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Insecticidal Effects of Two Plant Extracts of (*Bidens pilosa* and *Rumex dentatus*) and Neem Oil Against Certain Stored Grains Insects.

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ABSTRACT

The insecticidal activities of methanolic extracts of two weedy plants leaves *Bidens pilosa* L. and *Rumex dentatus* L. at three different concentrations 3, 5 and 7% were tested against three stored grain pests, Rice weevil *Sitophilus oryzae* L. Saw-toothed grain beetle *Oryzaephilus surinamensis* L., and Bean weevil *Acanthoscelides obtectus* Say, compared to Neem oil as a commercial product at the same concentrations for exposure periods 24, 48, 72 and 96 hrs of treatment under laboratory conditions. Results revealed that different concentrations and exposure periods of the two extracts and Neem oil had significant lethal effects against these tested grain pests. In general, *R. dentatus* extract was found to be the most toxic against the tested grain pests and caused the highest mortality (100.00%) for the three pests *S. oryzae*, *O. surinamensis* and *A. obtectus* when applied at its highest concentration (7%) for an exposure time of 96 hrs (the highest time), followed by Neem oil 90.0±10.0, 70.00±13.3 and 90.00±2.8%, then *B. pilosa* extract 75.83±5.8, 79.33±5.46 and 87.50±7.2%, respectively at the same concentration and exposure time, indicating that the obtained mortality percentages were concentration and exposure time-dependent. Finally, these results demonstrate that, *R. dentatus* extract can be used as a promising pesticide against the three stored products insect pests investigated under this study, and strongly recommended to be used as stored grain protectants in the future.

INTRODUCTION

The group of insect species associated with postharvest products is commonly called stored product pests. Approximately 1,660 insect species or more related to about 120 families have been investigated in stored products during storage, processing, transportation, and marketing (Hagstrum and Subramanyam 2009). The main damage and product loss of these stored pests are caused by feeding and contamination by insects themselves or their cast skins and immature stages of the pupa. They can cause reductions in weight, quality, commercial value and seed viability. These insects can cause losses of 9-10 % in developing countries while, the losses can be more than 50 % in undeveloped countries (Pimentel, 1991) and (Wolpert, 1967). The majority of these pests are

coleopterans and the most damaging species of storage insects belong to the genera *Sitophilus*, *Oryzaephilus*, *Acanthoscelides* and *Tribolium* (Marsans, 1987, Khan and Selman, 1988, Vinuela *et al*., 1993, Pinto *et al*., 1997, Mowery, *et al*., 2002 and Beckel, *et al*., 2007). Among these pests, rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae), saw-toothed grain beetle *Oryzaephilus surinamensis* Linnaeus (Coleoptera: Silvanidae) and the bean weevil *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) are three important store product pests that destruct stored grain pests resulting in quantitative and qualitative damage. Massive and repeated use of chemical pesticides and fumigants for controlling stored products has led to many problems including insect resistance, disrupted natural biological control systems, toxic residues in food grains, undesirable effects on non-target organisms and environmental pollution. For this reason, effective, safe and eco-friendly alternatives to protect stored grain products and decreasing chemical pesticides use are necessary. In recent years, there is a growing interest in the use of plant extracts as alternatives to chemical pesticides in integrated pest management (IPM) (Baba Tierto, 1994 and Golob *et al*., 1999). Botanicals and/or their constituents demonstrated efficacy against a range of stored product pests. The Hairy Beggar ticks, *Bidens pilosa* (Linn.) is a cosmopolitan weed, belonging to the family Asteraceae, originating from South America and is common in all tropical and subtropical areas of the world climates (Geissberger and Séquin, 1991; Alvarez *et al*., 1999). *B. pilosa* plant is reported as a noxious weed to crops in Egypt and over 40 countries that cause high reduction of different crop yields. (Holm., *et al*., 1977 and Boulos, 2002). In addition, *B. pilosa* has a long tradition as folk medicine worldwide to treat various ailments and it is known to possess insecticidal properties against tea pests (Mamun and Ahmed, 2011), and to control the bruchid pests (*A. obtectus* and *Zabrotes subfasciatus*) of common beans in storage (Renuka *et al*., 2014). The toothed dock, *Rumex dentatus* L. is a plant of the Polygonaceae family that is widely distributed in many countries including Egypt and possesses antifungal, antibacterial, insecticidal and allelopathic activities (Hussain *et al*., 1997;2010 and Umer *et al*., 2010).

To date, numerous studies have documented the effectiveness of plant extracts against insect pests Nevertheless, few studies have evaluated the bio-efficacy of *R. dentatus* and *B. pilosa* against the stored products pests therefore, the present study was carried out to investigate the insecticidal activities of the two plant weeds extracts, compared with Neem oil as a commercial product, against the three stored-product insect pests, *A. obtectus (Say), S. oryzae L.* and *O. surinamensis* L.

MATERIALS AND METHODS

1. Plants Materials Collection:

Naturally growing populations of *B. pilosa* and *R.dentatus* plants were identified and individually collected after growing season from the different arable zones of fields located in Giza Research Station (GRS) that affiliated to Agricultural Research Center (ARC), Giza, Egypt.

2. Preparation of Botanical Leave Extracts:

The freshly collected leaves of the two plant species were cleaned by tap running water then washed with distilled water and placed on paper for semi-drying and finally, hung vertically for complete air drying for 4 weeks in shade at room temperature (25°C). The dried plant materials were then pulverized into a coarse powder and sieved by 40 meshes to give equal particle size. The powdered leaves were successively extracted with methanol (98%) at room temperature for 48 hrs. The extracts were concentrated under low pressure using a rotary evaporator. The crude extracts were weighed and stored in the refrigerator. For comparing results, a commercial product of Neem oil (Nimbecidine that

contains 0.03% azadirachtin as an active ingredient and produced by Al Ahram Mining Company, Giza, Egypt), was used as a reference. Finally, three concentrations (3, 5 and 7%) of the two plant extracts and Neem oil were prepared in methanol for further investigation.

3. Insects Culture:

The culture of the three tested insect species was obtained from a stock colony maintained at the Stored Grain and Product insect laboratory, Economic entomology Department, Faculty of Agriculture, Cairo University. Adults of *A. obtectus* were reared on dry beans and maintained in glass bottles. Newly laid eggs were transferred onto fresh kidney beans to develop into subsequent stages until they reach the adult stage. Adults of *S. oryzae* were reared on whole wheat in continuous darkness while *O. surinamensis* were reared on oat seeds. All species were kept in a dark incubator under laboratory conditions (27± 1°C and 65 ± 5% RH).

4-Bioassays:

Laboratory bioassays were conducted to evaluate the toxicity of methanol extracts of (*R. dentate* and *B. Pilosa*) and Neem oil against the tested species. Different concentrations (3,5, 7%) of the two extracts and Neem oil were applied to a Petri dish of 9cm diameter. Two ml of each concentration was introduced into Petri dish then left for about 5-10 min until evaporation and dryness at room temperature. For each concentration, three replicates were prepared and negative control was prepared using methanol alone. For each prepared replicate and control dish, 10 newly emerged adults of each tested species were introduced into treated and control petri dishes. Mortality of the adult insects has recorded after 24, 48, 72 and 96 hrs.

Statistical Analysis:

All the data concerning mortality were corrected by using Abbott's formula (Abbott, 1925). Tests for insecticidal activity were performed in triplicate and the data presented are mean ± SE. The mean values were compared by one-way ANOVA and Tukey's multiple comparison tests using software SPSS, version 11.5.

RESULTS AND DISCUSSION

The insecticidal effects resulted from *R. dentatus* extract, *B. pilosa* extract and Neem oil, applied at different concentrations and exposure periods, on the adults of *O. surinamensis, S. oryzae* and *A. obtectus* are given in Tables (1,2 &3) and Figs. (1, 2 and 3).

Insecticidal Effect of Plant Essential Oils Against *O. surinmensis*:

The obtained data of the mean mortality of *R. dentatus* extract, *B. pilosa* extract and Neem oil against *O. surinmensis* adult showed in Table 1 and Figure 1 revealed that *R. dentatus* was the most effective that exhibit maximum mortality (100.00%) with its highest concentration (7%) after 96hrs of exposure; while *B. pilosa* and Neem oil-induced their highest mortality of 79.33±5.46 and 70.00±13.3% respectively with the same concentration and exposure time. On the other hand, The mortalities of *B. pilosa, R. dentatus* and Neem oil after 24hr of exposure, (The lowest exposure time), were 28.0±1.10, 40.83±5.83 and 30.0±7.63%, respectively, which then significantly increased to 79.33±5.46, 100.00, and 70.00±13.3% after 96hr (The highest exposure time) when the highest concentration (7%) was applied.

Insecticidal Effect of Plant Essential Oils Against *S. oryzae*:

Insecticidal efficiencies of all tested extracts of *R. dentatus, B. pilosa,* and Neem oil were found significant against *S. oryzae* adults (Table 2 and Fig. 2). The highest mean mortality (100.00%) was exhibited by *R. dentatus* followed by Neem oil (90.0±10.0%) and *B. pilosa* (75.83±5.8%) with the highest concentration (7%) at 96hrs of exposure. Exposure

of *S. oryzae* to *B. pilosa*, *R. dentatus* and Neem oil at their highest concentration (7%) and exposure period of 24 hrs caused mortality of 29.16±4.1, 64.16±5.83and 56.67±8.3%, respectively, while the highest mortalities 75.83±5.8, 100.00 and 90.0±10.0% were recorded at the same concentration but, after 96hrs, respectively.

Table 1: Corrected mortality of different concentrations of *Bidens pilosa* and *Rumex dentatus* extracts and Neem oil against *Oryzaephilus surinamensis* (L.) under different time intervals (24, 48 ,72 and 96 hours).

CONC. (%)	*Bidens pilosa*				*Rumex dentatus*				Neem oil			
	Corrected mortality ±SE (%)											
	Exposure Time (hrs.)											
	24	48	72	96	24	48	72	96	24	48	72	96
3	10.00± 0.00^{a}	10.00± 0.00^{a}	33.30± 3.30^{a}	65.00± 2.04^{a}	5.25± 0.47^{a}	22.50± 4.33^{a}	26.25± 3.75^{a}	51.2± 3.75^{a}	3.50± 0.28^{a}	20.00± 10.00^{a}	32.50± 4.78^{a}	57.50± 6.29^{a}
5	16.67± 0.00^{b}	41.67± 1.70^{b}	41.67± 1.70^{a}	76.67± 3.33^{ab}	38.3± 1.67^{b}	38.30± 1.60^{a}	46.66± 6.66^{b}	58.3± 0.33^{a}	15.0± 0.00^{ab}	21.25± 2.39^{a}	65.0± 15.00^{b}	65.00± 15.00^{b}
7	28.0± 1.15^{c}	28.0± 1.10^{c}	70.00± 5.00^{b}	79.33± 5.46^{b}	40.83± 5.83^{b}	64.16± 8.20^{b}	81.66± 5.83^{c}	100.0± 0.00^{b}	30.0± 7.63^{b}	50.0± 10.00^{b}	70.00± 13.33^{b}	70.00± 13.30^{b}
F	208.93	320.43	24.64	4.78	37.63	14.10	34.31	77.60	10.29	5.88	8.65	2.26
P. value	0.000	0.000	0.001	0.057	0.000	0.005	0.001	0.000	0.012	0.039	0.017	0.180

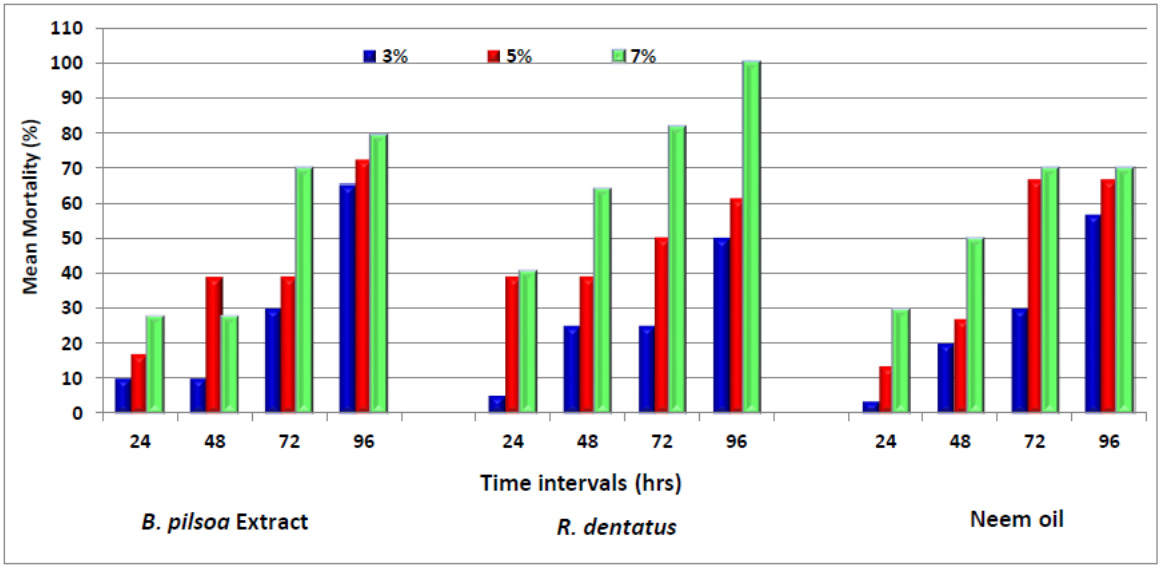

Fig. 1. Mean Mortality of *O. surinamensis* against different concentrations of *B. pilsoa, R. dentatus* and Neem oil under different time intervals (24,48, 72 and 96Hours).

Table 2: Effect of different concentrations of *Bidens pilosa extract, Rumex dentatus* extract and Neem oil on percentage mortality *of S. oryzae* under different time intervals (24,48 ,72and 96 hours).

CONC. (%)	*Bidens pilosa*				*Rumex dentatus*				*Neem oil*			
	Corrected mortality ±SE (%)											
	Exposure Time (hrs)											
	24	48	72	96	24	48	72	96	24	48	72	96
3	10.00± 0.00^{a}	15.00± 0.00^{a}	32.50± 4.70^{a}	67.50± 14.30^{a}	25.0± 8.30^{a}	43.16± 6.83^{a}	71.25± 7.18^{a}	92.50± 4.33^{a}	11.00± 0.30^{a}	25.08± 3.03^{a}	30.08± 4.15^{a}	49.17± 7.46^{a}
5	20.16± 5.10^{ab}	20.16± 5.10^{ab}	46.67± 10.00^{b}	66.67± 13.30^{a}	26.25± 3.75^{a}	52.5± 4.30^{a}	100.00± 0.00^{b}	100.00± 0.00^{a}	29.17± 4.16^{b}	70.00± 10.00^{b}	70.30± 6.60^{b}	70.00± 10.00^{ab}
7	29.16± 4.10^{b}	29.16± 4.10^{b}	52.50± 8.60^{b}	75.83± 5.80^{a}	64.16± 5.83^{b}	70.00± 0.00^{b}	100.00± 0.00^{b}	100.0± 0.00^{a}	56.67± 8.30^{c}	85.00± 2.88^{b}	90.00± 0.00^{c}	90.00± 10.00^{b}
F	12.107	6.577	2.382	0.148	7.239	9.100	8.906	1.667	23.711	56.036	67.274	5.871
P. value	0.008	0.031	0.173	0.866	0.003	0.015	0.016	0.266	0.001	0.000	0.000	0.390

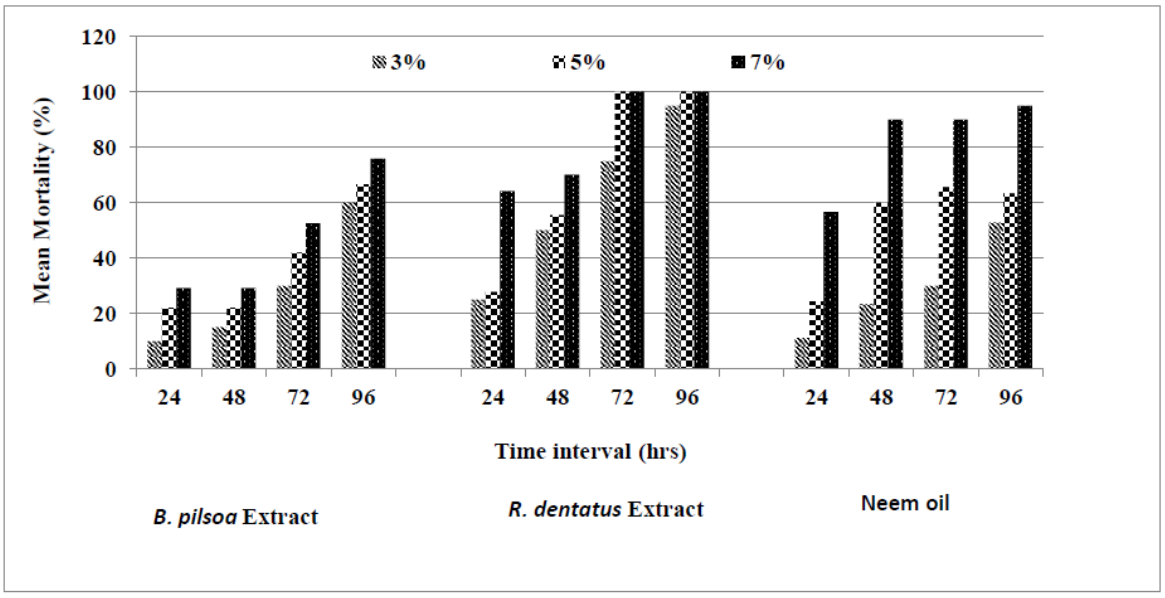

Fig. 2: Mean Mortality of *S. oryzae* against different concentrations of *B. pilsoa*, *R. dentatus* and Neem oil under different time intervals (24,48, 72 and 96Hours).

Insecticidal Effect of Plant Essential Oils Against *A. obtectus*:

As shown in Table3 and Figure 3, it is obvious that the tested two plant extracts and Neem oil proved to be effective in inducing mortality for *A. obtectus* at the highest concentration (7%) after 96hrs. Regarding lethal effects, *R. dentatus* gave maximum mortality (100.00%), while Neem oil and *B. pilosa* gave approximate toxic effect values of (90.0±2.80%) and (87.50±7.20%) respectively against *A. obtectus*. *R. dentatus* leave extract gave the maximum mortality ranging from 40.8±5.80% after 24hr to 100.00% after 96 hrs with the highest concentration (7%). While maximum mortality of Neem oil was 30.0±5.70 at 24 hrs then increased to 90.0±2.80 % at 96hrs with the same concentration. *B. pilosa* had a weak lethal effect (23.3±1.60%) after 24hr but with the increase of exposure time to 96hrs the lethal effect increased to (87.50±7.20%). In conclusion, the two plant extracts and Neem oil showed maximum mortality against *A. obtectus* in the following ascending order of preference *R. dentatus* > Neem oil > *B. pilosa*.

Table 3: Mean Mortality of *Acanthoscelides obtectus* Say against different concentrations of *Bidens pilosa* extract , *Rumex dentatus* extract and Neem oil under different time intervals (24,48 ,72and 96 hours)

CONC. (%)	*Bidens pilosa*				*Rumex dentatus*				*Neem oil*			
	Corrected mortality ±SE (%)											
	Exposure Time (hrs)											
	24	48	72	96	24	48	72	96	24	48	72	96
3	10.00± 0.00^{a}	20.00± 2.00^{a}	33.70± 3.70^{a}	45.00± 4.00^{a}	18.75± 3.75^{a}	41.25± 3.70^{a}	55.60± 10.60^{a}	59.37± 10.00^{a}	0.00± 0.00^{a}	15.050± 0.0^{a}	23.75± 5.50^{a}	21.25± 3.70^{a}
5	11.30± 0.60^{a}	22.50± 2.80^{a}	41.60± 3.30^{a}	61.60± 11.60^{a}	26.50± 3.80^{ab}	52.50± 6.10^{b}	61.60± 0.00^{ab}	75.00± 0.00^{ab}	7.50± 2.50^{a}	27.5± 7.50^{ab}	50.0± 10.00^{b}	70.00± 10.00^{b}
7	23.30± 1.60^{b}	23.30± 1.60^{a}	64.10± 5.80^{b}	87.50± 7.20^{b}	40.80 ±5.80^{b}	55.30± 5.30^{b}	93.30± 3.63^{b}	100.0± 0.00^{b}	30.0± 5.70^{b}	50.0± 11.50^{b}	50.0± 11.50^{b}	90.00± 2.80^{c}
F	57.181	0.756	12.351	11.852	6.268	2.154	5.433	6.328	22.412	5.220	3.356	70.075
P. value	0.000	0.510	0.007	0.008	0.034	0.197	0.045	0.033	0.002	0.049	0.105	0.000

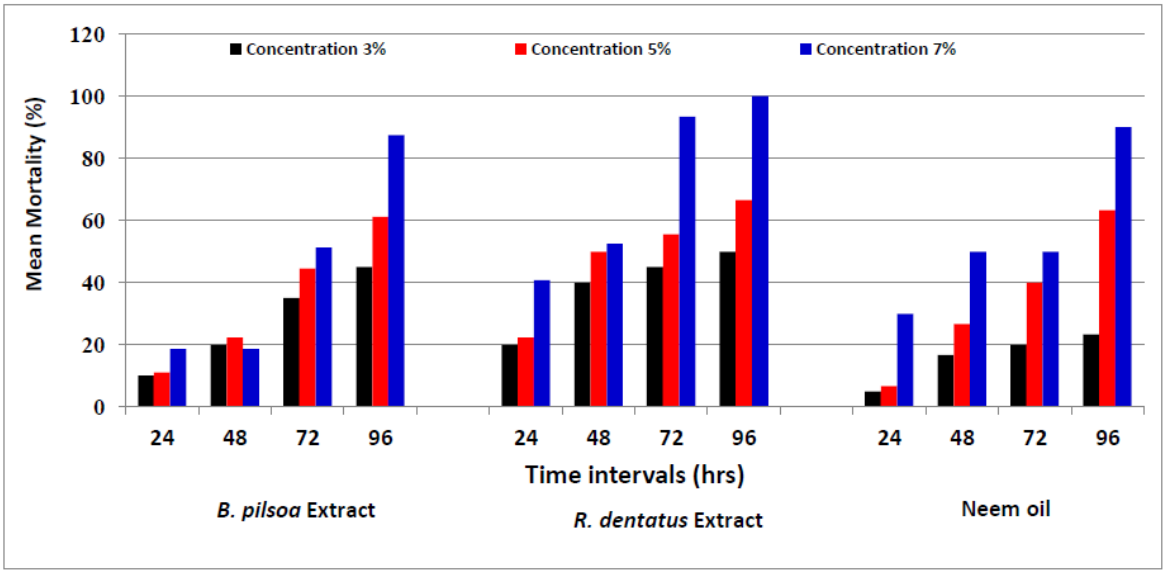

Fig. 3: Mean Mortality of *A. obtectus* against different concentrations of B. *pilsoa*, *R. dentatus* and Neem oil under different time intervals (24,48, 72 and 96Hours).

The medium lethal concentration (LC_{50}) of plant extracts on the *O. surinamensis*, *S. oryzae* and *A. obtectus* after 96hrs of exposure are presented in Table (4) and Figures (4 and 5). The results showed that the methanol extracts from *R. dentatus*, *B. pilosa* and Neem oil had LC_{50} values of 5.008, 4.558 and 4.208% against *O. surinamensis*, respectively, while, LC_{50} values of the same botanicals against *S. oryzae* and *A. obtectus* were (5.837, 2.593 and 3.937%) and (5.038, 3.338and 6.949%), respectively.

In general, the obtained data indicate that *R. dentatus* extract was the most toxic and has high potency compared to other tested botanicals extracts. It achieved the least lethal activity (50% mortality, LC_{50}) after 48 hrs. at concentration 7% and after 72hrs at concentration 5% when applied against the three tested pests. Also, the present study revealed that the mortality percentages of the two extracts and Neem oil against the three tested stored products were increased with increasing concentration and exposure time, indicating that the mortality percentages were concentration and exposure time-dependent.

Consistent with our results, Renuka *et al*., (2014) studied the bio-efficacy of methanol and acetone extracts of *B. pilosa* L. against *A. obtectus* and *Zabrotes subfasciatus* at different concentrations under laboratory conditions. They stated that different concentration of acetone extract gave 100% mortality for both pest species after 2.66±0.66 days of treatment, while mortality of adult bruchids treated with methanolic plant extract was directly proportional to the concentration levels and the exposure time of the pest., whereas, methanol extract at the concentration of (8%), gave 100% mortality after 5.66±0.33 days and gave 100% mortality of pest species on 7.66±0.66 days after treatment with its concentration of 2, 4 and 6%. In a related study, essential oil of *B. pilosa* was toxic to Bambara groundnut (BG) weevils *Callosobruchus maculatus* at different concentration, but, the effective concentrations of *B. pilosa* that kill 50% (LC_{50}), 80% (LC_{80}), and 99% (LC_{99}) population of *C. maculatus* were 2.48, 3.46 and 4.07 mg/ml respectively, (Goudoum *et al*., 2016). In other work, it appears from the results of Mamun and Ahmed (2011) that *B. pilosa* caused significant adult mortality and can be used to control tea pests.

Table 4: LC_{50} and LC_{90} values, together with their confidence limits, for *O. surinamensis*, *S. oryzae* and *A. obtectus* adults exposed to different concentrations of *Bidens pilosa* extract , *Rumex dentatus* extract and Neem oil combined with several exposure periods.

Treatments	Insect	LC_{50} (%)			LC_{90} (%)			Slope ± SE	Chi-square (χ^2)
		Value	Confidence limits (%)		Value	Confidence limits (%)			
			Lower	Upper		Lower	Upper		
O. surinamensis	*Bidens pilosa*	5.008	4.414	5.764	14.714	10.723	28.246	2.74± 0.5	3.7041
	Rumex dentatus	4.558	4.163	4.965	9.298	7.955	11.908	4.14±0.53	2.91
	Neem oil	4.208	4.071	5.342	11.335	9.125	18.647	2.98±0.50	4.03
S. oryzae	*Bidens pilosa*	5.837	4.771	8.804	35.098	16.521	591.568	1.64± 0.49	1.23
	Rumex dentatus	2.593	2.002	2.8	3.421	3.209	4.132	10.65±3.24	$-3.01*10^{-13}$
	Neem oil	3.937	3.59	4.256	7.325	6.529	8.672	4.75±0.55	0.9
A. obtectus	*Bidens pilosa*	5.038	4.209	6.261	22.755	13.325	105.684	1.95± 0.49	1.73
	Rumex dentatus	3.338	2.894	3.694	6.931	6.094	8.475	4.04±0.55	3.14
	Neem oil	6.949	5.674	11.409	34.709	17.169	343.664	1.83±0.5	$8.61*10^{-3}$

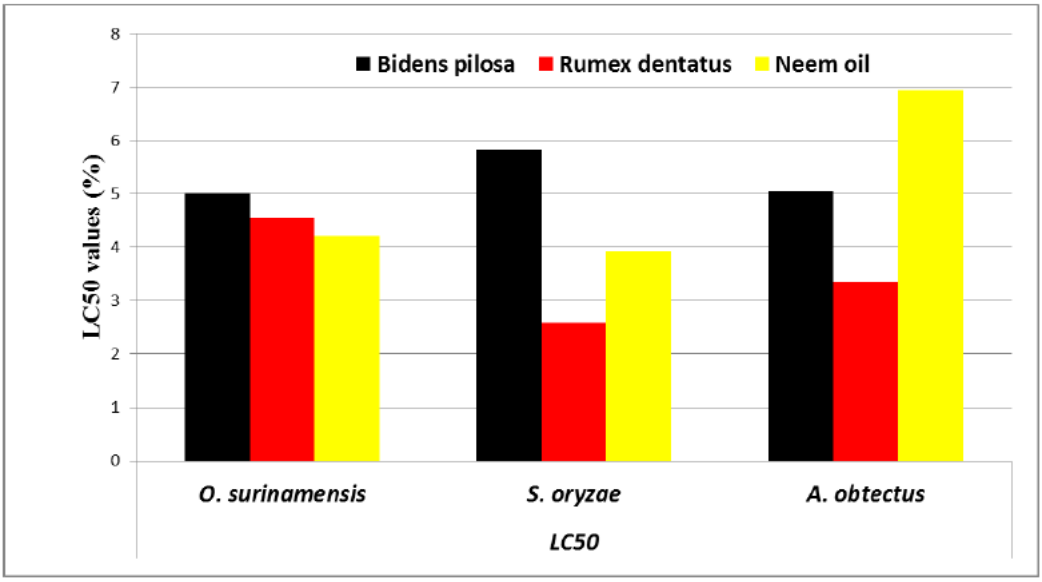

Fig.4: LC_{50} values for *O. surinamensis*, *S. oryzae* and *A. obtectus* adults exposed to different concentrations of *Bidens pilosa* , *Rumex dentatus* extract and Neem oil combined with several exposure periods

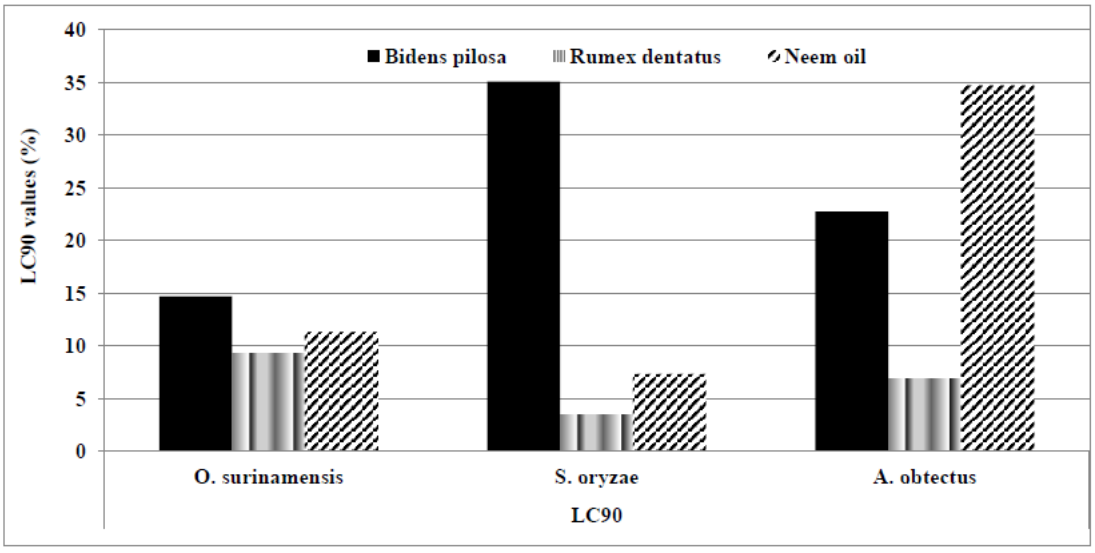

Fig.5: LC_{90} values for *O. surinamensis*, *S. oryzae* and *A. obtectus* adults exposed to different concentrations of *Bidens pi losa* , *Rumex dentatus* extract and Neem oil combined with several exposure periods

Our results of using *R. dentatus* against the three tested pests are supported by Hussain *et al*. (2010) who studied the insecticidal activities of the methanolic crude extracts of *Rumex hastatus, R. dentatus* and *R. nepalensis* against certain stored products pests found that *R. dentatus* shows high insecticidal activity against *Callosbruchus analis* and *S. oryzae*; moderate activity against *Rhyzopertha dominica* and low mortality against *Trogoderma granarium.* While *R. nepalensis* extract showed a high mortality rate against *S. oryzae, R. dominica, C. analis* and *T. granarium,* and finally, the methanolic extract of *R. hastatus* showed high insecticidal activity against *C. analis* and *S. oryzae;* moderate insecticidal activity against *R. dominica*, *T. granarium*. Also, our findings coincide with those of Shoukry *et al*. (2003) who showed that treatment with three fixed oils including *R. dentatus* affects the biochemical activities of *Plodia interpunctella* larvae, leading to disturbances in carbohydrate, lipid and protein levels in the haemolymph and protein fractions and they stated also that various plant oils cause morphological aberrations among which malformation of larval-pupal intermediates and formed pupae. Finally, our present work is in line with several previous studies that were conducted using different plant extracts and essential oils for controlling insect pests including stored grain pests such as; the bean weevil *A. obtectus* (Koona and Bouda 2006, Jovanovic *et al*., 2007 and Çetin *et al*., 2014); confused flour beetle *Tribolium confusum du* Val., and *A. obtectus* (Gokturk *et al*., 2020); the Mediterranean flour moth *Ephestia kuehniella* Zeller, the Indian meal moth *Plodia interpunctella* Hübner and *A. obtectus* (Ayvaz *et al*., 2010); three stored grain pests *O. surinamensis L., Tribolium castaneum.* Herbst and *Callosobruchus chinensis L.* (Manzoor *et al*., 2011); *O. surinamensis* L., *Rhyzopertha dominica* F. and *Sitophilus zeamais* Mots. (Moreira *et al.*, 2007 and Mulungu *et al.*, 2007) and *S. oryzae* (Yankanchi and Gadache 2010).

Conclusions and recommendations

Weeds are unwanted plants in certain places or times nevertheless; they should be continuously investigated and regarded as plants that have potentialities and significance. From the present study, it is concluded that the methanolic extracts of *R. dentatus* and *B. pilosa* possess toxic efficacy with significant insecticidal effects against *O. surinmensis, S. oryzae* and *A. obtectus*. The toxic effect of *R. dentatus* extract was higher than Neem oil (commercial product) against the three tested pests. Hence, these plants are easily available and widely distributed in many countries including Egypt, it could present promising opportunities to be invested among different IPM programs, as a potential botanical insecticide for stored grain in general and *O. surinmensis, S. oryzae,* and *A. obtectus* in particular.

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