Botanical Effect of *Euphorbia Prostrata* Ait. and *Oxalis Corniculata* L. Extracts on Some Crops and Vegetables

Khalil, N.A., A.S. Marzouk and A.M. Mohamed
Cent. Agric. Pest. Lab., Agric. Res. Cent. Dokki, Giza

ABSTRACT

The phytotoxicity effects of two common weeds; the prostrate spurge; *Euphorbia prostrata* Ait. (libbeina) and the creeping weed sorrel, *Oxalis corniculata* L. (homaid) extracts were studied on seed germination of wheat (*Trititicum aestivum*), maize (*Zea mays* L.) cucumber (*Cucumis sativus*), tomatoes (*Solanum lycopersicum*) and field pea (*Pisum sativum*). The results showed that both extracts inhibited both root and shoot length and reduced the weight of all studied plants. Also, *Oxalis* extract had more inhibitory effects on seed germination of all tested plants than *Euphorbia* extract. Tomatoes root length showed the height sensitivity, while, field pea showed the least when they were treated with *Euphorbia* extract. The LC_{50} values were 17.22 and 157.94 ppm, respectively. The LC_{50} values of *Oxalis* extract were 8.93 and 23.11 ppm, when it was added to tomatoes and cucumber, respectively. The highest inhibition effect on cucumber shoot length was gained when the seeds were treated with *Euphorbia* extract, while opposite findings were found in tomatoes. The LC_{50} values were 24.53 and 134.42 ppm, respectively. On the contrary, LC_{50} values were 8.7 and 56.42 ppm in tomatoes and maize, respectively when *Oxalis* was used. Diethyl ether + methanol (1:1 v/v) fraction revealed the most inhibition effects against seed germination of the tested plants. According to GC/MS analysis, *Euphorbia* extract contained the following compounds: [2-furancarboxaldehyde, 5-hydroxy methyl], phenol, 2-methoxy-4-vinyl, fatty acids [hexadecanoic], oleic, octadecanoic, linolenic and pentadecanoic. On the other hand, [phenol, 2-6-bis (1,1-dimethyl-4-methyl), octadecanoic hexadecanoic, oleic and pentadecanoic acids were the predominant compounds in *Oxalis* extract. The findings of bioassay for methanol extracts indicated that the inhibitory effect of the tested extracts was related to the level of allelochemicals found in plant extracts.

INTRODUCTION

Allelpathic compounds are produced in crops and weeds as a defense mechanism against attacking organisms. Previous work showed that weed species such as *Euphorbia prostrata* Ait. and *Panicum repens* L., produced allelopathic chemicals which inhibit the competing plants either a crop or weed (Zaki *et al.*, 1994). Growth of tomato seedlings *Lycopersicon esculentum* Mill and *Digitaria sanguinalis* L., were inhibited by 60% when leafy spurge *Euphorbia escula* L. leaves or roots extracts were added to soil (Steenhagen and Zimdahl, 1978). Root and foliage aqueous extracts of *Euphorbia prostrata* c`aused significant reduction in seed germination percentage (root and shoot lengths and fresh and dry weights) of all tested ornamental plants, turf grass and weeds (Mansour, 1991). Extract of

Euphoriba prostrata contain some inhibitory compounds which are phenolic in nature (Alsaadawi *et al.*, 1992) or phenolic and flavonoide compounds (Saleh, 1997). A significant relationship was observed between the weed above ground biomass and the allelopathic activity of exudates from *Oxalis* spp. (Sayka *et al.*, 2005). The compounds identified by GC/MS in hexane of *C. fistula* extract were: Linoleic acid (as a dominant with high percent area, 77.61%), hexadecanoic acid, Oleic acid and Octadecanoic acid. The main ester was Octadecanic acid, methyl ester (6.44%), while hexadecanoic acid was the main fatty acid detected (22.69%) (Soliman, 2001). Allelopathy, where plants release toxic substances into the soil either through root exudation or from decaying plant material can be exploited not only for sustainable cropping system in crop rotation to overcome the out-toxic problems but also for weed management system. (Rice, 1984).

The objective of this research is: (a) to investigate the effect of crude extracts of *Euphorbia prostrata* Ait. and *Oxalis corniculata* L. on seed germination of some crops i.e. wheat (*Triticum aestivum*) and maize (*Zea mays* L.), vegetables i.e. tomatoes (*Solanum lycopersicum*) and cucumber (*Cucumis sativus*) or field pea (*Pisum sativum*) as test plants, and (b) to identify the causative allelopathic compounds from the plant extracts.

MATERIALS AND METHODS

Sampling and extracts preparation

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

Euphorbia prostrata Ait. or *Oxalis corniculata* L. tissues were weighted (100 g) and then they were cut into small pieces and covered with 200 ml methanol 80% and left overmight. Tissues were blended in a 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 a final volume of 50 ml in a 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 was placed in each 9 cm diameter glass Petri dish. Ten grains of wheat and maize and seeds of tomatoes, cucumber and field pea 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 plant extracts:

The aqueous methanol extracts of *Euphorbia* and *Oxalis* 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 rinsed using ethanol 5% to reach a volume of 10 ml in a measuring flask.

The procedure of partitioning and fractionationing the plant extracts was carried out by transferring the extracts to chromatographic column (35 X 3.2 cm). A piece 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. Then, the solvent was allowed to percolate down at slow rate until the column was entirely moistened. The solvents used to remove relatively non - polar and polar compounds from the column were 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 against 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. 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 was carried out using the Ld-p Line program based on the probit analysis described by Finney (1971).

RESULTS AND DISCUSSION

Euphorbia prostrata Ait. and *Oxalis corniculata* L. extracts were assayed against wheat *(Triticum aestivum)*, maize *(Zea mays* L.*)*, cucumber *(Cucumber cucumis)*, tomatoes *(Solanum lycopersicuni)* and field pea *(Pisum sativum)* to determine its inhibition effect. Results showed that both extracts inhibited root and shoot length and reduced the weight of all tested plants. The degree of inhibition increased as the extract concentration increased. The extract concentration. The more powerful effect was found when the seeds were treated with *Oxalis* extract than *Euphorbia* extract.

Root length:

The effect of *Euphorbia* and *Oxalis* extracts on root length of the tested plants is presented in Table (1). It is evident that tomatoes showed the highest sensitivity to *Euphorbia* extract, followed by cucumber, wheat, maize and field pea, giving LC_{50} values 17.22 and 157.94 ppm, respectively the inhibition index of tomato was 9.17 fold as that of field pea, since inhibition ratios (IR) were 0.109 and 1.0, respectively. Tomatoes showed the highest sensitivity to *Oxalis* extract, followed by maize, wheat, field pea and cucumber. LC_{50} values of tomatoes and cucumber were 8.93 and 23.11 ppm. The inhibition index of tomatoes was 2.6 folds as that of cucumber and IR were 0.386 and 1.0 of tomatoes and cucumber, respectively.

Shoot length:

The obtained results (Table 2) showed that *Euphorbia* extract inhibited shoot length of cucumber and tomatoes giving LC_{50} values of 24.53 and 134.61 ppm, respectively. The inhibition index of cucumber was 5.5 fold as that of tomatoes, and IR were 0.18 and 1.0, respectively. The LC_{50} values of *Oxalis* extract against tomatoes and maize were 8.7 and 56.42, respectively. The inhibition index of tomatoes was 6.5 fold as that of maize, and IR were 0.154 and 1.0, respectively.

Root weight:

The results showed that root weight was inhibited in all tested plants when *Euphorbia* extract was used (Table 3). The LC_{50} values of maize and field pea were 46.92 and 195.85 and IR were 0.2 and 1.0, respectively. The inhibition index of maize was 4.2 fold as that of field pea. Also, *Oxalis* extracts giving similar effects. The LC_{50} values were 7.37 and 42.25 ppm

and IR were 0.174 and 1.0, respectively. The inhibition index of maize was 5.7 fold as that of field pea.

Shoot weigh:

The results presented in Table 4 showed a highest inhibition effect on field pea shoot weight, while, cucumber was found to be the least sensitive tested crop, when *Euphorbia* extract was treated. The LC_{50} values were 44.13 and 154.5 ppm, and IR were 1.0 and 3.52, respectively. Inhibition index of field pea was 3.5 fold as that of cucumber. The LC_{50} values of *Oxalis* extract against field pea and cucumber were 20.41 and 34.91 ppm and IR were 0.59 and 1.0, and the inhibition index of field pea was 1.7 fold as that of cucumber.

These results are in agreement with those obtained by Dharmaras *et al.* (1988), Zaki *et al.* (1994) and Saleh (1997), who reported that the ethanol crude extract of *Euphorbia* decrease root and shoot length of *Eruca sativa* L. to 47.1 and 56.6% as compared with control. Moreover, Gonzalez *et al.* (2002) and Sayaka *et al.* (2005) observed that the leachates and exudates from *Oxalis* spp., caused 78% inhibition of the radical elongation of lettuce seedlings. As the concentration of weed extracts increased, root growth of the tested plants was significantly reduced. The results may have value in enabling weed control based on natural plant extracts or crop residues in the fields (Chon and Kim, 2004).

GC/MS analysis showed that *Euphorbia* extract contained the following predominant compounds: [2-furancarboxaldhyde,5-hydroxymethyl], [phenol, 2-methoxy-4-vinyl], fatty acids [oleic, linolenic, hexadecanoic, octadecanoic and pentadecanoic] and ester methyl propionic acid. *Oxalis* extract contained the following compounds: [phenol 2.6-bis(,1-dimethyl ethyl)-4-methyl], hexadecanic, octadecanoic, pentadecanoic and oleic acids.

These findings are in harmony with those obtained by Alsaadawi *et al.* (1992), who observed that the aqueous extract of *Euphorbia prostrata* Ait. contained some inhibitory compounds which were phenolic in nature. Also, Saleh (1997) concluded that *Euphorbia prostrate* Ait. extract might contain galic and ferulic acids as phenolic compounds. Moreover, Kotob (2002) reported that the compounds of *C. fistula* extract which have been identified by GC/MS in hexane were: linoleic acid dominating with high percent area of 77.6, hexadecanoic, oleic and octadecanoic acid. The main ester identified was octadecanoic acid, methyl ester (6.44%), while

hexadecanoic acid was the main fatty acid detected (22.69%), (Soliman, 2001).

Allelpathy as a mechanism and future strategy for agricultural pests control and farm management and the potential use and development of some allelochemicals as a natural pesticides or plant growth regulators might be considered for the integrated pest management programes.

Table (1). Effect of *Euphorbia prostate* Ait. and *Oxalis corniculata* L. on root length of some crops and vegetables.

Crops	*Euphorbia prostate* Ait.			*Oxalis corniculata* L.		
	LC_{50} (ppm)	Index*	IR*	LC_{50} (ppm)	Index**	IR**
Wheat	29.720	531.417	0.188	17.326	133.395	0.750
Maize	73.018	216.299	0.462	15.747	146.771	0.681
Tomatoes	17.220	917.172	0.109	8.929	258.842	0.386
Cucumber	26.372	598.881	0.167	23.112	100.000	1.000
Field pea	157.937	100.000	1.000	23.102	100.043	1.000

* Index compared with field pea, and Inhibition Ratio (IR) compared with Field pea (IR = 1)
** Index compared with Cucumber, and Inhibition Ratio (IR) compared with Cucumber (IR = 1)

Table (2). Effect of *Euphorbia prostate* Ait. and *Oxalis corniculata* L. on shoot length of some crops and vegetables.

Crops	*Euphorbia prostate* Ait.			*Oxalis corniculata* L.		
	LC_{50} (ppm)	Index*	IR*	LC_{50} (ppm)	Index**	IR**
Wheat	78.584	171.289	0.584	33.820	166.821	0.599
Maize	85.358	157.696	0.634	56.419	100.000	1.000
Tomatoes	134.606	100.000	1.000	8.696	648.793	0.154
Cucumber	24.532	548.696	0.182	13.353	422.519	0.237
Field pea	89.411	150.547	0.664	23.653	238.528	0.419

* Index compared with tomatoes, and Inhibition Ratio (IR) compared with tomatoes (IR = 1)
** Index compared with maize, and Inhibition Ratio (IR) compared with maize (IR = 1)

Table (3). Effect of *Euphorbia prostate* Ait. and *Oxalis corniculata* L. on root weight of some crops and vegetables.

Crops	*Euphorbia prostate* Ait.			*Oxalis corniculata* L.		
	LC_{50} (ppm)	Index*	IR*	LC_{50} (ppm)	Index**	IR**
Wheat	79.861	246.495	0.406	25.663	164.622	0.607
Maize	46.921	419.541	0.238	7.371	573.152	0.174
Tomatoes	67.474	291.746	0.343	10.134	416.884	0.240
Cucumber	52.256	376.709	0.265	25.561	165.279	0.605
Field pea	196.853	100.000	1.000	42.247	100.000	1.000

* Index compared with peas, and Inhibition Ratio (IR) compared with Field pea (IR = 1)
** Index compared with peas, and Inhibition Ratio (IR) compared with peas (IR = 1)

Table (4). Effect of *Euphorbia prostate* Ait. and *Oxalis corniculata* L. on shoot weight of some crops and vegetables.

Crops	*Euphorbia prostate* Ait.			*Oxalis corniculata* L.		
	LC_{50} (ppm)	Index*	IR*	LC_{50} (ppm)	Index**	IR**
Wheat	74.981	206.072	1.699	31.545	110.645	0.904
Maize	74.661	206.955	1.692	24.531	142.281	0.703
Tomatoes	91.600	168.684	2.076	28.016	124.582	0.803
Cucumber	154.515	100.000	3.502	34.903	100.000	1.000
Field pea	44.125	350.176	1.000	20.407	171.034	0.585

* Index compared with cucumber, and Inhibition Ratio (IR) compared with Field pea (IR = 1)
** Index compared with cucumber, and Inhibition Ratio (IR) compared with cucumber (IR = 1)

REFERENCES

Alsaadawi, I.S.; S.J.H. Rizvi (ed.) and V. Rizvi. 1992. Allelopathic research activity in Iraq. Allelopathy: Basic and Applied Aspects, 53: 251-269.

Chon, S.V. and Y.Kim 2004. Herbicidal potential and quantification of suspected allelochemicals from four grass crop extracts. J. Agron. Crop Sci., 190 (2): 145-150.

Duke, S.O. 1986. Naturally occurring chemical compounds as herbicides. Rev. Weed Sci., 2: 15-44.

Duke, S.O.; F.E. Dayan; A. Hernandez; M.V. DMKe and H.K. Abbas 1997. Natural products as leads for new herbicide modes of action Brighton Crop Protection Conference, Weeds 2: 579-586.

Farrukh, H. 2004. Allelopathic effects of Pakistan weeds. *Euphorbia granulate* Forssk. Springer link Journal Article Biomedical and life sciences and Earth and Environmental Science, Journal, Oecologia, 45 (2): 267-269.

Finney, D.J. 1971. Probit analysis. 3rd edition. Cambridge University Press, Cambridge, U.K. PP. 333.

Gonzalez, G.H.; G.E. Zavala; R.K. Maiti; S. Moreno Limn; D.E. Lozano del. Rio and S. Martinez-lozano 2002. Effect of extracts of *Cynodn*

dactylon L. and *Sorghum halepenses* L. on cultivated plants. Crop Res. Hisar, 23 (2): 382-388.

Kotob, M.M.E.I. 2002. Phytochemical and Toxicological effect of plant extractives against *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hufnagel). M.Sc. Thesis, Faculty of Agriculture, Cairo University.

Mansour, A.A.1991. Effect of certain herbicides on some weeds, ornamental and turf plants with reference to Allelopathic effects. Ph.D. Thesis, Faculty of Agriculture, Cairo University.

Rice, L. 1984. Allelopathy, 2nd edn, Academic Press, New York, 422 PP.

Saleh, I.A.A.1997. Studies on allelopathic effects of certain weeds on some vegetable crops. M.Sc. Thesis, Faculty of Agriculture, Cairo University.

Soliman, M.M.M. 2001. Phytochemicals and Toxicological studies of some plant extracts against Aphis craccivora Koch. Ph.D. Thesis, Faculty of Agriculture, Cairo University.

Steenhagen, D.A. and R.L. Zimdahl.1978. Allelopathy of leafy spurge, *Euphorbia esula*. Weeds Science, 27 (1): 1-3.

Sayaka, S.; I. Watanabe, K. Kuno and Y. Fujii 2002. Allelopathic activity of leaching from dry leaves and exudates from roots of ground cover plants assayed on agar. Weed Biology and Management, 2 (3): 133.

Sayaka, S.; I. Watanabe, K. Kuno and Y. Fujii 2005. Evaluation of the allelopathic activity of five oxalidaceae cover plants and the demonstration of potent weed suppression by *Oxalis* species. Weed Biology and Management, 5 (3): 128.

Vyvyan, M.A.; H. El-Metwally and A.A. Mansour 1994. Allelopathic effects of certain weeds on some ornamental and turf plants. 5th EWRS Mediterranean Symposin. Weed control in sustainable agriculture in the Mediterranean area, Italy, Peragia: 171-178.

الملخص العربي
التأثير النباتي لمستخلصات الأيوفوربيا والأكساليس على بعض المحاصيل والخضر

ناجح عبد النور خليل – علاء سعد مرزوق – علاء الدين محسن محمد

قسم بحوث سمية المبيدات للنباتات – المعمل المركزي للمبيدات

مركز البحوث الزراعية – الدقي – الجيزة

تم استخلاص المجموع الخضري لحشائش الأيوفوربيا والأكساليس بمذيب الميثانول ٨٠% معمليا لدراسة تأثيرها السام على إنبات بذور بعض المحاصيل مثل القمح والذرة وبعض الخضروات مثل الخيار ، الطماطم ، البازلاء . أوضحت النتائج أن كلا من هذين المستخلصين ذو تأثير مثبط لإنبات النباتات المختبرة وأن مستخلص الأكساليس كان الأكثر تثبيطا من مستخلص الأيوفوربيا . عند دراسة صفة طول الجذر في النباتات المعاملة بالمستخلصات المختبرة كان مستخلص الأكساليس ذو التأثير الأكثر سمية على الطماطم بينما كان هذا التأثير قليلا على البازلاء حيث أعطى مستخلص الأيوفوربيا قيم LC_{50} قدرت بـ ١٧,٢٢ ، ١٥٧,٩٤ جزء في المليون على التوالي ومقدار دليل التثبيط للطماطم ٩,١٧ مرة مقارنة بالبازلاء ، بينما كانت قيم LC_{50} هي ٨,٩٣ – ٢٣,١١ جزء في المليون لمستخلص الأوكساليس على الطماطم الأعلى ضرراً والخيار الأقل تأثراً وكان مقدار دليل التثبيط للطماطم ٢,٦ مرة مقارنة بالخيار . وبالنسبة للتأثير على صفة طول الساق ، كان الخيار الأكثر تاثرا بينما الطماطم كانت الأقل حساسية لفعل مستخلص الأيوفوربيا حيث كانت قيم LC_{50} هي ٢٤,٥٣ ، ١٣٤,٦١ جزء في المليون على التوالي ومقدار دليل التثبيط للخيار وصل إلى ٥,٥ مرة مقارنة بالطماطم . أما مستخلص الأوكساليس ، فقد كان أكثر تأثيرا على الطماطم بعكس الذرة وأعطى قيم LC_{50} ٨,٧ ، ٥٦,٦١ على التوالي . ومقدار دليل التثبيط للطماطم ٦,٥ مرة مقارنة بالذرة . وقد أظهرت نتائج التحليل بجهاز GC/MS لهذه المستخلصات أن الاستخلاص بنظام داي ايثيل إيثر + ميثانول بنسبة ١-١ كان الأكثر تثبيطا 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 ، أوكتاديكاتيك ، لينولينك ، بنتاديكانيك واسترميثيل بروبيونيك آسيد . بينما مستخلص الأكساليس احتوى على المركبات التالية : فينول – ٦,٢ – داي ١,١ – داي ميثيل ايثيل -٤- ميثيل ، اكتاديكانيك ، هكساديكانيك أوليك وبنتا ديكانيك آسيد . وقد وجد أن التأثير المثبط لهذه المستخلصات له علاقة بمحتوى هذه النباتات من مركبات Allelochemicals والتي ربما تكون المسئولة عن هذا التأثير .