PHYTOTOXICITY OF SWEET CLOVER (*MELILOTUS INDICA* L.) EXTRACT ON GERMINATION AND SEEDLING GROWTH OF SOME PLANT SPECIES

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ABSTRACT

The present investigation was carried out to study the inhibitory effect (phytotoxicity) of sweetclover, *Melilotus indica* L. ethanol extract on seed germination and seedling growth of some plant species in addition to the isolation and identification of the chemical groups of sweetclover using Infrared (IR) spectrum.

The results showed that wildoat was the most sensitive species to the ethanol extract of sweetclover with 80.5% inhibition in germination as well as 66% and 50.9% inhibition in root and shoot growth, respectively. In contrast, sweet clover extract had no effect on seed germination and seedling growth of lentil. Also, germination of onion seeds and seedlings growth of chicory were not significantly affected by the extract.

In general, root growth was more sensitive than shoot growth in affected plants.

The crude ethanol extract of sweetclover exhibited five fractions having R_f values of 0.14, 0.29, 0.53, 0.68 and 0.90. Two fractions with R_f values of 0.53 and 0.68 were the most inhibitory effects on germination and growth of wildoat. Both active fractions contained chemical groups indicated the presence of Isocyanate (NCO), Methoxy group (OCH_3), saturated sulfoxide (S=O), acetylene (C=C), phenols (ph.OH), carbonyl group (C=O) and free OH.

Key words: Phytotoxicity, Plant extract, Sweet clover (*Melilotus indica* L.), Growth species.

INTRODUCTION

Weeds vigorously compete with crop plants for water, light and nutrients in addition to produce chemical groups act as strong inhibitors for germination and growth of weed and crop plants. Although natural products isolated from plants have been less active than synthetic herbicides owing to detoxication or degradation but modifying these natural products, the end product could be more active, selective or persistent (Duke *et al* 2000).

Consequently, the natural products is a rich source of new herbicides that might prove useful in this respect.

Phytotoxic action of plant extracts on crops and weeds was investigated by many workers (Abdallah, 1997; El-Mutlaq *et al* 2002; Iqbal *et al* 2003; Norsworthy, 2003; Laine *et al* 2004; El-Mahy, 2004 and Ali, 2005).

The objective of the present work was to study the phytotoxic effect of sweetclover *Melilotus indica* L. ethanol extract on the germination and growth of five crop and weed plants as well as identification of the functional groups of the more active fraction using IR.

MATERIALS AND METHODS

Plants used: The annual winter weed sweetclover, *Melilotus indica* L. was obtained from Egyptian clover fields, Faculty of Agriculture, Fayoum University to study its inhibitory effect on germination and growth characters of five plant species; wildoat (*Avena fatua* L.), onion (*Allium cepa* cv. Giza-20), chicory (*Cichorium pamilum* Jacq.), carrot (*Daucus carota* cv. Redcore chantenay) and lentil (*Lens esculenta* L.).

Preparation of crude extract: Whole plant of *Melilotus indica* was washed with distilled water and dried under room temperature. 100 g of the dried plant material was ground in a grinder and extracted with 95% ethanol at the rate of 2 ml / g plant material as described by Su and Horvat (1981). After 24 hours, the extract was filtered through anhydrous sodium sulfate and evaporated to dryness. The crude extract was kept in the refrigerator till it was assayed.

Bioassay technique: Ten seeds of each tested species were placed on Whatman No.1 filter paper in a 9 cm diameter glass petridish. After ethanol evaporation under room conditions, 5 ml distilled water / petridish were added. In the control only distilled water was added. Four replicates were made for each treatment as well as for the control.

The seeds of chicory, lentil and wildoat were incubated at 25°C. Whereas onion and carrot at 20°C. The germination percentage of seeds were recorded after adequate period of each plant species according to Abdallah *et. al.* (2002).

The root and shoot length was measured after ten days of incubation. Inhibition percentages were calculated as ((control - treatment) / control) x 100.

Isolation and identification of the active chemical groups: Glass plates (20 x 20 cm) were coated with silica gel (GF_{254}), 0.75 mm. thickness and left for dryness at room temperature. Ethanol extract was applied as a band and developed in solvent system consisting of chloroform – ethanol – acetic acid (92 : 4 : 4 v/v/v).

The chromatogram was exposed to UV lamp at 254 nm. To calculate rate of flow (R_f values) for each band (fraction). Each band (fraction) was separately scraped from chromatogram and eluted with acetone, then evaporated and subjected to bioassay tests. According to this procedure the crude extract of sweetclover was fractionated to five bands. Two active bands with R_f values of 0.53 and 0.68 were selected and subjected to analysis by IR to identify its active chemical groups.

Statistical analysis: Data were statistically analyzed according to Snedecor and Cochran (1980). Means were compared using the least significant difference (L.S.D.) test at 0.05 significance level.

RESULTS AND DISCUSSION

1. Seed germination:

Data in Table (1) indicate that wildoat was the most sensitive plant to sweetclover ethanol extract followed by carrot and chicory. In contrast, no significant inhibition of germination was found for onion and lentil seeds.

The five tested plants could be arranged in ascending order according to inhibition percentages as follows: wildoat (80.5%), carrot (59%), chicory (28.9%), onion (10.7%) and lentil (0.0%).

2. Root and shoot length:

Data presented in Table (2) show that sweetclover ethanol extract inhibited seedling growth of wildoat more than carrot followed by onion and chicory. Also, root length was more sensitive than shoot length. In root length, inhibition percentages were 66%, 38.4%, 30% and 16% for wildoat, carrot, onion and chicory, respectively. In shoot length, inhibition percentages were 50.9%, 24.6%, 19.5% and 0.0% for the same mentioned plants. Both root and shoot lengths of chicory and lentil were not significantly affected by sweetclover extract.

The present results were in harmony with those obtained by many investigators. El – Mutlaq *et. al.* (2002) showed that the alkaloidal extract isolated from *Rhazya stricta* decreased seedling growth of alfalfa, wild radish, wheat and Italian ryegrass. Alfalfa was more sensitive to the extract than other plants and root growth was more sensitive than shoot growth in all tested plants.

Norsworthy (2003) indicated that wild radish aqueous extract suppress seed germination and radicle growth of wheat, corn and cotton. Wheat was most tolerant to the extract followed by corn and then cotton.

Abdallah *et. al.* (2002) assayed water extract of *Cyperus rotundus* and El–Mahy (2004) tested ethanol extract of *Euphorbia prostrata* and reported that both extracts inhibited seed germination and reduced growth of onion, carrot and cabbage.

Table (1): Effect of sweet clover ethanol extract on seed germination of tested plants.

Tested plant	Germination %	Control	L. S. $D_{(0.05)}$
Wild oat	18.7 (80.5)*	96.0	14.6
Carrot	36.0 (59.0)*	88.0	11.5
Chicory	64.2 (28.9)*	90.3	5.0
Onion	73.2 (10.7)*	82.0	NS
Lentil	100.0 (nil)*	100.0	NS

*Inhibition percentage (%) = ((control - treatment) / control) x 100.

Table (2): Effect of sweet clover ethanol extract on seedling growth of tested plants.

Tested plant	Length (cm)	Control	L. S. $D_{(0.05)}$
Wild oat Root	5.04 (66.0)*	14.85	3.07
Wild oat Shoot	6.23 (50.9)*	12.70	2.38
Carrot Root	1.96 (38.4)*	3.18	0.41
Carrot Shoot	3.71 (24.6)*	4.92	0.86
Chicory Root	2.56 (16.0)*	3.05	NS
Chicory Shoot	5.80 (nil)*	5.73	NS
Onion Root	3.57 (30.0)*	5.10	0.79
Onion Shoot	6.69 (19.5)*	8.32	0.92
Lentil Root	4.25 (nil)*	4.29	NS
Lentil Shoot	7.00 (nil)*	6.82	NS

*Inhibition percentage (%) = ()control - treatment) / control) x 100.

3. <u>Identification of chemical groups:</u>

Data in Table (3) reveal that ethanol extract of sweetclover exhibited five fractions having R_f values of 0.14, 0.29, 0.53, 0.68 and 0.90. Two fractions with R_f values of 0.53 and 0.68 showed inhibitory effect on germination and growth of wild oat. The other three fractions with R_f values of 0.14, 0.29 and 0.90 appeared to have no effect. The first active fraction (R_f = 0.53) significantly inhibited germination of wild oat seeds by 75%. Also, the same fraction caused significant inhibition in root and shoot length by 62% and 48.3%, respectively, as shown in Table (3).

Table (3): Effect of different components isolated from sweet clover ethanol extract on seed germination and seedling growth of wild oat.

R_f value	Germination %	Length (cm)	
		Root	Shoot
0.14	100.0 (nil)*	16.28 (nil)*	12.73 (nil)*
0.29	92.0 (2.1)*	14.57 (8.5)*	12.46 (nil)*
0.53	23.5 (75.0)*	6.05 (62.0)*	6.39 (48.3)*
0.68	0.0 (100.0)*	0.00 (100.0)*	0.0 (100.0)*
0.90	78.3 (16.7)*	13.93 (12.6)*	11.08 (10.5)*
Control	94.0	15.94	12.38
L. S. $D_{(0.05)}$	**25.31**	**3.07**	**2.24**

*Inhibition percentage (%) = ((control - treatment) / control) x 100.

Fig. 1

Fig. 2

The infrared (IR) spectrum of the same fraction, Fig. 1, indicates the presence of the following functional groups: CH – saturated (3000 cm^{-1}), ph. OH (1100 – 1150 cm^{-1}), Isocyanate NCO (2240 – 2275 cm^{-1}) and aldhyde or ketone (1660 – 1680 cm^{-1}).

The second active fraction (R_f = 0.68) gave complete inhibition in germination and seedling growth of wildoat. The IR spectrum of this fraction, Fig.2, was characterized by the following functional groups: Free OH (3500 cm^{-1}), acetylene C = C (2100 – 2150 cm^{-1}), methoxy group O – CH_3 (2900 cm^{-1}), carbonyl group C = O (1700 – 1740 cm^{-1}), aromatic ring (1350 – 1450 cm^{-1}) and saturated sulfoxide S = O (1070 cm^{-1}).

These results agree with those obtained by Abdalla (1997) who demonstrated that the crude extract of *E. prostrata* yielded four fractions having R_f values as follows: 0.18, 0.42, 0.64 and 0.82. The only fraction with R_f value of 0.82 was the most inhibitory, indicating 26.7% seed germination. *E. prostrata* ethanol extract may contain the following function groups as recorded by IR spectrum: ether, CH_2 or CH_3, HCO, P – O – ethyl, 1,3 disubstitution aromatic ring and free OH.

Iqbal *et. al.* (2003) mentioned that aqueous and ethyl acetate extracts of the aerial parts of buck wheat inhibited the root and shoot growth of lettuce seedlings. The ethyl acetate extract showed maximum activity and plants grown in the presence of the ethyl acetate extract showed severe root browning. The allelopathic constituents identified as gallic acid and (+) – catechin.

Laine *et. al.* (2004) reported that vetiver oil distilled from the roots of vetiver grass *Vetiveria zizanioids* may possess herbicidal activity. Vetiver oil inhibited germination and seedling expansion of *Chenopodium album, Amaranthus retroflexus and Ambrosia trifida.*

Ali (2005) found that aqueous extract and residue of *Cyperus rotundus* had allelopathic effect on the germination and growth characters of *Echinochloa crus – galli* and *Phalaris minor*. Allelopathic inhibitors identified as flavonoid and phenolic compounds such as rutin, kaempferol, quercetin and myricetin flavonoids as well as p. hydroxybenzoic, chlorogenic and ferulic phenolic acids.

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التأثير السام لمستخلص الحندقوق على انبات ونمو بادرات بعض الأنواع النباتية

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اجرى هذا البحث لدراسة التأثير السام للمستخلص الايثانولى لحشيشة الحندقوق على انبات ونمو بذور خمس أنواع نباتية وهى الزمير- الجزر- البصل- الشيكوريا- العدس، بالاضافة الى فصل وتعريف المجاميع الكيميائية الفعالة ذات التأثير المثبط لمستخلص الحندقوق باستخدام التحليل الكروماتوجرافى بالطبقة الرقيقة (TLC) وجهاز الأشعة تحت الحمراء (IR).

وقد أظهرت النتائج المتحصل عليها أن حشيشة الزمير كانت من اكثر الأنواع النباتية حساسية للمستخلص يليه الجزر فى حين لم يتأثر معنويا انبات بذور العدس أو نمو بادراته، كذلك لم يؤثر المستخلص الايثانولى للحندقوق تاثير معنوى على انبات بذور البصل ونمو بادرات الشيكوريا مقارنة بالكنترول. وبصفة عامة كان نمو الجذير أكثر حساسية للمستخلص من نمو الريشة. من ناحية أخرى أعطى مستخلص الحندقوق خمس بقع مفصولة وكان معدل السريان (قيم R_f) لها كالتالى: ٠.١٤- ٠.٢٩- ٠.٥٣- ٠.٦٨- ٠.٩٠، وكان اثنان منهـا(٠.٥٣- ٠.٦٨) ذات تأثير مثبط للانبات ونمو بادرات حشيشة الزمير لذلك تم تعريف المجاميع الكيميائية الفعالة فى البقعتان (٠.٥٣- ٠.٦٨) وهى مجاميع الاستيلين والميثوكسى والكربونيل، بالاضافة الى الأيزوسيانات والفينولات، وقد يعزى التأثير المثبط لمستخلص الحندقوق الى وجود هذه المجاميع الكيميائية النشطة.