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WATER EXTRACTS OF LEAVES OF SOME *Morus alba* L. VARIETIES AS A POSSIBLE BOTANICAL PESTICIDE AGAINST COTTON LEAF WORM, *Spodoptera littoralis* BOISD.

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ABSTRACT

First and fourth larval instars of a sensitive laboratory strain of the cotton leaf worm; *Spodoptera littoralis* Boisd. were reared on castor *Ricinus communis* L. leaves; treated with three concentrations (5.0, 10.0 and 15.0%) of water extracts prepared from three different varieties of mulberry leaves; *Morus alba* var *rosa*, *M. alba* var *morittiana* and *M. alba* var *kokuso* -27. Different biological aspects were determined to evaluate the insecticidal effect of the three mulberry varietyleaves. The *M. alba* var *rosa* was significantly more lethal to both larval instars of *S.littoralis* than the *M. alba* var *morittiana* and *M. alba* var *kokuso*-27 The fourth larval instar was more tolerant to the lethal effect of all mulberry leaf extracts than the first one. The higher the concentration of mulberry leaf extracts the greatest the lethal effect obtained with all treatments. Protein profile of haemolymph samples was monitored for the sixth larval instar of *S.littoralis* leaves by the 15.0% concentration of all mulberry varieties from the fourth larval instar. Electrophoretic protein analysis showed disappearance of some protein bands in haemolymph samples from treated larvae by *M. alba* var *rosa*.

***Key words**: botanical pesticide, Morus alba, Spodoptera littoratis*

1. INTRODUCTION

The Egyptian cotton leaf worm; *Spodoptera littoralis* Boisd is a polyphagous defoliate insect and one of the most destructive pests on vegetable and field crops. Resistance against many insecticide groups appeared in field strains as a result of extensive use of chemical control and this resistance is accumulative (Kamel and Moustafa, 1969; Zeid *et al.*, 1972; El-Sebae, 1977 and El-Sinary, 1987). The use of traditional agrochemicals for such pest control is undesirable because of the resistance problems and other harmful effects on beneficial insects, fish and wild life (El-Sinary, 1995). So, there is an urgent need for safe but effective, biodegradable pesticides with no toxic effects on non-target organisms. This has created a world wide interest in the development of alternative strategies including the search for new types of insecticides and re-evaluation and use of age-old traditional botanical pest control agents (Heyde *et al*., 1983; El-Sinary and Rizk , 2001).

The present study depends on the long history of mulberry trees defense against the herbivorous insects and lepidoptera larvae other than the silkworm (Konno *et al*., 2005; Rama Kant *et al*., 2005 and Shree and Mahadeva, 2005b). Three preparations from three varieties of mulberry leaves were examined as botanical pesticide against the first and fourth larval instars of the *M. alba* var *rosa* cotton leaf worm, *S.littoralis*.

2. MATERIALS AND METHODS

2.1. Insect preparation

Sensitive laboratory strain of cotton leaf worm, *S.littoralis* was obtained from the apiary yard of the Agricultural Experiment and Research Station, Faculty of Agriculture, Cairo University, Giza and reared at 25-32^{o}C and relative humidity of

60-70%. Egg masses were collected daily and placed in a clean glass jar of 250 ml capacity. The hatched larvae were provided with fresh and clean castor leaves, *Ricinus communis* L.

2.2. Mulberry leaf preparations

Three different Mulberry varieties; *M*orus *alba* var. *morittiana*; *M.alba* var. *kokuso -27* and *M.alba* var. *rosa* were obtained from the Faculty of Agriculture, Cairo University, Giza. Samples were collected from fresh mature leaves of uniform size and free from physiological defects and insect infestation in April 2007.

Leaves were cleaned with tap water , dried with current air till dryness then minced in electric machine to be powder. Three concentrations of mulberry / water extract were prepared; 5.0, 10.0 and 15.0% (leaves powder (gm) / water (ml)). Castor leaves were treated by spraying filtrated mulberry /water extracts and left till dryness then offered to *S.littoralis* larvae for feeding.

2.3. Developmental stages of the cotton leaf worm treatments

Newly hatched larvae (the first larval instar) and the fourth larval one of *S.littoralis* were fed on castor leaves treated with the three concentrations (5.0, 10.0, 15.0%) of the three different varieties of mulberry leaf extract two *M.alba* var. *rosa*; *M. alba* var. *morittiana* and *M.alba* var. *kokuso -27* . Extracts of different mulberry were sprayed on castor leaves till dryness then offered to the larvae. Biological aspects; survived larvae, matured larvae, pupation ratio and adult emergence percentage were determined. Pupal weight was also calculated. All treatments were triplicated in a completely randomized design.

2.4. Electrophoretic separation of haemolymph

Haemolymph samples were extracted from six larval instars of *S.littoralis* fed with castor leaves treated with 15.0% conc. of the mulberry varieties from the fourth instar. Samples were extracted with 0.03 M Tris-HCI (PH 7.2) buffer and untreated ones at room temperature to yield whole buffer extrat as described by Iwabuchi and Yamauchi (1987), then centrifuged at 10000 g f a 15 min at 15^{o}C. Soluble protein was estimated using the method of comassie Brillent Blue. G-250 according to Bradford (1976). SDS PAGE electrophoresis was carried out according to the method of Laemmli (1970).

2.5. Statistical analysis

The obtained data were statistically analyzed by the least significant differences, whenever, the calculated "F" values were significant at 5% and 1% level (Snedecor and Cochran, 1980).

3. RESULTS AND DISCUSSION

Table (1) shows the insecticidal effect of the three varieties of mulberry leaves used in the three different concentrations of leaves / water extract. The first larval instar of the cotton leaf worm showed highly sensitive reaction for the *M. alba* var *rosa* leaf extract in all concentrations compared to *M.alba* var *morittiana* and *M.alba* var *kokuso*-27, respectively. Data revealed less survived larvae, matured larvae, pupation and adult emergence percentage also less pupal weight (mg) in all treatments with the *M.alba* var *rosa*. The higher the concentration of mulberry extracts the more lethal effect on the *S.littoralis* first instar larvae. The concentration of 15.0% of *M. alba* var *rosa* leaf extract had a completely lethal influence for *S.littoralis* larvae followed by 10.0% conc. which scored 18.67 , 14.00, 9.33 , 6.00% as survived larvae, matured larvae, pupation and adult emergence percentage comparing with 96.00, 93.33 , 92.00, 90.67% with the untreated control, respectively. The lowest pupal weight was also determined being 204.75 mg compared to 290.73 mg. in untreated control. The 5.0% concentration of *M.alba* var *rosa* leaf extract monitored 25.33, 20.00, 18.67 and 13.33% survived larvae, matured larvae, pupation and adult emergence percentage and 221.56 mg for pupal weight. *M. alba* var *morittiana* has an intermediate lethal effect on the first larval instar of *S.littoralis*. The concentration of 15.0% gave better results than 5.0 % and 10.0% concentrations. Percentages of survived larvae, matured larvae pupation and adult emergence were 74.67, 60.67, 47.33 and 35.33, respectively. Pupal weight average was 243.88 mg. *M. alba* var *kokuso*-27, in all extract concentrations, did not give promising results. The obtained results with 5.0 and 10.0% concentrations

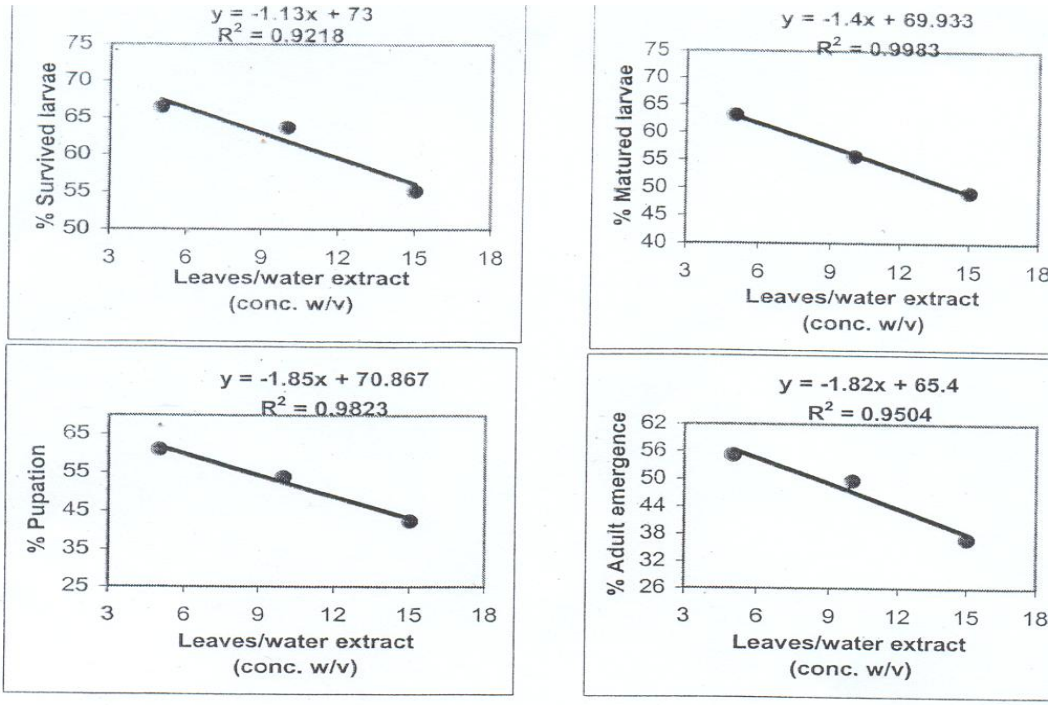


Fig. 1: Coefficient of determination and linear regressions between leaves/water extract concentrations and the biological aspects of the first larval inistar.

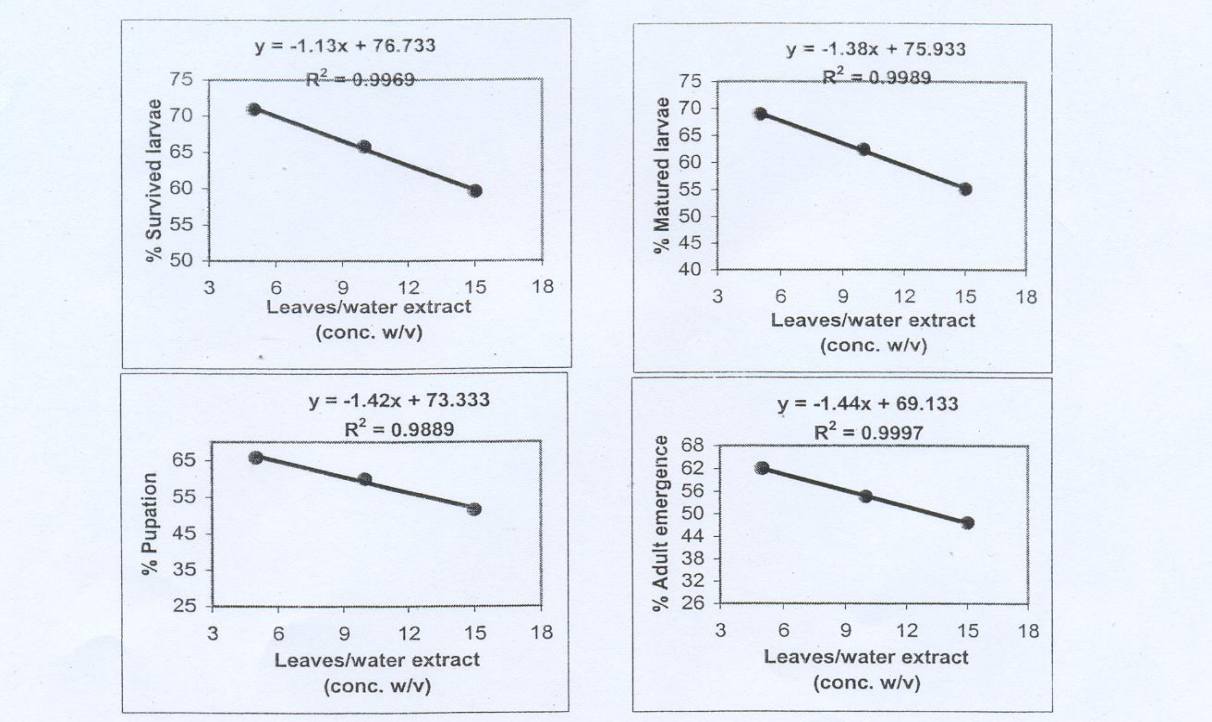
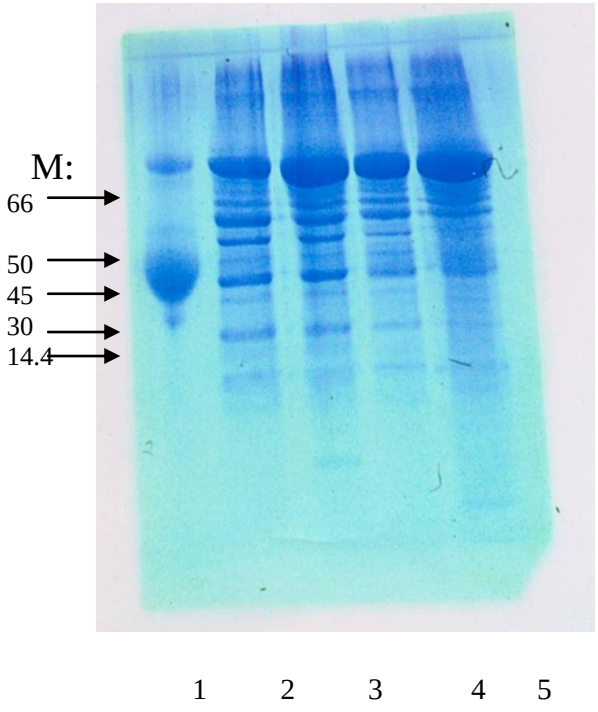


Fig. 2: Coefficient of determination and linear regressions between leaves/water extract concentrations and the biological aspects of the fourth larval inistar.

of *M. alba* var *kokuso* -27 were not significantly different in comparison with the untreated control. Table (2) shows the insecticidal effect of the three different mulberry varieties, on the fourth larval instars of *S.littoralis* treated with the three concentrations. In general, the fourth larval instar was more tolerant than the first larval one. *M. alba* var *rosa* leaf extract of all concentrations scored the highest lethal effect against the larvae of *S.littoralis* than the two other varieties. Survived larvae, matured larvae, pupation and adult emergence percentage were 8.67, 6.67, 6.0 and 0.0 with 15.0% concentration of *M.alba* var *rosa* leaves extract, comparing with 97.33, 95.33, 94.0 and 93.33 with the untreated control, respectively and pupal weight; was 226.0 compared with 301.15 mg in the untreated control. The same concentration with the *M. alba* var *morittiana* leaf extract resulted in 77.33, 68.67, 60.0 and 58.0% of survived larvae, matured larvae matured larvae, pupation and adult emergence percentage and pupal weight 266.59. The obtained results revealed that, in the first larval instar, the *M. alba* var *kokuso*-27 gave less lethal effect with no significant differece with the untreated control. In all treatments, there were positive correlations between the concentration of mulberry leaf extracts and their insecticidal effect against both the first and fourth larval instars. This was expressed by the coefficient of determination values (ranged between 0.9218 and 0.9989) and authenticated by the linear regressions illustrated in Figures (1) and (2). The differences in the insecticidal effect between the three varieties of mulberry leaves may be due to the variations in components between mulberry varieties. The chemical analysis proved that the highest values of soluble sugars, crude proteins, high moisture were detected with the *M. alba* var *kokuso* -27 leaves; *Morus alba* var *kokuso* -27 as mentional by Mahmoud (2002) and Ashour (2005 a &b). The reduction in pupal weight was highly significant as compared with the untreated control in *M. alba* var *rosa* and *M. alba* var *morittiana*. This reduction in pupal weight was in harmony with the findings obtained by Schmidt *et al.* (1998); Amr (2001), Bandyopadhyay *et al.* (2001), Cotter and Wilson (2002), Dutta *et al.* (2005) and Amin *et al.* (2007). These authors stated that the treatment of insects with strange extracts inhibited the nutrients uptake in the insect midgut and the extracts may bind with the carbohydrate part of the brush border membrane vesicle receptor protein and that may decrease the permeability of the membranes and lead to weight reduction and lethal abnormalities. Roman (2004) treated larvae of *S.littoralis* with unfavorite plant extract; *Tagetes erecta* and discovered that it caused prolongation of larval retarded growth and caused reduction in both larval and pupal weights and may lead to 100% mortality.

Figure (3) shows the electrophoretic separation of protein subunits in tricoglycine buffer pH 8.3. Samples were taken from the haemolymph of the sixth larval instar which were fed on castor leaves treated with the heighest concentration of the three different mulberry leaves; 15.0%.



1: Marker 2: Control 3: *M. alba* var *kokuso* 4: *M. alba* var *morittiana* 5: *M. alba* var *rosa* M= Molecular weight protein markers, 66.0 50.0, 45.0, 30.0 and 14.4 K Da.

It was noticeable that *M. alba* var *kokuso*-27 samples were similar to the untreated control samples followed by *M. alba* var *morittiana* samples. There were reductions in many protein bands in haemolymph samples which were taken

from the larvae reared on *M. alba* var *rosa* leaves extract. Protein content as mg/ml haemolymph was estimated; the least level was recorded with haemolymph samples taken from larvae fed on *M. alba* var *ros a* leaves (47.4) followed by *M. alba* var *morittiana* sample (49.6) and *M. alba* var *kokuso*-27 sample (51.0) compared with 52.0 for the untreated control. Molecular weight protein markers scored 66.0, 50.0, 45.0, 30.0 and 14.4 KDa, for the respective samples. These results are in conformity with the findings recorded by many authors when they used mulberry leaves varieties in rearing different insects and registered changes in the biochemical constituemts in various pests haemolymph, Shree and Umesh Kumar (1989) with meal bugs; Satya *et al*., (2002) with thrips; Narayanaswamy (2003) with leaf rollers; Shree and Mahadeva (2005 a&b) with jassids and concluded that there were alteration in the free amino acids, total soluble protein, total reducing sugars, total soluble sugars, starch and phenols. Other investigations were carried out by Aly and Haiba (1997), El-Degwi *et al*. (2001), who stated that when the insect was treated with unpreferable

Table (1): Insecticidal activity of the three different mulberry leaf extracts on the first larval instar of cotton leaf worm, *S.littoralis*.

	Survived larvae (%)	Matured larvae (%)	Pupation (%)	Pupal weight (mg)	Adult emergence (%)
Control, 0	96.00	93.33	92.00	290.73	90.67
M. alba var *rosa*					
5.0	25.33	20.00	18.67	221.56	13.33
10.0	18.67	14.00	9.33	204.75	6.00
15.0	0.00	-	-	-	-
M. alba var *morittiana*					
5.0	80.00	76.67	72.67	259.88	64.67
10.0	78.67	70.67	62.00	259.32	56.67
15.0	74.67	60.67	47.33	243.88	35.33
M. alba var *kokuso* -27					
5.0	94.00	92.67	91.33	288.85	87.33
10.0	93.33	92.00	90.00	282.76	86.00
15.0	90.67	86.67	80.00	266.63	75.33
L.S.D.					
0.05	13.4	12.0	13.02	6.2	10.5
0.01	18.7	17.2	18.5	7.8	16.6

Table (2): Insecticidal activity of the three different mulberry leaf extracts on the fourth larval instar of cotton leaf worm, *S.littoralis*.

	Survived larvae (%)	Matured larvae (%)	Pupation (%)	Pupal weight (mg)	Adult Emergence (%)
Control					
0	97.33	95.33	94.00	301.15	93.33
M. alba var *rosa*					
5.0	33.33	30.00	26.67	233.61	24.00
10.0	20.67	19.33	15.33	235.00	9.33
15.0	8.67	6.67	6.00	226.00	0.00
M. alba var *morittiana*					
5.0	84.00	82.67	77.33	270.21	70.67
10.0	82.67	75.33	73.33	261.33	65.33
15.0	77.33	68.67	60.00	266.59	58.00
M. alba var *kokuso* -27					
5.0	95.33	94.00	93.33	300.07	91.33
10.0	94.00	92.67	91.33	293.00	89.33
15.0	92.67	90.00	88.67	288.72	84.67
L.S.D.					
0.05	12.7	12.4	14.00	5.8	11.1
0.01	18.0	17.9	18.3	8.3	17.1

media such as *Phthorimaea operculella* with less toxic insecticides and the plant extract of *Artimesia absinthium* and *Callosobruchus maculatus* with pepper powder, they detected variable protein bands and found that the number of bands was reduced after larval treatment.

From the obtained results, it is strongly recommended to use mulberry leaf water extracts (especially the *M. alba* var *rosa*) successfully as a botanical pesticide for cotton leaf worm control programmes because of its high lethal effect, safe and has no deleterious effects on the environment or human health, easy to prepare, has no risk during usage, available from the surrounding nature and cheaper than other pesticides.

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المستخلص المائي لأوراق بعض أصناف التوت كمبيد نباتي ضد دودة ورق القطن

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ملخص

تم تربية يرقات عمر يوم و أربعة أيام لسلالة معملية حساسة لدودة ورق القطن على ورق الخروع المعامل بالمستخلص المائي لثلاثة أصناف من أوراق التوت (البلدي – الرومي – الياباني) بتركيزات ثلاث (5.0 ، 10.0 ، 15.0%). تم تقدير عدة قياسات بيولوجية لدراسة مدي سمية أوراق التوت للأصناف الثلاثة المختلفة. كان الصنف البلدي فى كل المعاملات هو الأكثر سمية لكلا العمرين ليرقات دودة ورق القطن يليه الرومي ثم اليابانى بفارق معنوي كبير.

كان بصفة عامة العمر اليرقي الرابع ليرقات دودة ورق القطن أكثر مقاومة لفعل المستخلص المائي لنبات التوت للأصناف الثلاثة مقارنة العمر اليرقي الأول . كلما زاد تركيز المستخلص كلما زاد تأثيره السام على اليرقات بالنسبة للعمرين الأول والرابع على السواء فى كل المعاملات. أثبت التحليل البروتيني لدم يرقات العمر السادس والتي تمت تغذيتها من العمر الرابع على المستخلص المائي تركيز 15.0% للأصناف الثلاثة لأوراق التوت حدوث اختفاء لبعض أشرطة البروتينين ، فى المعاملة بمستخلص أوراق التوت البلدي يليه الرومي ثم الياباني والذي كان متشابهاً للعينة غير المعاملة مما يثبت سمية التوت البلدي عن الأصناف الأخرى وإحداثه خللاً فى التركيب البروتيني فى اليرقات التي تتغذي عليه.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (59) العدد الثانى (ابريل 2008):148- 155.