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ALLELOPATHIC BEHAVIOUR OF *CYPERUS ROTUNDUS* L. ON BOTH *CHORCHORUS OLITORIUS* (BROAD LEAVED WEED) AND *ECHINOCHLOA CRUS-GALLI* (GRASSY WEED) ASSOCIATED WITH SOYBEAN

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Abstract: Purple nutsedge (*Cyperus rotundus*) foliage and tubers were tested for allelopathic potential against the following weeds; jute *Chorchorus olitorius* (broad leaved weed), and barnyard grass *Echinochloa crus-galli* (grassy weed) associated with soybean. In the greenhouse, during 2008 and 2009, foliage and tubers of *C. rotundus* were mixed with soil surface at 20, 40, 60 and 80 g/kg. *C. rotundus* negatively affected those of jute and barnyard grass particularly at 80 g. Jute was more susceptible to allelopathy by *C. rotundus* than barnyard grass. Inhibition in weed dry matter was higher with tuber than foliage residues. Tuber residues reduced the dry weight of jute and barnyard grass by 85.96% of the control and by 58.28% with 80 g, respectively. On the other hand, soybean growth and yield showed a high significant increase compared with unweeded pots. A high-performance liquid chromatography analysis showed that *C. rotundus* foliage contained the following phenolic acids: caffeic, ferulic, coumaric, benzoic, vanelic, chlorogenic and cinnamic. Tubers contained hydroxybenzoic, caffeic, ferulic, vanelic and chlorogenic.

Key words: *Cyperus rutandus*, allelopathy, soybean, phenolic acids, *Chorchorus olitorius*, *Echinochloa crus-galli*

INTRODUCTION

Soybean (*Glycin max*) is one of the most important summer leguminous crops, extensively successful in Egypt and worldwide. Soybean contains a larger amount of protein and fat than any other type of pulses. Soybean contains all eight essential amino acids necessary for the human body to make protein. This characteristic separates it from the other types of pulses. Soybean is an excellent source of protein (Krishnan 2005). Soybean contains the chemical known as "Westin" Lecithin known to help break down the cholesterol in the blood (Polichett *et al.* 1996).

Weeds are serious pests that can cause damage to most crops. Weeds present in crop fields, compete with crop plants for light, moisture and other essential nutrients (Hussein 2001). As a result weeds reduce the quality and yield of crops and increase the cost of production.

The use of herbicides causes health problems and environmental contamination. Allelopathy can be defined as the effect of one plant on another plant through the release of chemical compounds in the environment (Rice 1984), or the growth suppression of one plant species by another due to the release of toxic compounds (Yongqing 2005). Many plant products are known to inhibit germination and growth of other plants. This is the property of all herbicides. Therefore, the plant product can be a possible alternative to synthetic herbicides and these may be used as natural herbicides (Mahmood and Cheema 2004). The idea of natural herbicides is the occurrence of the allelopathic phenomenon, which refers to bio-chemical interactions between all types of plants. Several workers reported the possibility of using allelopathy in weed control (McLaren 1986; Tawaha and Turk 2003; El-Rokiek *et al*. 2006).

Allelopathic substances are most commonly found in plant extracts and in plant residues in soil (Singh *et al*. 2003).

Many noxious annual and perennial weeds have been regarded as species with allelopathic potential and can severely affect crop survival and productivity (Qasem 1994, 2001). Purple nutsedge *(Cyperus rotundus)* is a perennial weed, widespread in cultivated fields in Egypt. It invades field crops as well as landscape.

This weed has been reported to possess high allelopathic activity against crops such as wheat (Alam and Azmi 1991), corn (Porwal and Mundra 1992; Chou Chang and Ming Yang 1992; Hamayun *et al*. 2005), rice (Quayyum *et al*. 2000); banana (Singh *et al*. 2009) and weeds. Ali (2005) reported that mixing ground tubers and leaves of *C. rotundus* with soil, inhibited barnyard grass and canary grass growth. In general, nutsedge species were found to posses allelopathic activity; Dirk and Jerry (1980) reported that *C. esculentus* residue reduced soybean and corn growth. Scott *et al*. (1992) found that *C. kyllingia* and *C. brevifolius* reduced the growth of *Cynodon dactylon*.

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The objective of the present work was to investigate the allelopathic effect of foliage and tubers of purple nutsedge on both *Chorchorus olitorius* (broad leaved weed) and *Echinochloa crus-galli* (grassy weed) associated with soybean.

MATERIALS AND METHODS

Preparation of plant materials

The stock of purple nutsedge (*C. rotundus* L.) used as a source of tubers was collected from a dense stand at the National Research Centre garden. One hundred plastic pots (30 cm height, 30 cm diameter) were filled with a soil mixture (clay/sand, 2 : 1) and the collected tubers placed inside. There were 2 tubers per pot. Sowing was carried out on August of 2007 and 2008. The tubers were left to grow for 2 months. Irrigation was carried out when needed. Two months later the plants were harvested. The foliage and tubers (underground parts) were then separated and oven dried at 40°C for 48 hours. The dried foliage and tubers were ground to a fine powder (passed through a 1.5 mm mesh). The prepared powder was kept in Pyrex vacuum desiccators for use when needed.

The investigation was carried out in the greenhouse of the National Research Center, Dokki, Cairo, Egypt, for two successive summer seasons 2008 and 2009. Soybean seeds cv. Giza 111 were obtained from the Agricultural Research Center, Ministry of agriculture, Giza, Egypt. Ground, dried foliage or underground materials (tubers) were mixed thoroughly with the surface of the potted soil mixture at a rate of 20, 40, 60 and 80 g/kg. The ground, dried foliage or underground materials were placed in pots which had a 30 cm diameter (17 cm height). They were frequently irrigated with tap water for one week to allow natural decay of the foliage or tubers of *C. rotundus* residues. Then, the pots were sown with soybean seeds (5 seeds/pot) in June under average maximum and minimum temperature 35.5±1 and 18.5±1°C. The pots were infested with *C. olitorius* (broad-leaved weed) and *E. crus-galli* (grassy weed) at a consistent seed weight. All pots were infested with the same weight of the weed seeds. Weed seeds were sown simultaneously and mixed thoroughly at a 2 cm depth in the soil. Two weeks later, thinning of soybean was done so that two homogenous soybean seedlings were left per pot. Ammonium nitrate and super phosphate (2 : 1 w/w) were added for each pot during plant growth.

The experiment consisted of 10 treatments including two control treatments. The control treatments were without the addition of *C. rotundus residues* (free-weed and unweeded). In each treatment 9 pots were used. The pots were arranged in a completely randomized design. Samples of weeds as well as soybean plants were taken from three pots at each stage.

Weed samples

The infested weeds were collected from each pot after 30 and 60 days from sowing. Weed samples were taken from three pots at each stage (all weed samples in each pot were pulled up). The data on fresh and dry weight of grown weeds were recorded.

Soybean

Data on Soybean growth were recorded as the two plants in each pot were pulled up (three pots in each stage). Some morphological and growth characteristics of soybean plants were recorded for each individual plant after 30 and 60 days from sowing. The recorded characteristics included plant height (cm), number of leaves/plant, and fresh and dry weight/plants (g). At the end of the season, soybean yield and its components including, number of pods/ plant, weight of seeds/pod, weight of seeds/plant and weight of 100 seeds were also calculated for each treatment.

Determination of some chemical changes

Determination of total carbohydrate contents in soybean seeds

Total carbohydrate contents were extracted from dry finely ground soybean seeds (powdered). Total carbohydrates were extracted according to Herbert *et al*. (1971) and estimated colourimetrically by the phenol-sulphuric acid method as described by Montogomery (1961).

Determination of protein contents in soybean seeds

Protein contents were determined in dried seeds according to the method described by Lowery *et al*. (1951).

Determination of oil contents in soybean seeds

Oil contents were determined in soybean dried seeds according to the method described by AOCS (1964). In this method a known weight of ground sunflower seeds was imbedded in 50 ml of petroleum ether. Oil was extracted using a Soxhlet apparatus.

Phenolic acid contents by HPLC of the experimented extracts

Phenolic acids in the tested aqueous extracts of foliage and tubers of purple nutsedge were extracted as follow: 2 g of dried foliage or tubers of *C. rotundus* were immersed in 100 ml distilled water for 7 days, and filtered. The filtrate was subjected to separation by HPLC with the following condition: mobile phase acetonitrile (86%), Buffer 14% (pot. dihydrogen phosphate: phosphoric acid, 2 : 1v/v), flow rate 1 ml/min; Agilent 1 100 series (Waldborn, Germany), quaternary pump (G1311A), Degasser (G1322A), Thermostated Autosamples (G1329A), variable wave length detector (G1314A); and column: Zorbax 300SB C18 column (Agilent Technologies, USA). Injection was carried out at wave lengths 254, 280, and 320 nm for separation of different phenolic acids.

Statistical analysis

The data were subjected to standard analysis of variance by means; LSD was obtained when F values were significant at 5% level (Snedecor and Cochran 1980).

RESULTS

Weed growth

All applied rates of foliage or tuber residues of *C. rotundus* delayed growth of both broad leaved weed (*C. olitorius*) and grassy weed (*E. crus-galli*) compared with the untreated control (Table 1). Inhibition in weed growth showed different responses depending on the type of residue, their rates and the type of weed.

In general, growth inhibitions of both weeds were higher in response to tuber residues. There was a maximum significant inhibition in weed growth recorded by 80 g tuber residues in comparison to the untreated control. However, control of *C. olitorius* was consistent during the experimental period, while control of *E. crus-galli* was inconsistent. The rate of reduction in dry weight of *C. olitorius* increased during the experimental period, while there was a decreased in the case of *E. crus-galli* compared with the untreated control. The reduction in fresh weight averaged from 32 to 80% in *C. olitorius* and from 21 to 71% in *E. crus-galli* 30 days after sowing. The reduction in dry weight reached 85.96% in *C. olitorius* by applying 80 g of tuber residues, and 58.28% in *E. crus-galli*, 60 DAS (days after sowing).

Soybean growth

The phytotoxic effects of the *C. rotundus* residues on weed growth were concomitant with a significant enhancement effect on soybean growth. Plant height significantly increased with applying foliage or underground residues of *C. rotundus*. The response to tubers was greater. The highest significant increase was 64.3% over the unweeded control with the application of tuber residues at 80 g (60 DAS) (Table 2). Number of leaves exhibited a highly significant increase with the application of 80 g of *Cyperus* residues. Number of leaves was less variable with other rates compared to the unweeded control. In addition, the increase in fresh weight was persistent in recording a high significant increase with 80 g residues. The tuber residues were more effective. The response in dry matter contents was more or less similar, maximum content was 114.26% over unweeded control. Weed-free pots had the highest significant increase in dry matter content (133.5% over the unweeded control).

Table 1. Allelopathic effects of foliage or tubers of *C. rutundus* on growth of *E. crus-galli* (narrow weed) and *C. olitorius* (broad leaved weed). (Average of the two seasons)

Treatments [g/ kg soil]		FW [g]				FW [g]		DW [g]	
		broad	narrow	broad	narrow	broad	narrow	broad	narrow
Controls	weed free	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	unweeded	15.16	18.03	2.11	5.05	26.36	40.43	5.34	9.66
Foliage parts	20	10.36	14.23	1.22	3.82	16.13	31.00	1.33	8.66
	40	7.23	9.96	0.88	3.47	12.16	30.50	0.93	8.44
	60	5.60	7.63	0.80	2.81	8.20	29.20	0.88	8.01
	80	4.55	7.13	0.71	2.14	6.76	27.40	0.86	7.42
Tubers	20	9.13	12.56	1.15	4.04	15.70	29.20	1.62	7.12
	40	6.93	8.60	0.84	3.31	11.93	27.50	0.89	6.83
	60	5.06	6.06	0.75	1.56	8.16	26.40	0.83	6.52
	80	3.06	5.20	0.36	1.40	6.60	20.80	0.75	4.03
LSD at 5% level		0.874	0.905	0.074	0.379	2.873	3.426	0.145	0.543

FW – fresh weight, DW – dry weight, DAS – days after sowing, Broad – broad-leaved, weed – *C. olitorius*, Narrow – grassy weed – *E. crus-galli*

Table 2. Allelopathic effects of foliage or tubers of *C. rutundus* on growth of soybean. (average of the two seasons)

Treatments [g/ kg soil]		30 DAS				60 DAS			
		pl. height [cm]	No. leaves/ pl.	FW/pl. [g]	DW/pl. [g]	pl. height [cm]	No. leaves/ pl.	FW/pl. [g]	DW/pl. [g]
Controls	weed free	64.72	6.33	6.56	2.87	142.1	17.6	62.5	14.08
	unweeded	43.00	3.33	3.33	1.44	79.3	7.0	24.0	6.03
Foliage parts	20	43.00	4.00	3.76	1.60	84.00	7.7	25.4	9.70
	40	52.66	4.66	5.70	1.71	110.4	8.3	35.8	9.86
	60	61.50	5.00	5.80	2.21	115.6	10.5	42.6	10.75
	80	60.00	5.66	6.53	2.51	125.2	13.1	50.6	11.78
Tubers	20	46.25	4.00	4.06	1.90	101.1	8.0	26.0	7.43
	40	53.50	4.66	4.50	2.00	112.1	8.0	44.0	11.38
	60	56.00	5.33	6.20	2.45	116.3	11.0	45.6	11.89
	80	67.00	6.00	6.80	2.60	130.3	16.6	51.8	12.92
LSD at 5% level		3.454	0.649	0.471	0.227	6.754	1.223	6.327	1.078

pl. – plant; DAS – days after sowing; FW – fresh weight; DW – dry weight days after sowing

Soybean yield

Foliage and tuber residues of *C. rotundus* induced a significant increase in soybean yield and its components over the untreated control. A marked significant increase was observed with higher rates especially tubers. However, a significant increase was observed to a lesser extent with lower rates of residues (Table 3). The increase in number of pods/plant and number of seeds/pod recorded by 80 g tuber residue increased significantly reaching 67.34 and 90.58% over the unweeded control. In addition, weight of pods/plant, weight of seeds/pod, weight of seeds/plant and weight of 100 seeds recorded a high economic return in response to application of different rates of foliage and tuber residues. The increase in weight of pods/plant averaged from 40.6 to 184.6 over the unweeded control. Moreover, weight of seeds/pod ranged from 40 to 96.36% over the unweeded control.

Furthermore, seed yield/plant recorded a high net return which reached 222.6 over the untreated control with 80 g tuber residue. A similar trend was observed in the weight of 100 seeds in which there was a marked significant increase over the untreated control (43.6%). It is worth mentioning that all yield parameters in weed-free pots exceeded the recorded values due to different rates of foliage or tuber residues (Table 3).

Table 3. Allelopathic effects of foliage or tubers of *C. rutundus* on yield of soybean. (average of the two seasons)

Treatments [g/kg soil]		No. of pods/ plant	Wt. of pods/ plant	No. of seeds/ pod	Wt. of seeds/ pod	Wt. of seeds/ pant	Wt. of 100 seeds
Controls	weed free	50.00	39.51	4.09	1.14	44.20	23.20
	unweeded	28.60	10.40	2.55	0.55	13.50	15.81
Foliage parts	20	29.06	14.62	2.96	0.77	16.23	16.36
	40	31.60	18.60	3.07	0.91	17.14	17.02
	60	41.60	21.5	4.53	0.93	21.31	19.30
	80	45.00	24.3	4.64	1.00	25.12	21.33
Tubers	20	30.33	18.04	3.58	0.88	15.71	16.90
	40	34.00	19.16	3.67	0.90	20.53	17.30
	60	44.06	21.68	4.42	0.99	24.52	20.66
	80	47.86	29.60	4.86	1.08	43.55	22.70
LSD at 5% level		1.865	1.273	0.228	0.060	1.955	1.286

Wt – weight

Some chemical constituents in the yielded seeds

Carbohydrate content (%)

It is important to estimate carbohydrate, protien and oil contents in the yielded seeds for evaluating seed quality. The extract of foliage or tuber residues in the soil could have variable effects starting from nonsignificant, with 20 g foliage, to a highly significant increase with 80 g of tubers (Table 4). Carbohydrate contents in the yielded seeds get maximum benefits when applying 80 g of tubers. Carbohydrate contents reached 99% over the value recorded in the yielded seeds in unweeded pots.

Table 4. Allelopathic effects of foliage or tubers of *C. rutundus* on some chemical constituents of the yielded soybean seeds. Average of the two seasons

Treatments [g/kg soil]		% of carbohydrate	% of protein	% of oil
Controls	weed free	35.13	42.48	18.39
	unweeded	17.24	27.69	11.62
Foliage parts	20	17.89	29.26	15.74
	40	22.02	31.57	17.80
	60	28.22	33.61	18.54
	80	29.49	34.24	19.07
Tubers	20	22.64	31.58	18.06
	40	26.33	32.81	18.39
	60	30.11	37.07	18.86
	80	34.31	38.34	19.65
LSD at 5 % level		1.171	2.868	0.464

Protein content (%)

A significant increase in seed protein contents was detected with most of the treatments. An additional increase in protein occurs with the increase in the rates of foliage and tuber residues (Table 4). Accumulation of protein in the yielded seeds in weed free pots reached maximum level (53.4 over the unweeded control) followed by the seeds treated with 80 g of tuber residues (38.55% over the unweeded control).

Oil contents

Total oil contents extracted from the yielded soybean seeds exhibited a significant increase in all rates of foliage and tuber residues of *C. rotundus* over oil extracted from the yielded seeds in the unweeded pots. Furthermore, oil contents recorded with 80 g of tubers exceeded that recorded in seeds of weed free pots (69% over unweeded control).

DISCUSSION

The plant is a vast source of naturally occuring and selective herbicide. Selective herbicide may be extracted from the powdered portions of plants such as the flower, leaves, stem and roots. The extract could control or kill the growth of weeds that will absorb the nutrients of the soil which is needed by crops. Some of these plants inhibit seed germination and growth of other plants by means of producing toxic allelochemicals. Allelochemicals are the secondary metabolites produced by plants (Chon *et* *al*. 2003). Therefore, understanding plant interactions is important to be able to reduce the dependency on herbicides in future cropping systems. (Olofsdotter *et al*. 2002).

The results of the present investigation indicate that different rates of the extract of *C. rotundus* in soil, inhibited the growth of weeds (Table 1). The results indicate that this inhibition depended on the rate of application. The data also point out that *C. olitorius* (broad leaved weed) was more susceptible to allelopathic extract of *Cyperus* foliage and tubers than *E. crus-galli* (grassy weed). The reduction in weed growth of *C. olitorius* was consistent during the experimental period. In general, inhibition in the growth of many weed species in response to different plant extracts is well documented by many investigators (Olofsdotter *et al*. 2002**;** Duke *et al*. 1998; El-Rokiek *et al*. 2006, 2009). Aqueous extracts of *Cyperus* were also documented (Alam and Azmi 1991; Porwal and Mundra 1992; Chou Chang and Ming Yang 1992; Quayyum *et al*. 2000; Hamayun *et al*. 2005) and recently by Singh *et al*. (2009). The negative effect of *C. rotundus* extracts on weed growth is similar to that obtained by Ali (2005). The great suppression in weed growth may be attributed to the presence of toxin compounds. In our work the toxin compound is phenolic acid (Table 5). Similar results in plant extracts were found by Chon *et al*. (2003); Chung *et al*. (2003); Singh *et al*. 2003; Chon and Kim 2004 who attributed the highly allelopathic herbicidal potential in plant extracts to the presence of allelopathic substances e.g. coumarin, *o*-coumaric acid, *p*-coumaric acid, benzoic acid, P-hydroxybenzoic acid, and ferulic acid. The results confirmed by El-Rokiek 2007.

Table 5. Constituents of the phenolic acids in the extracts of foliage and tubers of *C. rotundus*

Phenolic acids	% in foliage	% in tubers
Ferulic	8.74	35.81
Coumaric	12.01	0.00
Benzoic	24.14	0.00
Vanelic	25.04	33.67
Chlorogenic	15.61	3.17
Caffiec	4.63	21.73
Gallic	0.37	0.00
Hydroxybenzoic	0.00	5.60
Cinnamic	9.42	0.00

The results of the present work indicate that aqueous extracts of foliage and tubers of *C. rotundus* had a great influence on the growth of soybean (plant height, number of leaves, fresh and dry weight).

The above mentioned positive effects on the growth of soybean would explain the increased net return (Table 3) as number of pods/plant and number of seeds/pod, weight of pods/plant, weight of seeds/pod, weight of seeds/pant and weight of 100 seeds.

It should also be noted that the increase in soybean growth and yield treated with different aqueous extracts of *C. rotundas*, was accompanied by a corresponding decrease in weed growth. Weed competition reduced yield in untreated pots, while the allelopathic negative effect of *Cyperus* extract on both broad and grassy weeds reduced weed competition and increased soybean yield (Table 3). Consequently, there was an increased net return in treated pots; number of pods/plant, weight of pods/plant, number of seeds/pod, weight of seeds/pod, weight of seeds/pant and weight of 100 seeds.

In general, these results coincided with those of other workers who reported that controlling weeds decreased yield loss and increased net return (El-Rokiek *et al*. 2006; El-Metwally and El-Rokiek 2007; Abdelhamid and El-Metwally 2008). Hence, increasing growth and yield were concomitant with an increase in the different metabolic activity such as total carbohydrates, protein and oil contents in the yielded seeds (Table 4).

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POLISH SUMMARY

ALLELOPATYCZNE ZACHOWANIE SIĘ *CYPERUS ROTUNDUS* L. NA *CHORCHORUS OLITORIUS* I *ECHINOCHLOA CRUS-GALLI* ZWIĄZANYMI Z SOJĄ

Badano allelopatyczny potencjał liści i bulw *Cyperus rotundus* przeciwko następującym chwastom: *Chorchorus olitorius* (juta) i *Echinochloa crus-galli* (chwastnica jednostronna) związanych z soją. W latach 2008 i 2009 w szklarni ulistnienie i bulwy *C. rotundus* mieszano z powierzchniową warstwą ziemi w proporcji – 20, 40, 60 i 80 g/kg. *C. rotundus* negatywnie wpływał na jutę i *E. crus-galli*, szczególnie stosowany w ilości 80 g/kg. Juta była wrażliwsza na allelopatię *C. rotundus* niż *E. crus-galli*. Inhibicja suchej masy chwastu była niższa w przypadku bulw niż resztek ulistnienia. Resztki bulw, w ilości 80 g/kg, redukowały suchą masę juty i chwastnicy jednostronnej odpowiednio o 85,96 i 58,28%. Z drugiej strony wzrost soi oraz plon wykazywały wysoce istotny wzrost w porównaniu z poletkami nieodchwaszczonymi. Analiza wykonana metodą wysoce wydajnej chromatografii płynnej wykazała, że ulistnienie *C. rotundus* zawierało kwasy fenolowe i kwas kofeinowy.