Remarkable Influence of *Euphorbia Prostrata* Ait. and *oxalis Corniculata* L. Extracts on Seed Germination of Certain Weeds

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ABSTRACT

Euphorbia prostrata Ait. *and Oxalis corniculata* L. weeds tissue were extracted by methanol 80% and their effect against smooth pigweed *(Amaranthus hybridus* L.), oat *(Avena sativa* L.) chicory, *(Chichorium pwnpilum* Jacq.), and medic, (*Medicago intertexta,* L.) Mill) were investigated against seeds garmination in laboratory. The results showed that the two extracts inhibited root length of all tested weed seeds. *Euphorbia* extract inhibited root length of the smooth pigweed and medic giving LC_{50} of A10.92 ppm and 205.2 ppm, raspectively. *Oxalis* extract inhibited oat and medic reaching LC_{50} values 16.62 ppm and 79.76 ppm, respectively. *Euphorbia* extract inhibited shoot length of medic and chicory reaching LC_{50} values 89.3 ppm and 445.9 ppm, respectively. The LC_{50} values of *Oxalis* extrect against medic and smooth pigweed were 22.91 ppm and 66.82 ppm, respectively. GC/MS analysis for diethyl ether + methanol, (1 : 1) fraction showed that *Euphorbia* extract contained [2-furancarboxaldehyde, 5-(hydroxy methyl)], [phenol, 2-methoxy-4-vinyl], fatty acids [hexadecanoic, oleic, octadecanoic, linolenic and pentadecanoic and the ester of methyl propionic acid are the predominant compounds. In *Oxalis* extract, the following compounds were found: [phenol,2,6-bis(l,l-dimethylethyl-4-methyl)],octadecanoic, hexadecanoic, oleic and pentadecanoic acids. *Euphorbia* and *Oxalis* extracts exert an inhibitory effect explored the tendency of using it as herbicides for future weed management strategies.

INTRODUCTION

Allelopathic compounds occur in crop and weed plants in such ways that protect the producing plant against attacking pests. Weed species such as *Euphorbia prostrata* Ait. and *Panicitm repensl,* produced allelopathic chemicals which inhibited the competing plants either a crop or weed (Zaki *et al.,* 1994).

The aqueous extracts of *Euphorbia granulata* Forsk, significantly inhibited germination and radical growth of *Oxalis corniculata* L., *Cynodon dactylon, Setaria italica* and *Lactuca sativa* in laboratory bioassay (Farrukh, 2004). The extracts of different plants were found to inhibit the germination of *Amaranthus ratroflexus, Avena sterills, Rumex crispus* and *Trifolium repens,* (Kadioglu and Yanar, 2004). Allelochemicals are important potential source for new herbicides and agrochemicals, since they offer

new modes of action, more specific interactions with weeds and potentially less environmental damage (Vyvyan, 2002, Qasem and Foy, 2001, Batish *et al.*, 2002, Duke, 1986 a & b, Duke *et al.*, 1997 and Rice, 1995).

The objective of this research is (a): to investigate the influence of the crude extracts of *Euphorbia prostrata* Ait. and *Oxalis corniculata* L., against seed germination of some weeds, smooth pigweed (*Amaranthns hybridits* L.) oat (*Avena saliva* L.), chicory *(Cichorium pumpilum* Jucq*)* and medic *(Medicago intertexta* L, Mill) as test plants, (b): To identify the causative allelopathic compounds of the plant extracts. Furthermore, to start a new channel for weed management system using natural plant.

MATERIALS AND METHODS

Sampling and preparation of extracts:

Euphorbia prostrata Ait. and *Oxalis corniculata* L. weeds were obtained from the Faculty of Agriculture farm, Cairo Univ., Giza. Flora and phyto-taxonmoy were identified by the Herbarium Agricultural Research Center.

Euphorbia prostrata Ait., or *Oxalis corniculata* L. tissue was weighted (100 g), then they were cut into small pieces and covered with 200 ml methanol 80% and left overnight. The tissues were blended in blender at 400 rpm. The extracts were filtered through Buchner funnel under vacuum. The filtrates were evaporated at 40°C under vacuum in rotary evaporator near dryness. The residues were rinsed several times using ethanol 5% to reach 50 ml volume in measuring flask. Each stock extract was diluted appropriately with sterile distilled water to give the final concentration of 100, 50, 25, 12.5 and 6.25%. Distilled water was used as control. A Whatman No. 1 filter paper was placed in each 9 cm diameter glass Petri dish. Ten seeds of chicory, oat, and medic, and 20 seeds of smooth pigweed were placed in each Petri dish. Diluted extract of 5 ml were added by a pipette to the filter paper. Roots and shoots lengths and weight of the tested plants of all seedlings in each Petri dish were measured after 5 days from planting. The Petri dishes were covered and incubated according to the plant type.

Partitioning and fractioning of plant extracts:

The aqueous methanol extracts of *Euphorbia* and *Oxelis* tissues were prepared as described previously. The methanolic extracts (50 ml each) were mixed with an equal volume of ethyl acetate using separating

funnel (250 ml). The upper layer of ethyl acetate was taken and evaporated at 40°C under vacuum in rotary evaporator near dryness. The residues were extracted using ethanol 5% to reach 10 ml volume in measuring flask.

The procedure of partition and fractionation of plant extracts was carried out by transferring the extracts to chromatographic column (35 X 3.2 cm). A peace of glass wool was tamped down into the bottom of the column and 25 g silica gel (40 - 120 mesh) activated at 125°C for 4 h. was added. The column was filled with adequate quantity of petroleum ether (100 ml) and another quantity of sodium acetate (5 g) was added on the top of silica gel layer. The solvent was allowed to percolate down at slow rate until the column was entirely moistened. The solvents were used to remove relatively non-polar and polar compounds from the column are: petroleum ether (100%), petroleum ether + diethyl ether (50:50 %), diethyl ether (100%), diethyl ether + methanol (70 : 30 %), diethyl ether + methanol (50 : 50 %) and methanol (100%). The eluted samples (6 fractions/ 30 ml each) were concentrated to 2 ml in vacuum rotary evaporator. The residues were transferred using ethanol 5% to reach 10 ml volume in measuring flask. Each fraction compounds were examined for their germination inhibition of wheat grains in vitro (Petri dish bioassay technique). Diethyl ether + methanol (50 : 50 %) system was more proper for separating the phytotoxic compounds from *Euphorbia* and *Oxalis* extracts, respectively. These fractions were analyzed by GC/MS to identify those phytotoxic compounds. The more power phytotoxic fraction (diethyl ether/ methanol (1:1) of *Euphorbia* and *Oxalis* extracts were analyzed by GC/MS.

Statistical analysis:

Statistical analysis of all data wes carried out using the Ld-p Line program as that described by Finney (1971).

RESULTS AND DISCUSSION

Euphorbia prostrata Ait. and *Oxalis corniculata* L., extracts were assayed against germination of smooth pigweed, oat, chicory and medic weeds as shown in Tables 1-4. Both extracts inhibited germination of all tested weeds. *Oxalis* extract had more inhibitory effects against weeds than *Euphorbia* extract.

Root length:

The obtained results (Table 1) showed that the highest phytotoxicity as affected by *Euphorbia* extract was recorded with smooth pigweed, followed by oat, chicory and medic, respectively. *Euphorbia* extract inhibited root length of smooth pigweed and medic, giving LC_{50} values 10.92 ppm and 205.2 ppm. The inhibition ratio (1R) were 1.0 and 18.8, respectively. Phytotoxicity effect on the smooth pigweed was 18.79 fold as medic. The highest effect of *Oxalis* extract was more powerful on oat followed by chicory, smooth pigweed and medic, respectively. That highest inhibition effect was found on oat root length, while, the lowest inhibition effect was noticed on medic. The IR was 1.0 and 4.8, respectively. The LC_{50} values were 16.62 ppm and 79.76 ppm. Phytotoxicity on oat was 4.8 times compared to medic.

Shoot length:

The results in Table 2 revealed that *Euphorbia* extract inhibited shoot length of medic and chicory giving LC_{50} values 89.3 ppm and 446 ppm, respectively. Phytotoxicity effect of medic was 4.9 fold as chicory and the IR were 1.0 and 4.9, respectively. The LC_{50} values of *Oxalis* extract against medic and smooth pigweed were 22.91 ppm and 66.82 ppm, respectively. Phtotoxicity effect of medic was 2.9 fold as smooth pigweed and the IR were 1.0 and 2.9, respectively.

Rot weight:

The results showed that root weight was inhibited in all tested weeds as reference of using *Euphorbia* extract (Table 3). The LC_{50} values against smooth pigweed and medic root weight were 16.06 ppm and 196.2 ppm, and IR were 1.0 and 10.9, respectively. *Oxalis* extract inhibited smooth pigweed and midic reaching LC_{50} values of 5.11 ppm and 53.9 ppm, respectively. Phytotoxic effect f smooth pigweed was 10.5 fold as medic. The IR were 1.0 and 10.5, respectively.

Shoot weight:

The results presented in Table 4 indicated that highest inhibition effect by *Euphorbia* was fund in oat shoot weight, while, the lowest inhibition effect was noticed on smooth pigweed. The LC_{50} values were 33.97 and 63.29 ppm and IR were 1.0 and 1.9, respectively. Phytotoxic effect on oat was 1.9 fold compared to smooth pigweed. The LC_{50} values of *Oxalis* extract against oat and sooth pigweed were 16.12 ppm and 32.6

ppm and IR were 1.0 and 2.0, respectively. Phytotoxic effect of oat was 2.0 fold as smooth pigweed.

These results are in agreement with those obtained by Gonzalez *et al.* (2002) and Sayaka *et al.* (2005), who noticed that *Oxalis* spp. significantly reduced the weed population. Furthermore, a significant relationship was observed between the weed above ground biomass and the allelopathic activity of exudates from *Oxalis* spp. A Petri dish assay showed that plant extracts significantly reduced root growth of *Medicago setiv* L., *Echinochloa crus gali* and *Eclipta prostrate* L. The results may have value in enabling weed control based on natural plant extracts or crop residues in the fields, (Chon and Kim, 2004). Root and foliage aqueous extract of *Euphorbia prostrate* caused significant reduction in seed germination percentage (root and shoot lengths and fresh and dry weights) of all ornamental plants, turf grass and weeds (Mansour, 1991).

GC/MS analysis of diethyl ether + methanol (1:1) fraction showed that *Euphorbia prostrate* Ait. extract contained the predominant compounds: [2-furancarboxaldhyde,5-hydroxymethyl], [Phenol, 2-methoxy-4-vinyl], Fatty acids [hexadecanoic, oleic, octadecenoic, linolenic and pentadecanoic] and ester of methyl propionic acid. On the other hand, *Oxalis corniculata* L. extract contained the following compounds: [phenl 2.6-bis(1,1-dimethyl ethyl)-4-methyl], octadecanoic, hexadecanoic, oleic and pentadecanoic acids. These findings are in agreement with those obtained by Alsaadawi *et al.* (1992), who indicated that the aqueous extract of *Euphorbia prostrate* Ait, contained some inhibitory compounds, phenolic in nature. Additionally, Saleh (1997) concluded that *Euphorbia prostrate* Ait. extract might contain galic and ferulic acids as phenlic compounds. Kotob (2002) reported that the compounds of *C. fistula* extract identified by GC/MS in hexane were: linoleic acid which was dominating with high percent area 77.6, hexadecanoic, oleic and octadecanoic acid. The main ester identified was octadecanoic acid, methyl ester 6.44%, while hexadecanic acid was the main fatty acid detected (22.69%), (Sliman, 2001).

Therefore, the study reveals that *Euphorbia* and *Oxalis* extracts gave an inhibitory effect on seeds germination for all tested weeds. These results could lead to further detailed study to explore the herbicidal effect of these *Euphorbia* and *Oxalis* against seed germination of certain weeds. Moreover, they could lead for future new weed management strategies.

Table (1). Biological performance of *Euphorbia prostate* Alt. and *Oxalis corniculata* L. on root length of weed seeds

Crops	*Euphorbia prostate* Alt.			*Oxalis corniculata* L.		
	LC_{50} (ppm)	Index*	IR*	LC_{50} (ppm)	Index**	IR**
Smooth pigweed	10.910	1878.958	1.000	28.014	284.729	1.686
Oat	36.400	563.739	3.333	16.619	479.957	1.000
Shecory	45.381	452.174	4.155	18.672	425.136	1.129
Medic	205.201	100.000	18.790	79.764	100.000	4.800

* Index compared with medic, and Inhibition Ratio (IR) compared with smooth pigweed (IR = 1)
** Index compared with medic, and Inhibition Ratio (IR) compared with oat (IR = 1)

Table (2). Biological performance of *Euphorbia prostate* Alt. and *Oxalis corniculata* L. on shoot length of weed seeds

Crops	*Euphorbia prostate* Alt.			*Oxalis corniculata* L.		
	LC_{50} (ppm)	Index*	IR*	LC_{50} (ppm)	Index**	IR**
Smooth pigweed	119.453	373.336	1.337	66.816	100.000	2.916
Oat	276.651	161.200	3.097	58.425	114.362	2.550
Shecory	445.961	100.000	4.992	24.136	276.631	1.053
Medic	89.331	499.223	1.000	22.912	291.62	1.000

* Index compared with sheco, and Inhibition Ratio (IR) compared with medic (IR = 1)
** Index compared with smooth pigweed, and Inhibition Ratio (IR) compared with medic (IR = 1)

Table (3). Biological performance of *Euphorbia prostate* Alt. and *Oxalis corniculata* L. on root weight of weed seeds

Crops	*Euphorbia prostate* Alt.			*Oxalis corniculata* L.		
	LC_{50} (ppm)	Index*	IR*	LC_{50} (ppm)	Index**	IR**
Smooth pigweed	18.062	1086.270	1.000	5.110	1054.110	1.000
Oat	86.647	226.438	4.797	30.820	174.773	6.031
Shecory	117.738	166.643	6.519	12.182	442.169	2.384
Medic	196.202	100.000	10.863	53.865	100.000	10.541

* Index compared with medic, and Inhibition Ratio (IR) compared with smooth pigweed (IR = 1)
** Index compared with medic, and Inhibition Ratio (IR) compared with smooth pigweed (IR = 1)

Table (4). Biological performance of *Euphorbia prostate* Alt. and *Oxalis corniculata* L. on shoot weight of weed seeds

Crops	*Euphorbia prostate* Alt.			*Oxalis corniculata* L.		
	LC_{50} (ppm)	Index*	IR*	LC_{50} (ppm)	Index**	IR**
Smooth pigweed	63.289	100.000	1.863	32.797	100.000	2.034
Oat	33.965	186.336	1.000	16.122	203.430	1.000
Shecory	35.348	179.045	1.041	25.397	129.137	1.575
Medic	46.436	136.293	1.367	21.593	151.887	1.339

* Index compered with smooth pigweed, and Inhibition Ratio (IR) compared with oat (IR = 1)
** Index compared with smooth pigweed, and Inhibition Ratio (IR) compared with oat (IR = 1)

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الملخص العربي

تأثيرات جيدة لمستخلصات الأيوفوربيا والأكساليس على إنبات بذور بعض الحشائش

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أجريت دراسة معملية للمستخلص الميثانولي ٨٠% من الأيوفوربيا *Euphorbia prostrate* Ait. والأوكساليس *Oxalis comiculata* L. لمعرفة تأثير سمية هذه المستخلصات على إنبات بذور بعض الحشائش (عرف الديك ، الزمير ، الشيكوريا ، النفل) وقد أوضحت النتائج أن كلا المستخلصين لهما تأثير مثبط لإنبات بذور جميع الحشائش المختبرة . وبدراسة صفة طول الجذور : أعطى مستخلص الأيوفوربيا تأثير ضار على حشيشة عرف الديك بينما أظهر تأثيراً أقل على حشيشة النفل حيث كانت قيم LC_{50} هي ١٠,٩٢ جزء في المليون و ٢٠٥,٢ جزء في المليون على التوالي . بينما كان لمستخلص الأوكساليس تأثيراً ضاراً على حشيشة الزمير عن حشيشة النفل حيث كانت قيم LC_{50} ١٠,٦٢ جزء في المليون و ٧٩,٧٦ جزء في المليون على التوالي . ولصفة طول الساق : كان تأثير مستخلص الأيوفوربيا أكثر سمية على حشيشة النفل بعكس حشيشة الشيكوريا التي كانت الأقل تأثراً مسجلاً قيم LC_{50} ٨٩,٣ جزء في المليون و ٤٤٥,٩ جزء لي المليون . أما مستخلص الأوكساليس فكان أكثر سمية على حشيشة النفل وأقل سمية على حشيشة عرف الديك فكانت قيم LC_{50} ٢٢,٩١ جزء في المليون و ٦٦,٨٢ جزء في المليون على التوالي. أظهرت نتائج التحليل GC/MS لهذه المستخلصات أن الاستخلاص بنظام داي ايثيل أثير + ميثانول بنسبة ١ – ١ كان الأكثر تثبيطاً للنباتات المختبرة وأوضحت النتائج أن مستخلص الأيوفوربيا يحتوي على المركبات السائدة التالية : ٢ فيوران كربوكسي ألدهيد–٥–هيدروكسي ميثيل ، فينول–٢–ميثوكسي–٤– فينيل ، الأحماض الدهنية لكل من هكساديكانيك . أوليك ، أوكتاديكانيك ، لينولينك ، بنتاديكانيك وأسترميثيل بروبيونيك أسيد . بينما مستخلص الأكساليس احتوى على المركبات التالية : فينول –٦,٢–داي١,١–داي ميثيل ايثيل –٤–ميثيل ، أكتاديكانيك ، هكساديكانيك أوليك وبنتا ديكانيك أسيد

ويعتبر هذا التأثير المثبط لكل من الأيوفوربيا والأوكساليس هو خطوة تمهيدية قوية لاستخدام هذه المستخلصات كمبيدات حشائش في مستقبل إدارة مكافحة الحشائش .