Turnip Seed (*Brassica Napus*) Extracts as Grain Wheat Protectants Against the Granary Weevil, *Sitophilus Granarius* L.

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Abstract. Turnip seeds, *Brassica napus,* were extracted by different solvents, and tested for their toxicities to the granary weevil, *Sitophilus granarius* L. infesting wheat. Petroleum ether, chloroform and acetone extracts, at a rate of 9 ml/kg seeds, showed 100% mortality after 7 days. Petroleum ether extract was the most potent, at both LC_{50} and LC_{95} levels, whereas other extracts were slightly less. Reproductive capacity of the weevil was strongly afffected, at the two levels of the tested extracts. Complete protection (100%) to grain wheat against *S. granarius* L. was achieved. The residual effect was studied after different periods (from 1 to 8 weeks) from the time of treatment. Results showed that chloroform extract gave a good protection for up to 7 weeks. All the extracts reduced the germination of wheat grains at both their LC_{50} and LC_{95} values, at the time of treatment and after 7 weeks. No marked effect of the tested extracts on the water inhibition by the grains was observed. The initial effect of the extracts on the chlorophyll contents was variable; after 7 weeks the results were more constantly high. The tested extracts prove effective in controlling of *S. granarius* L., with some adverse effect on the germination of the grains and on the chlorophyll contents of the wheat leaves.

Key Words: Plant products control, *Sitophilus granarius* L., grain protectants.

Introduction

Wheat is one of the principal food crops in Saudi Arabia and in many other countries. The extensive damage caused to this food crop during storage due to various insect pests is a serious problem. Control of these insects by chemical insecticides has created several problems such as, pollution of the environment, insect resistance and high mammalian toxicity.

Use of biopesticides of plant origin, is a good trend that preserves the environment from contamination with harmful toxicants. Several studies suggested the use of certain plant dusts and extracts. Golob and Webly, (1980) found that *C. maculatus* adults failed to produce progeny when exposed to cowpea seeds, treated with oils extracted from the peal of citrus fruits. Similar results were obtained by using different plant materials against different insect *Pests* (Makanjuola, 1989, Ahmed, 1992, Mostafa, *et al.*, 1996, and Mahgoub, *et al.*, 1998).

The granary weevil, *Sitophilus granarius* L. is an important and worldwide grain pest, the purpose of the present work is to study the efficacy of turnip-rape (*Brassica napus)* extracts against *S. granarius* L. infested wheat grains.

Materials and Methods

Turnip seeds, *Brassica napus* were thoroughly grounded to fine powder (50 mesh). An amount of 200g of the resulting powder was soaked in different organic

Table 1. Effect of *Brassica napus* seeds extracts against adults of *Sitophilus granarius* L.

Extract	% ml/kg seeds	% mortality after indicated time of exposures				LT_{50} Value
		1	3	7	14 days	
Petroleum Ether	5	8	20	34	53	3.051
	6	16	31	56	62	
	7	19	50	72	90	
	8	42	72	100	100	
	9	72	95	100	100	
Chloroform	6	12	24	50	61	5.823
	8	15	28	64	70	
	9	21	40	100	100	
	10	42	64	100	100	
	11	54	79	100	100	
	12	80	95	100	100	
Acetone	6	10	20	38	58	2.522
	7	14	46	51	63	
	8	28	58	72	80	
	9	49	70	100	100	
	11	57	84	100	100	
Control		-	--	6	8	

*This median lethal time estimated for 7% dosage of pet-ether and 8% dosage of chloroform and acetone.

solvents of different polarities: petroleum ether (B.P. 40-60 °C), chloroform and acetone, (at a ratio of one gram powder to 3 ml of solvent) for 48 hours, as described by Su (1985). The flask was then shaken and its contents were passed through a column containing anhydrous sodium sulphate, the solvent was then evaporated on a water-bath at 40 °C. The resultant crude extracts were kept in a refrigerator for further work (Islam, 1983, Afifi *et al.*, 1988).

Adults (1-2 weeks old) of the granary weevil, *S. granarius*, reared on wheat grains were used as test insect.

Serial concentrations from each extract were prepared by dissolving the appropriate weights in 10 ml of the solvent of extraction.

Each solution was mixed with 10 grams of the tested grains, then the solvent was evaporated at 40 °C.

Insecticidal Activities of the extracts were conducted in test tubes, contained ten grams of treated grains each, and 25 adults of the weevil. Each treatment was replicated three times. Control treatments containing grains treated with the corresponding solvents only and untreated grains were included. Tubes were covered with muslin fixed with rubber band. Mortality counts were recorded after 1,3,7 and 14 days. Percentages of insects mortality were corrected for the natural mortality by means of Abbott's formula (1925), and the values of LT_{50} were estimated.

The corrected mortality percentages were statistically computed according to

Table 2. Toxicity of petroleum ether, chloroform, and acetone extracts to granary weevil.

Extracts	Lethal concentration and their 95% confidence limits ml/kg		Slope ±SE
	LC_{50}	LC_{95}	
Pet-ether	6.6481* (6.0751-7.2148)	10.2683 (8.9515-13.9005)	8.712±0.753
Chloroform	8.6994 (7.4108-9.8427)	14.8844 (12.2007-27.7745)	7.652±0.611
Acetone	7.5771 7.3064-7.8369	13.2863 (12.2287-14.9096	6.744±0.712

-SE: Standard error of line.
-Average of 3 replicates, 25 insects per replicate.
*Significantly different between pet-ether and acetone.
* Significantly different between pet-ether and chloroform.

Finney (1971). Computed mortality percentages after 72 hrs. of exposure were plotted versus the corresponding concentration on log-probability paper, and the mortality concentrations of 50% and 95%, and slopes of the curves were determined.

Egg deposition test was accomplished by the addition of five grams of treated grains (at LC_{50} and LC_{95} values) and five couples of adults. After 14 days insects were removed and the number of deposited eggs in the grain were calculated according to Frankenfeld (1948) and Howe (1952). Three replicates were made for each concentration, beside three replicates of untreated grains were used as control treatment.

Number of progeny of adults test, was conducted by placing ten couples of (1-2) weeks old of *S. granarius* adults in glass tubes, each containing 10 grams of wheat grains treated with LC_{50} and LC_{95} values of each of the tested extracts. Each treatment was replicated three times.

After 14 days, the insects were removed. The total number of the emerged adults was recorded after 6 weeks from the infestation, and percentages of reduction in progeny were calculated according Abbott (1925). Data were analyzed, using analysis of variance, and means were separated by the least significant difference (LSD) test, $P \geq 0.05$.

Residual activity measurements were conducted in tubes, containing 10g of the grains, previously treated with the LC_{50} concentration of each extract. They were divided into several groups. Each group consists of three replicates for each storage period. The tubes were kept under laboratory conditions. Twenty-five adults of *S. granarius* were introduced into each tube (3 replicates) at every 7 days for 8 weeks. Similar three replicates of untreated wheat were used as control. In all cases, mortality counts were carried out 72 hours after introducing the insects. Mortality percentages were corrected according to Abbott's formula (1925). Then the values of LT_{50} were estimated.

Germination and water absorption of seeds for each extract at its LC_{50} and LC_{95} levels were carried. Germination was tested 1 day and 7 weeks after treatment, in petri dishes, (9 cm), lined with two layers

Table 3. Effect of turnip seed extracts on the number of eggs and progeny of granary weevil adults.

Extracts	% Concentration ml/kg grains	Mean no. of eggs/5 pairs	% Reduction than control	Mean no. of progeny emergence	% Reduction than control
Pet-ether	6.7 (LC_{50})	9.7*	88.3	00.00	100
	10.3 (LC_{95})	3.3*	96.0	00.00	100
Chloroform	8.7 (LC_{50})	7.7*	90.7	0.33	99.6
	14.9 (LC_{95})	2.0*	97.6	00.00	100
Acetone	7.6 (LC_{50})	8.0*	90.4	00.00	100
	13.3 (LC_{95})	0.7*	99.2	00.00	100
Control		83		16.5	

- Tabulated F ($P<0.05$) = 4.07
- LSD = 8.56 and 8.13 (LC_{50} and (LC_{95}), respectively)
* Each significantly different from control at $P<0.05$ based on LSD.

of filter paper, soaked with 4 ml of water. Twenty five treated grains were placed on the surface of paper. Germination counts were recorded 7 days later. Each treatment was replicated three times. Untreated grains were tested for comparison.

Ten grams from each treatments were weighed and submerged in water. Moisture absorption was measured after 1,5 and 24 hrs as percentage of weight increase after drying the grains with paper towels, (according to Yuntai, and Wendell, 1981).

Determination of chlorophylls was carried out by weighing shoot samples (1gm) ground in cold aqueous acetone (80%). The paste was filtered under suction through a Whatman No. 2 filter paper.

The concentration of chlorophyll a,b and the total were determined colourimetrically, by measuring the optical density of the chlorophyll extracts, using the wave lengths 645, 663 and 652 um, respectively. Chlorophylls concentrations were calculated by the equation of Machinney (1941).

Results and Discussion

Insecticidal activities of the extracts are recorded in (Table 1) that indicate that, three days after exposure to the highest concentrations of petroleum ether, chloroform and acetone extracts of *Brassica napus* seeds were the most effective (95,95 and 84% kill) respectively, while, after 7 days of exposure, 9 ml/kg of each tested extract gave 100% mortality. The LT_{50} values were estimated for 7% dosage of petroleum ether, and 8% dosage of each of chloroform and acetone, using probit analysis, for clear expression of relative toxicity of all tested extracts. It is clear that, acetone extract was the most rapid extract in killing the insect (LT_{50} = 2.522 weeks), followed by petroleum ether, and chloroform extracts (LT_{50}=3.051, 5.823 weeks respectively). Meanwhile, on the basis of LC_{50} and LC_{95} values (Table 2) petroleum ether extract was the most effective at both levels, while other extracts were slightly less.

Table 4. Estimated initial residual activity of LC_{95} tested extracts on granary weevil adult mortalities.

Period after treatment weeks	% Mortality of exposed insects		
	Pet-ether	Chloroform	Acetone
Initial	96	95	96
1	96	96	95
2	95	96	96
3	95	96	89
4	97	95	54
5	90	95	50
6	81	93	42
7	62	88	30
8	33	52	28
LT_{50}	13.775	56.27	5.266
LT_{95}	2.057	2.394	1.451

The slopes showed that petroleum ether and chloroform extracts were the most potent against *S. granarius* L.

Similar results were obtained by (Mahgoub and Ahmed, 1996; Ahmed and Mahgoub, 1996) who worked on the toxicity of castor oil against *Sitophilus oryzae* L. and *Callosobruchus maculatus* F. and found that pet-ether extract was more effective than chloroform and acetone extracts.

Exposing granary weevil adults to surface treated wheat grains with LC_{50} and LC_{95} values of the extracts resulted in highly significant reduction in egg production compared with the control, based on least significant difference (at 5% probability level, L.S.D. = 8.56 and 8.13 (LC_{50} and LC_{95}, respectively), without significant difference between extracts (Table 3).

There was a highly significant reduction in progeny emergence which marked 99.6 less than control at LC_{50} of chloroform extract, while no adults emergence was noticed, at all other levels of all extracts, therefore, 100% relative protection was achieved against *S. granarius* in wheat grains at all levels.

These results are in agreement with Sing *et al.*, (1978) with grounded oil at 5 ml/kg cowpea seeds which prevented emergence of *C. maculatus* progeny.

Prakash *et al.*, (1979, 1980 and 1982) found that garlic extract, bell leaves and undicake proved to be promising protectants against some stored grain insects.

Also Golob and Webly (1980) and Zeway (1987) stated that many vegetable and mineral oils prevent ovipositions, at rates ranging between 5 to 12 ml/kg of grains.

Data combined (Table 4) indicate the influence of the high concentrations (LC_{50} 's) of the three extracts on the tested insect. It is clear that mortality percentages of the exposed *S. granarius* adults to treated wheat grains with chloroform extract were highly effective (LT_{50} = 56.27 weeks), while incase of acetone extract the deterioration was higher (LT_{50} = 5.266 weeks).

Table 5. Germination of wheat grains after *Brassica napus* seeds extracts treatment and water absorption for 1,5 and 24 hrs. stored for 7 weeks.

Extracts	Concentrations ml/kg	Initial (0)				After 7 weeks			
		Germination %	Water absorption increase %			Germination %	Water absorption increase %		
			1 hr	5 hr	24 hrs		1 hr	5 hrs	24 hrs
Pet-ether	6.7 (LC_{50})	77	25.5	36.5	56.2	53	22.2	35.2	61.0
	10.3 (LC_{95})	78	25.5	36.2	56.8	56	21.3	32.2	57.5
	Control	95	21.3	38.2	55.3	93	19.5	34.7	55.7
Chloroform	8.7 (LC_{50})	68	25.3	36.7	59.0	67	22.8	35.2	61.3
	14.9 (LC_{95})	63	24.0	35.0	54.5	35	22.8	34.8	60.2
	Control	95	21.3	38.2	55.3	93	19.5	34.7	55.7
Acetone	7.6 (LC_{50})	64	20.3	30.2	58.8	54	20.2	31.2	55.3
	13.3 (LC_{95})	63	16.5	27	52.2	57	20.8	31.5	57.8
	Control	96	18.2	32.2	55.5	93	17	29.7	55.0

* LC_{50} = the median lethal concentration.

** LC_{95} = the dose which caused 95% mortality.

Table 6. Effect of *Brassica napus* on chlorophyll content of germinated wheat grains.

Extracts	% concentration	Chlorophyll content at time intervals after treatments: Initial (0) Chlorophyll a	Initial (0) Chlorophyll b	Initial (0) Total Chlorophyll	After 7 weeks Chlorophyll a	After 7 weeks Chlorophyll b	After 7 weeks Total Chlorophyll
Control		0.2424	0.1604	0.4492	0.2615	0.1732	0.473
Pet-ether	6.7 (LC_{50})	0.1350	0.0962	0.2463	0.2020	0.1404	0.3623
	10.3 (LC_{95})	0.1460	0.1123	0.2898	0.1616	0.1043	0.2898
Chloroform	8.7 (LC_{50})	0.2329	0.1324	0.4292	0.1616	0.1163	0.3043
	14.9 (LC_{95})	0.2222	0.1404	0.4216	0.1414	0.0922	0.2463
Acetone	7.6 (LC_{50})	0.1515	0.1123	0.3333	0.1705	0.1150	0.3436
	13.3 (LC_{95})	0.1515	0.1003	0.3043	0.1432	0.1077	0.2813

* LC_{50} = the dose which caused 50% mortality.
* LC_{50} = the dose which caused 95% mortality.

Regarding the chloroform extract, it is found that, no deterioration occurred in its residual activity until 7 weeks.

Generally, chloroform extract was more effective than other extracts.

Germinations of the grains, treated with extracts at the LC_{50} 's and LC_{95} 's for 8 weeks are presented in (Table 5). All the tested extracts reduced the grain germination compared with the control, at the two levels, in the initial treatment, and after 7 weeks. The same results were reported by Ahmed (1992) who proved that extracts of *P. aromeniaca* or S. *saponaria* showed obvious adverse effect on germination of cowpea seeds. Ahmed and Mahgoub (1996) found that germination was adversely affected by *R. communis* extracts.

The percentage of water absorption by grains did not vary consistently with the amount of extract applied. Yuntai and Wendell (1981) reported that there was no obvious effect on water absorption of the wheat seeds, treated with some vegetable oils (cottonseed, soybean, maize and peanut).

Effect of the extracts on the chlorophylls contents, given in (Table 6) shows that, at the initial time petroleum ether extract decreases the amount of both chlorophylls a,b and the total, meanwhile, the other extracts show only a slight reduction.

After 7 weeks of exposure, the tested extracts gave a high reduction in the amount of both chlorophyll a,b and the total, at the two tested levels.

The initial effect is therefore variable, however, after 7 weeks of storage, the results were more constantly high. These results agree with those reported by Ahmed (1996) who found that cowpea seeds treated with *Menta longifolia* showed certain decrease in the chlorophylls content. The obtained results also confirm those of Mahgoub *et al.*, (1988) who used *Petroselium sativum* oil on wheat grains and mungbeen seeds, and decreased the amount of total chlorophyll, especially at LC_{95}'s level.

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مستخلصات بذور اللفت كمواد واقية لحبوب القمح ضد حشرة سوسة القمح *Sitophilus granarius. L.*

نادرة حمود المعجل

كلية التربية للبنات بالرياض – الأقسام العلمية – قسم علم الحيوان

أجرى هذا البحث بغرض تقييم التأثير السام لمستخلصات البتروليم إيثر والكلوروفورم والأسيتون لبذور اللفت ضد حشرة سوسة القمح. كما تم تقييم تأثير هذه المستخلصات على كل من حيوية البذور المعاملة عند الإنبات. ومعدل امتصاص البذور للماء، وكذلك كمية الكلوروفيل المتكونه في بادرات القمح.

وقد أظهرت النتائج أن التركيز ٩ مل/كجم لجميع المستخلصات المختبرة قد أعطى ١٠٠% موت وذلك بعد ٧ أيام من المعاملة.

كان مستخلص البتروليم إيثر بالتراكيز LC_{50} و LC_{95} أكثر فعالية من المستخلصات الأخرى. حيث أن الجرعة التي تسبب الموت كانت أقل من مثيلاتها من المستخلصين الآخرين. وقد منعت جميع المستخلصات المختبرة بتركيز LC_{50} و LC_{95} تكاثر أعداد حشرة سوسة القمح على حبوب القمح، مما أدى إلى حماية الحبوب حماية كاملة.

عند دراسة التأثير المتبقي لجميع المستخلصات المختبرة على فترات متتالية (من ١ – ٨ أسابيع) من بدء المعاملة، لوحظ أن مستخلص الكلوروفورم أعطى حماية كاملة لحبوب القمح من الإصابة بسوسة القمح وذلك لسبعة أسابيع متوالية.

كما تسببت مستخلصات بذور اللفت بتركيزاتها المختبرة إلى خفض حيوية البذور المعاملة. و كان للمستخلصات تأثير كبير على تكوين الكلورفيل في أوراق بادرات القمح حيث قلت كميتة المتكونة فية, خصوصاً عند مستوى ٩٥%، مقارنة بالبادرات النابتة من حبوب غير معاملة.

لذا يوصي بإستخدام جميع مستخلصات بذور اللفت بتركيز LC_{50} لمنع تكاثر أعداد حشرة سوسة القمح على حبوب القمح. إلا أنه لا يوصي بإستخدامها في حالة إستخدام هذه الحبوب كتقاوي.