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Preliminary pesticidal studies of willow bark (*Salix safsaf*)

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ABSTRACT

The bark of safsaf tree, willow (*Salix safsaf*) was collected and cut into small strips. These strips were dried and powdered, then extracted with aqueous acetone. After the complete evaporation of acetone, some fractions were separated from the resultant extract by using partitioning between different solvent systems. These fractions were tested for their phytocidal effects on wheat and squash seedlings, insecticidal effects on the rice weevil (*Sitophilus oryzae*) and molluscicidal effects on the terrestrial snail (*Theba pisana*). Fraction **II**, which was isolated from n-hexane layer, was found to be more active in the inhibition of root growth of wheat and squash seedlings with IC_{50}'s equal 170 and 230 ppm, respectively. Fraction **II** was effective against the rice weevil with LC_{50} equal 140 ppm during 2.4 days. It gave high lethal effects against the tested snail (*T. pisana)* with LC_{50} equal 56 ppm. The other tested fractions were relatively less effective.

INTRODUCTION

Willow trees are good fodder, livestock eats their leaves and humans developed various medicinal uses from this tree. Roots are used in medicines that help cure stomach pains, fever and headaches. Aspirin, for example, is a derivative of the willow species. Some other uses include, applying bark powder to burns, brewing tea from the leaves to treat rheumatism, which also acts as a mild laxative (Julkunen and Sorsa, 2001). In addition to the traditional uses of their woods in different respects. A new phenolic glycoside, 2'-cinnamoylsalicortin was found by Nichols-Orians *et al* (1992) in *Salix sericea* which likely to posses biological activity. Some water extracts from *S. caprea, Calluna vulgaris* and *Potentilla erecta* were found to be the most potent cyclooxygenase inhibitors and were active to inhibit the prostaglandin biosynthesis and platelet activity factor (PAF) induced exocytosis in vitro (Tunon *et al*, 1995). Some phenylpropanoids like triandrin and n- coumaric alcohol derived from *S. viminalis* showed stimulating action in mice as spontaneous motor and antihyprotic activities (Sokolov *et al*, 1990). Saturated fatty acids (C-15 and C-31) and flavonoids (apigenin, kaempherol and luteolin) were isolated and identified from *S.*

lindleyana, which has antipuretic properties (Thapliyal and Bahuguna, 1993). Feeding of some insects on leaves of some plants and insect mortality figures indicated that willow, aspen, bird cherry and apple had antibiotic effects (Ovcharov, 1984). Malterud *et al* (1985) found that the isolated flavonoids from the wood of *S. caprea* were potent inhibitors of wood-destroying fungi and inhibited some of rot-producing fungi. The blood coagulatory activity and biochemical composition of the extracts of dried ripe catkins of *S. daphnoides* were similar to those of thromboplastin isolated from rat brain (Kudryashov *et al*, 1985).

These different activities of willow (*Salix spp*) tree directed the present studies to investigate the effects of the separated fractions of the powdered dried extract of willow bark (*S. safsaf*) for their phytocidal effects on wheat seedlings (*Triticum aestivum*) and squash (*Cucarbita pepo*), their insecticidal effects on the rice weevil (*Sitophilus oryzae*) in addition to their molluscicidal effects on the terrestrial snail (*Theba pisana*).

MATERIALS AND METHODS

1-Methods of separation: Dried powdered bark of willow (*S. safsaf*), 500 gm, soaked for one month in 2.2 liter of aqueous acetone (70%) at room temperature. The acetonic mixture was filtered by using Buchner funnel. The precipitate was re-soaked four times each in 2.5 liter of acetone (90%) at room temperature for further one month, then filtered off. The mixtures of all filtrates were combined together as original extract. The original extract was concentrated by using rotary evaporator at 50-70°C under low pressure to 820 ml (77.15 gm oven dried extract). The concentrate was separated in petroleum ether (30-40° C) 7 times by using a separatory funnel, the aqueous layer was kept under cooling (4°C) for two days. A brown precipitate was filtered and dried as fraction I (15.14 gm), whereas the filtrate was separated 8 times in ethylacetate and the aqueous layer was concentrated and separated with n-hexane. The n-hexane layer (black layer, 900 ml) was separated with toluene (750 ml) which produced some resin material. The resin material was separated and was exposed to air dryer, kept under cooling (4°C), then dried over sodium hydroxide for 10-days giving a brown powder as fraction **II** (29.44 gm). However, the supernatant solution of resin passed through silica gel column (2.5 × 19 cm), the absorbed materials on silica gel were treated with solvents mixture of [Toluene: acetone: ethylacetate (2:1:2)] for 2-days and separated. The low layer was evaporated from acetone to give brown precipitate as fraction **III**

(1.48 gm). The aqueous layer of hexane separation was concentrated by using rotary evaporator and kept under cooling for 3-days, then filtered and a yellowish brown precipitate was dried as fraction IV (1.58 gm). The filtrate was concentrated and was treated with aqueous hydrochloric acid (10%) and was cooled to give black precipitate as fraction V (0.5 gm). These separation methods were carried out according to Desheesh *et al.*(2000) with modifications.

2-Pesticidal effects of the separated fractions:

A-Phytocidal effects: Phytocidal effects of the separated fractions against wheat seedlings (*T. aestivum*) and squash seedlings (*C. pepo*) were conducted by using the plain agar (0.4%) technique (Zemanek, 1963), in test tubes. The tested concentrations were 100, 1000, 2000 and 4000 ppm. Pre-germinated seeds were sown in the solidified agar. Three replicates were used for each treatment. Untreated control was carried out at the same conditions. The test tubes were watered with a constant volume and kept until the roots reach the bottom of the tube. The length of the root and shoot systems were measured and percentages of inhibition were calculated according to the following equation (Kaukinen, 1979).

$$\% \text{ Inhibition} = 100 - (\text{Treatment}/\text{Control} \times 100).$$

B-Insecticidal effects: Insecticidal effects of the tested fractions against the rice weevil (*S. oryzae*) were carried out according to Kenaga *et al.* (1965) with modifications. Whole-wheat seeds (2.2 gm, 50 seeds) were placed in each test tube. The tested concentrations (10, 50, 100, 500, 1000 and 10000 ppm) were dissolved in 1.5 ml acetone and added on wheat seeds in each test tube and mixed well. The test tubes were kept at room temperature in the dark until the acetone solution was evaporated. Rice weevils (6 insects/test tube) were added and the test tubes were sealed with a piece of fixed cloth with a rubber band. Control was concurrently conducted under the same conditions. The number of dead and alive insects was recorded. The mortality percentages were measured from which, lethal concentration, which caused 50% mortality (LC_{50}) and the required time to cause 50% mortality (LT_{50}) were determined by using semi log paper.

C- Molluscicidal effects: The used snails *T. pisana* were collected from the bark of citrus trees in Tawfik El-Hakeem village, EL-Behira Governorate, Egypt. The snails were kept under the laboratory conditions for ten days. They were fed on lettuce through the adaptation period. The used bait was prepared by adding the commercial blue paint at a ratio of 2% to the wheat bran. The water was added at a ratio of 2 ml/ 10 gram of the prepared bait. The tested fractions were mixed with the prepared bait at the concentrations of 1, 10, 50, 100 and 200 ppm. Ten grams of the toxic bait and 15 snails were placed in 9 cm petri dish as one replicate. The petri dish was placed in a plastic pot that was covered with a piece of fixed cloth with rubber band. Water (15 ml) was added in the bottom of the pot to keep the moistened media available. The cloth pieces were daily sprayed with water to keep the moisture. Three replicates were carried out for each treatment. Control was carried out under the same conditions. The dead snails were counted and the toxic effects were recorded as number of alive or dead snails after 5 days. The mortality percentages were calculated and LC_{50} values were derived from the drawn line on semi loge paper. This method was carried out according to Abdallah *et al.* (1998) with modifications.

RESULTS AND DISCUSSION

A-Phytocidal effects: Phytocidal effects of the tested fractions which were isolated from willow bark (*S. safsaf*) on roots and shoots of wheat (*T. aestivum*) and squash (*C. pepo*) seedlings are recorded in Tables (1 and 2). The inhibition percentages of roots and shoots of both wheat and squash seedlings were increased with increasing the tested concentrations of all fractions. Root system of wheat seedlings was more susceptible to the tested fractions than their shoot system. Fraction **II** caused high inhibition to roots of wheat seedlings, followed by the original extract, fraction **I**, and fraction **IV**. The IC_{50} values of these extracts equalled 170, 250, 400 and 990 ppm, respectively. However, wheat seedlings shoot system was found to be less susceptible than root system for all treatments. The IC_{50} values equalled 1450, 1700, 3700 and > 4000 ppm which were caused by the original extract, fractions **II**, **IV** and **I**, respectively. On the other hand, root system of squash was more inhibited than its shoot system. Original extract and fraction **II** were found to be more active against root system of squash seedlings than its shoot system. The IC_{50} values equalled 230 and 260 ppm, respectively. The less active fractions were fraction **IV** and fraction **I**. Their IC_{50} values were 1150 and 1175 ppm, respectively. Shoot system of squash

seedlings was less affected by all the tested treatments.

So, root systems of both wheat and squash seedlings were more affected by all the tested treatments. Original extract and fraction **II** were the highly active extracts in the inhibition of the root and shoot systems of wheat and squash seedlings. Stunting and formation abnormalities in the secondary roots were noticed to be accompanied with the inhibition effect in the growth.

Table (1): Phytocidal effects of the tested fractions of willow bark (*Salix safsaf*) on roots and shoots of wheat seedlings (*Triticum aestivum*).

Treatment	Part	(%) Inhibition at different concentrations (ppm)				IC_{50} (ppm)
		100	1000	2000	4000	
Original extract	Root	37.5	89.6	94.7	96	250
	Shoot	21	42.3	57	74.5	1450
Fraction I	Root	26.6	58.6	82.1	90	400
	Shoot	17	22.7	41.7	41.7	>4000
Fraction II	Root	41.7	79	91.6	96	170
	Shoot	3.6	32.8	55.4	74.4	1700
Fraction IV	Root	18	50.8	80.5	89.9	990
	Shoot	7.2	19.7	38.7	73.3	3700

Table (2): Phytocidal effects of the tested fractions of willow bark (*Salix safsaf*) on roots and shoots of squash seedlings (*Cucarbita pepo*).

Treatment	Part	(%) Inhibition at different concentrations (ppm)				IC_{50} (ppm)
		100	1000	2000	4000	
Original extract	Root	38	67	89.2	94	230
	Shoot	7	18	28.7	70	2700
Fraction I	Root	12.8	57.4	42.7	75.8	1175
	Shoot	11.2	39.2	21	49.7	>4000
Fraction II	Root	41.5	65	85.4	98	260
	Shoot	10.5	14.7	30.1	76.7	2600
Fraction IV	Root	12.8	47.2	77.8	93	1150
	Shoot	18.2	23.1	30.1	55	3400

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B-Insecticidal effects: Insecticidal effects of willow bark (*S. safsaf*) fractions against the rice weevil (*S. oryzae*) are shown in Table (3). Fraction **II** proved to be active against the weevil . It gave the lowest LC_{50} value (140 ppm) and LT_{50} equalled 2.4 days. Fraction **IV** caused the same effect on the rice weevil through the same lethal time with LT_{50} value 2.4 days but at higher concentration where its LC_{50} value was 400 ppm.

Table (3): Insecticidal effects of willow bark (*Salix safsaf*) fractions against the rice weevil (*Sitophilus oryzae*).

Treatment	Original Extract	Fraction I	Fraction II	Fraction III	Fraction IV
LT_{50} (days)	> 9.0	5.4	2.4	3.5	2.4
LC_{50} (ppm)	> 10000	520	140	600	400

On the other hand, the effects of fraction **I** needed higher time equalled to 5.4 days as (LT_{50}) and LC_{50} equalled 520 ppm. Fraction **III** caused its effects at relatively higher concentration. The LC_{50} value equalled 600 ppm and its LT_{50} value was with relatively lower equalled 3.5 days. However, the original extract was found to be the lowest effective extract against the weevil. It caused LC_{50} more than 10000 ppm with LT_{50} more than 9-days. These results emphasize that the active constituent might be distributed among the extracted fractions mainly fraction **II**. These results agreed with the results of Nichols-Orians *et al.* (1992) and Ovcharov, (1984). They mentioned that *Salix spp* extracts possess biological and insecticidal activities.

C-Molluscicidal effects: Molluscicidal effects of the tested fractions which were isolated from willow bark (*S. safsaf*) on the terrestrial snail, *T. pisana* were recorded in Table (4). Fraction **II** proved to be the active fraction against the tested snail. It gave LC_{50} equalled 56 ppm. The other tested fractions were less effective against the tested snail at the tested concentrations. They gave mortality percentage as high as 47%. However, their concentrations have to be increased more than the tested one.

From the mentioned results, it could be concluded that fraction **II** caused phytocidal effect on wheat and squash seedlings, insecticidal effect on rice weevil and molluscicidal effect against the tested snail. These biological effects may be due to the active materials in fraction **II**. More efforts of research to separate and identify the active compounds in fraction **II** to know their biological properties are needed.

Table(4): Molluscicidal efficacy of the willow bark (*Salix safsaf*) fractions against the terrestrial snail, *Theba pisana*.

Fraction	(%) mortality at different concentrations (ppm)						LC_{50}
	1	10	50	100	200	400	
Original Extract	0	0	7	23	40	30	> 400
Fraction I	0	10	-	33	47	-	>200
Fraction II	0	30	43	60	70	-	56
Fraction III	0	0	0	20	33	-	> 200

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دراسات أولية إبادية للحاء الصفصاف

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تم جمع وتقطيع لحاء أشجار الصفصاف إلى أجزاء صغيرة و بعد تجفيفه تم إستخلاصه بواسطة محلول الأسيتون المائي Aqueouas acetone وتبخير الأسيتون كاملاً. كذلك تم فصل ٤ أجزاء (قطفات) من المستخلص الأساسي للحاء بتوزيعه بين مذيبات مختلفة. تم تقييم القطفات التى تم فصلها مقارنة بالمستخلص الاساسى من حيث التأثيرات الأبادية على النبات Phytocidal activity بطريقة الأجار المائي على بادرات الكوسة *Cucarbita pepo* كمثال للنباتات عريضة الأوراق ونبات القمح *Triticum aestivum* كأحد أمثلة النباتات رفيعة الأوراق . وأيضا تم دراسة التأثيرات المميتة على سوسة الأرز *Sitophilus oryzae* (rice weevil)كمثال للتأثير الابادى الحشرى وكذلك التاثير على القواقع الزراعية مستخدما القوقع الأرضى *Theba pisana*.

وقد أظهرت النتائج أن القطفة II و التى تم عزلها بواسطة مذيب الهكسان العادى أكثر فاعلية لتثبيط نمو المجموع الجذرى لنباتي القمح والكوسة حيث كان التركيز المثبط لـ٥٠ % من نمو الجذور هو ١٧٠ و ٢٣٠ جزء في المليون على الترتيب وكذلك كانت نفس القطفة هى الأكثر كفاءة في إبادة سوسة الأرز حيث كان التركيز المميت لـ٥٠ % من العشيرة المعاملة قدره ١٤٠ جزء في المليون. أظهرت نفس القطفة تأثيراً مميتاً ضد القوقع المستخدم حيث كانت قيمة التركيز القاتل لـ ٥٠% من العشيرة المعاملة قدرة ٥٦ جزء في المليون بينما كانت القطفات الأخرى أقل نشاطاً في تأثيراتها نسبياً في جميع الحالات .