the common brackish water gastropod, *Tympanotonus fuscatus* **(Aleem 1987)**. So, the effective application rate is directly proportional to the nicotine content of the used tobacco dust extract. Relative toxicity of the used extracts was accomplished by calculating toxicity index. Toxicity index is a mean for comparing the relative toxicity of compounds. The same trend was found with this parameter.

Similar to other insecticides, tobacco dust extracts treatments have negative impacts on the growth and developmental, processes at sublethal concentrations. In the current study, the 2^{-nd} instars larvae of *S. littoralis* treated with LC_{50} of the tested extracts. Pupation and adult emergence percent were decreased after exposure to sublethal concentrations of the tested extracts. Similarly, mean pupal weight (mg) decreased as a result of larval sublethal exposure.

Treating the cotton leaf worm, *S. littoralis* with LC_{50} of the tested extracts resulted in significant reduction in certain nutrient components. Total carbohydrates showed the highest reduction in all treatments followed by lipids and proteins. The depletion of carbohydrate may due to increase their utilization as response to hyper activity resulted from pesticide treatment to produce extra energy to combat insecticidal stress **(**Saleem *et al.,* 1998).Similarly, declined lipids interpreted as a result to lipid catabolism due to insecticidal stress. While, Protein decline may due to interference with protein synthesis regulation **(Sharma *et al.,* 2011)**.**Li *et al.,* 1995** stated that treatment of *Spodoptera litura* with azadirachtin lowered significantly protein expression. Also, **Rao and Subrahmanyam, 1986** found that azadirachtin treatment caused hormonal disturbance in protein synthsis in *Schistocerca gregaria*.

In conclusion, the present study proved the efficacy of tobacco dust extracts against *S. littoiralis* and confirmed the importance of extraction method. But there is great need for further studies on this available inexpensive and easily degraded material to enhance their use in pest control.

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Table3. Total lipid, protein and carbohydrate content of 6^{-th} instars of *S. littoralis* after the treatment` the 2^{-end} larval instars with LC_{50} of different tobacco dust extracts.

Content	Control	Alcohol extract	Petroleum ether	Water extract	LSD
Total lipid (mg/g.b.wt.)	5.7 ±0.266d	5.11±0.05a	5.27±0.135b	5.55±0.213c	0.019
Total protein (mg/g.b.wt.)	21.48±0.95b	17.86± 0.41a	20.56± 0.64b	21.33±1.21b	1.17
Total carbohydrates (mg/g.b.wt.)	14.33±0.41d	8.3±0.43a	11.73±0.46b	12.40±0.15c	0.56

*The mean difference is significant at the 0.05 level .
*The same letters means no significant difference in the same row .

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التأثيرات الإبادية والبيوكيميائية لمستخلصات غبار الدخان ضد دوده ورق القطن

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الملخص العربى:

دودة ورق القطن آفة رئيسية تصيب العديد من محاصيل الحقل،الخضر والزينة.ويهدف هذا البحث إلي بحث إمكانية إستخدام غبار التبغ(منتج ثانوي لصناعة التبغ) كبديل للمبيدات وذلك لتقليل استخدام المبيدات والاتجاه للبدائل من خلال استخدام المخلفات وتدويرها للاستفاده منها فى مكافحه الحشرات.وكذلك للتغلب علي مشاكل المبيدات التقليدية مثل التلوث البيئي،تطور المقاومة،إنبعاث الآفات الثانوية والتأثير الضار علي الكائنات الغير مستهدفة. وتم دراسة التأثيرات البيولوجيةوالبيوكيميائية لثلاثة مستخلصات لغبار التبغ وهي المستخلص الكحولي ومستخلص البترولیم إيثير والمستخلص المائي وأظهر المستخلص الكحولي أعلي تأثير إبادي ضد يرقات العمر الثاني متفوقاً علي باقي المستخلصات متبوعا بمستخلص البترولیم إيثير وأخيرا المستخلص المائي. كذلك أظهرت هذة المستخلصات تأثيرات بيولوجية متأخرة تمثلت في خفض أوزان العذاري،خفض نسبة التعذيروخفض نسبة خروج الحشرات الكاملة.وأظهر التحليل الكيميائي لمعرفة أثر هذة المعاملات علي نسبة الكربوهيدرات ،البروتين والليبيدات الكلية إنخفاض نسبة هذة المكونات في جسم الحشرات المعاملة بما يفسر حدوث التشوهات في الحشرات المعاملة بسبب دخول هذة المكونات في تركيب الكيتين.