

EVALUATING THE PHYSIOLOGICAL INFLUENCE OF BENZOIC AND CINNAMIC ACIDS, ALONE OR IN COMBINATION ON WHEAT AND SOME INFESTED WEEDS COMPARING WITH THE HERBICIDE ISOPROTURON

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Keywords: Benzoic acid, Cinnamic acid, Isoproturon herbicide, Allelopathy

ABSTRACT

A Petri dish assay was carried out for screening different concentrations of the two phenolic acids, benzoic acid and cinnamic acid on germination and seedling growth of wheat, chard (*Beta vulgaris*, as an example of broad leaf) and oat (*Avena fatua*, as an example of grasses). Seed germination, seedling root and shoot lengths of wheat as well as the above mentioned weeds were completely inhibited with the treatments of benzoic acid at 1000 ppm in combination with cinnamic acid (500&1000 ppm). studies were conducted in the greenhouse of National Research Center, Egypt in the two winter seasons, 2004/2005 and 2005/2006 on the bases of the preliminary work (Petri dish assay) to assess the effects of the two phenolic acids benzoic and cinnamic acids alone or in combination on some infested associated weeds as well as the growth and establishment of wheat (*Triticum aestivum*) compared with the recommended dose of the herbicide Arelon (isoproturon). The length of the shoot and the fresh and dry weights of wheat were significantly increased as well as grain yield when wheat was sprayed with phenolic acids especially in mixture. While, the mixture of two phenolic acids (benzoic and cinnamic) at 500 + 1000 ppm was inhibitory. This inhibition was accompanied with

severe reduction in total amino acid contents in the yielded grains. However, the fresh and dry weights of infested broad (*Beta vulgaris*) and narrow (*Avena fatua*) weeds were significantly reduced by the herbicide Arelon and all the concentrations of the two acids used either alone or in combination as compared with the control. Even the lowest concentration of the individual acids was inhibitory, indicating phytotoxic effects. The effect of the herbicide was higher. To determine the possible contribution of the phenolic acids in controlling weeds, the studies involved estimation of the endogenous contents of total phenols in both broad and narrow weeds. These studies were also performed on wheat. With all treatments, the inhibitory effects on both weeds were correlated with accumulation of internal contents of total phenols compared to respective controls. A significant amount of the total phenols was found to be present in wheat tissues. The amount of phenols correlated well with growth performance especially in weeds. This study establishes the effect of the two phenolic acids benzoic and cinnamic acids to weeds in wheat, which may serve as possible tools in establishing their herbicidal potential.

INTRODUCTION

Unknown compounds in crop plants are inhibitory to seed germination and early seedling growth of weed plants (Chon and Kim, 2004). Allelopa-

thy can be defined as the effects of one plant on another plant (including microorganisms) 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 (Lambers *et al* 1998).

The evidence suggests that plant phenolics play a major role in allelopathy (Inderjit, 1996). The allelopathic potential of simple phenols, benzoic and cinnamic acid derivatives is well documented in the literature (Rice, 1984). Moreover, various phenolics have been implicated in allelopathic interactions among algae, fungi, bryophytes, pteridophytes, gymnosperms, and angiosperms. A significant amount of water-soluble phytotoxins (the phenolic) was found to be present in the soil infested with *Ageratum conyzoides* (Singh *et al* 2003). In addition, among nine phenolic compounds assayed for their phytotoxicity on root growth of alfalfa, coumarin, *trans*-cinnamic acid and *o*-coumaric acid were most inhibitory, hydro-cinnamic acid were detected as the highest amount in barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) extracts (Chon and Kim, 2002 and 2004). They reduced significantly root growth of alfalfa (*Medicago sativa* L.), barnyard grass (*Echinochloa crus-galli*, Beauv. var. *oryzicola* Ohwi.) and eclipa (*Eclipta prostrata* L.). Similar results were reviewed by (Barkes and Putman, 1987; Wu *et al* 2001 and Kato-Noguchi *et al* 2002). Chon *et al* (2003) attributed the highly allelopathic herbicidal potential of some plant extracts of the family *Compositae* to the presence of causative allelopathic substances e.g. coumarin, *trans*-cinnamic acid, *o*-coumaric acid and *p*-coumaric acid. As well, Chung *et al* (2003) found that *p*-hydroxybenzoic acid, *p*-coumaric acid and ferulic acid were highly produced from rice extract and showed the greatest inhibitory effect on barnyardgrass growth. Furthermore, Soybean growth was significantly reduced by 0.5 mM *p*HBA, or higher concentrations, with the degree of inhibition being concentration dependent (Barkosky and Einhellig, 2003).

So, Significance of mixtures should be considered when one seeks to illustrate phenolic allelopathy as an important ecological mechanism of plant interference. Blum, (1996) reported that mixture of phenolic allelochemicals and other organics present in substratum can cause inhibitory effects even though the concentration of individual allelochemical is below required levels to cause allelopathic effects. Various workers have shown

that additive or partially antagonistic activities are responsible for the observed effects in natural systems (Netzly *et al* 1988; Ben-Hammouda *et al* 1995), and chances of only one phenolic compound causing allelopathic effects are remote (Einhellig, 1995). In addition, Macias *et al* (1998) suggested that allelochemicals are biocommunicators that active in mixtures because of the increasing the number of findings in which single compound is not active as mixture. The use of natural compounds in plant protection has been also reviewed by (Macias, 1993; Reigosa *et al* 1999b; Inderjit *et al* 2002; Serantes *et al* 2002).

Since allelopathic activities in nature are largely due to the presence of several compounds in a mixture, the present study advances understanding of the joint action of binary combination of benzoic acid and cinnamic acid in a mixture that mostly extracted by plants and enabling weed control based on natural plant extracts or crop residues in the fields and evaluating these compounds as compared to the herbicide isoproturon.

MATERIALS AND METHODS

Laboratory screening

A Petri dish assay was carried out for screening different concentrations of benzoic acid, cinnamic acid or their mixtures on germination and seedling growth of wheat, chard (*Beta vulgaris*, as an example of broad leaf) and oat (*Avena fatua*, as an example of grasses).

Seeds were allowed to germinate in Petri dishes containing 1-layer filter paper Whatman No. 3 contained 6 ml of the following treatments: Benzoic acid at 250, 500 or 1000 ppm
Cinnamic acid at 250, 500 and 1000 ppm
Benzoic acid at 250 ppm + cinnamic acid at 250, 500 or 1000ppm.
Benzoic acid at 500 ppm + cinnamic acid at 250, 500 or 1000ppm.
Benzoic acid at 1000 ppm + cinnamic acid at 250, 500 or 1000ppm.
Distilled water

The germination was carried out in the laboratory on November with average maximum and minimum temperature 25.5 and 13.5°C. Each treatment was represented by five replicates; each Petri dish represented one replicate, five days later 2ml of the previous treatments were added. Records on percentage of germination, seedling root and shoot lengths of wheat plants and weeds were taken 10 days after germination.

Pot experiments

Pot experiments were conducted under greenhouse conditions at the National Research Centre, during two successive winter seasons (2004 /2005 and 2005 /2006) on the bases of the preliminary work (Petri dish assay). Wheat grains were obtained from Agricultural Research Centre, Egypt, were sown (8 grains / pot) in pots (30 cm diameter). The pots were filled with silty clay soil and infested with seeds of chard at 10 seeds / pot (*Beta vulgaris*) as an example of broad leaf and seeds of oat as an example of grass weeds (10 seeds/ pot). Weed seeds (10 seeds /pot) were sown simultaneously and mixed thoroughly at 2 cm depth from the soil. The pots were watered as required. Thinning was done after 2 weeks so that 3 homogeneous wheat seedlings were left per pot. Routine fertilizers were added as a mixture of calcium super phosphate and ammonium sulfate, representing sources of N, P...

The treatments were applied foliarly 30 days after sowing as follow:

Benzoic acid at 250 or 500 ppm

Cinnamic acid at 250, 500 and 1000 ppm

Benzoic acid at 250 ppm + cinnamic acid at 250, 500 or 1000ppm

Benzoic acid at 500 ppm + cinnamic acid at 250, 500 or 1000ppm

Arelon (isoproturon) at 1.25 L / feddan (3000ppm)

Hand weeded

Untreated unweeded control.

The experiment consisted of 14 treatments including the control and hand weeded.

Each treatment was represented by 9 pots. The pots were distributed at complete randomized design. Samples were taken from three pots at each stage.

The infested weeds were collected from each pot 50 and 70 days after sowing. Samples of all wheat plants were taken from each pot (three plants) 50 and 70 days after sowing to measure plant height (cm), fresh and dry weight (g). At the end of the season wheat yield and it's components including spike length (cm), number of spikes / plant, number of spikelets /spike, weight of 1000 grains (g) and weight of grains / plant were calculated.

Combined data of the two seasons were statistically analyzed according to **Little and Hills (1978)**.

Determination of some chemical changes

Total phenols

Total phenolic compounds in both wheat and weeds were extracted from dry finely ground tissues (powdered). Drying of tissues was carried out in an electric oven at 60 °C till constant weight.

Total phenols were determined colorimetrically according to the method defined by **Snell and Snell (1953)** using Folin and Ciocalteu phenol reagent.

Total amino acids in the yielded grains

Hydrolysis was carried out according to the method of **Spitz (1973)** as follow. A known weight of grinded grains was placed in a test tube with screw cap; 10ml of 6N HCl was added. The test tubes were transformed to an electric oven at 110 °C for 24 hours. The extract was filtrated, then transferred quantitatively to evaporating flask and allowed to remove HCl under vacuum

Total amino acid analyses were performed on an Eppendorf-Germany LC 3000 amino acid analyzer. The operating conditions were: flow rate, 0.2ml / min.; Pressure of buffer from 0 to 50 bars; pressure of reagent from 0 to 150 and reaction temperature 123°C.

RESULTS AND DISCUSSION

Germination bioassay test:

The data included in **Table (1)**: illustrate that the percentage of germination of both broad and narrow weed seeds as well as wheat grains negatively affected by benzoic acid, cinnamic acid at different concentrations alone and their mixtures as compared to untreated control. It is clear from **Table (1)** that benzoic acid at 1000ppm alone inhibited the germination of chard seeds (broad-leaved weeds) completely. In addition, the mixture of benzoic acid at all concentrations (250, 500 &1000 ppm) with high concentrations of cinnamic acid (500 &1000 ppm) inhibited completely the germination of chard seeds (broad-leaved weeds). The results recorded in oat (narrow-leaved weeds) are more or less similar. Meanwhile, germination of wheat grains was inhibited completely by combination of highest concentration of benzoic acid (1000 ppm) with cinnamic acid (250-1000 ppm).

Table 1. Effects of benzoic acid, cinamic acid alone or their mixtures on the percentage of germination; seedling root and shoot lengths of chard (*Beta vulgaris*), oat (*Avena fatua*) and wheat (*Triticum aestivum* L.) cv. Gimmiza 9.

Treatments	Concentration (ppm)	Chard (Broad leaved)			Oat (Narrow leaved)			Wheat cv. Gimmiza 9		
		% of germination	Root length (cm)	Shoot length (cm)	% of germination	Root length (cm)	Shoot length (cm)	% of germination	Root length (cm)	Shoot length (cm)
Benzoic acid	250	100.00	25.00	49.70	100.00	100.30	100.38	100.00	117.00	132.50
	500	33.50	13.50	26.40	47.80	81.68	81.65	100.00	86.68	120.55
	1000	0.00	0.00	0.00	20.00	2.93	4.93	100.00	29.03	45.45
Cinnamic acid	250	100.00	25.00	65.00	100.00	119.60	121.80	100.00	129.96	131.65
	500	33.85	23.30	61.15	33.85	112.10	114.10	100.00	117.20	111.20
	1000	33.30	5.00	30.00	25.00	3.70	6.38	90.00	64.06	73.20
Benzoic acid + Cinnamic acid	250+250	33.20	12.50	30.00	33.20	12.50	22.35	70.00	73.86	116.65
	250+500	0.00	0.00	0.00	0.00	0.00	0.00	70.00	58.15	98.05
	250+1000	0.00	0.00	0.00	0.00	0.00	0.00	60.00	49.96	86.65
Benzoic acid+ Cinnamic acid	500+250	0.00	0.00	0.00	0.00	0.00	0.00	60.00	34.40	76.65
	500+500	0.00	0.00	0.00	0.00	0.00	00.00	40.00	20.55	69.68
	500+1000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzoic acid + Cinnamic acid	1000+250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1000+500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1000+1000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0	100.00	45.00	90.00	100.00	141.75	133.35	100.00	181.70	183.45
LSD	at 5 % level	1.46	1.90	3.52	1.26	3.17	3.37	4.58	6.41	4.94
	at 1 % level	2.07	2.70	5.00	1.78	4.48	4.76	6.48	9.06	6.99

The results also show that root and shoot seedlings of both weeds significantly reduced under control due to benzoic and cinnamic acids alone or in mixtures. The reduction reached 100% (complete inhibition) in chard by benzoic acid alone at 1000 ppm or the mixtures of benzoic and cinnamic acids at all concentrations. This result was obtained in oat by the combination of benzoic acid and cinnamic acid at most concentrations used. Wheat seedlings recorded similar results by mixtures of both acids especially at high concentrations.

Pot experiments

Weeds

Table (2) shows that both fresh and dry weights of broad and narrow weeds were significantly reduced by the herbicide Arelon or phenolic acids alone or their mixtures after 50 and 70 days from sowing. The reduction caused by the herbicide in fresh weights of both broad and narrow weeds were higher (93.5 and 77.1 % inhibition, 50 days after sowing), the reduction reached 91.45 and 86.30 % 70 days after sowing. The greatest significant reduction caused by phenolic acids was recorded by 500 + 1000 of benzoic and cinnamic acids respectively in fresh weight of both broad and narrow weeds (86.23 and 70.0 %) after 50 days from sowing. This reduction in fresh weight recorded 86.16 and 80.13% after 70 days from sowing. The reduction in dry weight exhibited similar trend at both stages.

Changes in phenolic contents in weeds

It is clear from the results given in Table (3) that there were great differences between the contents of total phenols in weeds treated with different concentrations of benzoic acid or cinnamic acid alone or in combination and untreated weeds (broad and narrow). Accumulation of total phenols was detected with the concentration of 500 + 1000 ppm of benzoic and cinnamic acids respectively in both broad and narrow leaves weeds at both stages in comparison to the corresponding controls.

Wheat growth

Table (4) indicates that foliar application of wheat plants with the two phenolic acids alone didn't result in significant changes, in the number

of branches per plant, at any of the two recorded stages. In contrast, a significant increase of branching was obtained in response to treatment with the herbicide Arelon as well as mixtures of the two phenolic acids as compared to the control.

As illustrated in Table (4) no significant differences were detected in plant height due to foliar application of single phenolic acids at the first stage (after 50 days from sowing). Meanwhile, significant increases were detected by all treatments alone or in mixtures of both acids as well as the herbicide Arelon. Maximum increase was obtained with the herbicide treatment followed by mixture of benzoic acid and cinnamic acid at 500 + 500 ppm. However, significant reduction was obtained by the mixture at 500 + 1000 ppm. Fresh weights at both vegetative and flowering stages (Table 4) recorded great significant increases with the mixtures especially at the concentration of 500+500 ppm (81.55 and 121.55 %). The increase in fresh weight due to this treatment was accompanied by dry matter accumulation especially at the flowering stage (144.55%) as compared to the untreated control. On the other hand, the highest concentration of both benzoic and cinnamic acid mixtures (500+1000) inhibited both fresh and dry weights of wheat as indicated by the results in Table (4).

Yield and yield components

Table (5) shows that single application of benzoic or cinnamic acid had no significant effects on the number of spikes / plant. Significant responses of this criterion were realized starting from 1000 ppm cinnamic acid in addition to application of the mixtures at all concentrations used as well as the herbicide Arelon in comparison to the control. Maximum increase was detected with the treatment of the herbicide followed by mixture of benzoic and cinnamic acids at 500+ 500 ppm.

Generally, spike length exhibited significant increase due to treatments with benzoic at 500 ppm or cinnamic acid at 1000 ppm. Moreover, great significant increase in spike length was recorded with mixtures especially at 500 + 500 ppm.

The results in Table (5) illustrate that most treatments showed significant increases in number of spikelets / spike. Pronounced significant increase was observed with the herbicide followed by mixture treatments particularly at 500 + 500 ppm. in comparison to the control.

Table 2. Effects of benzoic acid, cinamic acid alone or their mixtures on the growth of chard (*Beta vulgaris*) and oat (*avena fatua*) after 50 and 70 days from sowing (Combined analysis of the two seasons)

Treatments	Concentration (ppm)	After 50 days from sowing						After 70 days from sowing					
		Fresh weight (g)			Dry weight (g)			Fresh weight (g)			Dry weight (g)		
		Broad leaved	Narrow leaved	Total weeds	Broad leaved	Narrow leaved	Total weeds	Broad leaved	Narrow leaved	Total weeds	Broad leaved	Narrow leaved	Total weeds
Benzoic acid	250	6.86	2.83	9.69	1.180	0.476	1.656	9.93	6.47	16.40	1.800	1.567	3.367
	500	4.25	1.75	6.00	0.943	0.333	1.276	8.36	4.56	12.92	1.396	1.333	2.729
Cinnamic acid	250	7.26	3.25	10.51	1.230	0.553	1.783	10.13	6.83	16.90	1.833	1.627	3.460
	500	5.10	3.00	8.10	0.950	0.460	1.410	8.53	6.00	14.53	1.616	1.433	3.049
	1000		2.60	6.76	0.803	0.301	1.104	7.16	4.13	11.29	1.293	1.270	2.563
Benzoic acid+ Cinnamic acid	250+250	4.08	2.30	6.38	0.735	0.251	0.984	6.33	4.03	10.36	1.033	1.234	2.267
	250+500	3.23	1.60	4.83	0.693	0.238	0.931	4.53	3.06	7.59	0.890	1.333	2.223
	250+1000	2.86	1.50	4.36	0.633	0.232	0.865	3.83	2.87	6.70	0.670	0.833	1.503
Benzoic acid+ Cinnamic acid	500+250	2.56	1.40	3.96	0.511	0.200	0.711	2.96	2.50	5.46	0.503	0.733	1.236
	500+500	1.46	1.27	2.73	0.293	0.188	0.481	2.40	1.96	4.36	0.483	0.656	1.139
	500+1000	1.06	1.05	2.11	0.216	0.114	0.330	1.83	1.45	3.28	0.453	0.466	0.919
Arelon	3000	0.50	0.80	1.30	0.051	0.090	0.141	1.13	1.00	2.13	0.346	0.387	0.733
Free weed control		0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.000	0.000	0.000
Unweeded control		7.70	3.50	11.20	1.323	0.657	1.980	13.23	7.30	20.53	2.566	2.034	4.600
LSD at 5% level		0.39	0.20	0.44	0.038	0.019	0.044	0.99	0.51	1.21	0.147	0.140	0.298

Table 3. Effects of benzoic acid, cinamic acid alone or their mixtures on endogenous total phenol contents in both broad and narrow weeds after 50 and 70 days from sowing

Treatments	Concentration (ppm)	Total phenols (mg / g dry weight)			
		Broad leaved weeds		Narrow leaved weeds	
		After 50 days	After 70 days	After 50 days	After 70 days
Benzoic acid	250	15.94	18.04	13.26	14.99
	500	16.09	18.91	14.04	15.14
Cinnamic acid	250	19.84	22.26	14.81	15.31
	500	20.37	23.16	15.91	16.60
	1000	21.08	25.10	17.13	17.90
Benzoic acid+ Cinnamic acid	250+250	21.3	26.02	19.21	19.42
	250+500	21.65	26.34	20.78	21.90
	250+1000	22.15	28.10	23.27	25.06
Benzoic acid+ Cinnamic acid	500+250	26.70	31.23	24.33	26.30
	500+500	27.23	32.68	27.24	28.16
	500+1000	40.51	41.30	33.08	35.58
Arelon	3000	38.47	39.41	31.01	33.06
Unweeded control	-	15.57	16.26	13.05	14.45
LSD at 5% level		1.15	1.88	0.94	1.68

Table 4. Effects of benzoic acid, cinamic acid alone or their mixtures on the growth of wheat (*Triticum aestivum* L.) cv. Gimmiza 9 after 50 and 70 days from sowing (Combined analysis of the two seasons)

Treatments	Concentration (ppm)	Days after sowing (DAS)							
		50days				70 days			
		Number of branches /plant	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Number of branches /plant	Plant height (cm)	Fresh weight (g)	Dry weight (g)
Benzoic acid	250	1.66	55.00	6.90	0.880	2.88	81.00	14.80	3.226
	500	2.00	58.50	7.73	0.960	2.99	84.00	16.90	3.700
Cinnamic acid	250	2.00	54.50	6.00	0.800	2.55	77.16	12.20	2.853
	500	2.00	55.73	6.20	0.850	2.88	83.00	16.20	3.500
	1000	2.33	55.83	6.26	0.950	3.00	84.50	18.30	4.300
Benzoic acid+ Cinnamic acid	250+250	2.33	60.00	6.68	0.950	3.88	85.00	19.50	5.000
	250+500	2.33	60.16	8.40	0.966	4.11	85.33	23.16	5.333
	250+1000	2.66	60.76	9.33	0.983	5.00	86.66	24.20	5.663
Benzoic acid+ Cinnamic acid	500+250	3.00	60.83	9.66	1.040	5.00	87.50	24.50	5.816
	500+500	3.00	62.00	10.53	1.168	5.00	89.00	25.70	6.400
	500+1000	1.33	47.00	4.80	0.450	2.00	64.00	11.00	2.213
Arelon	3000	3.33	62.10	10.80	1.300	5.00	91.16	27.00	6.500
Free weed control	-	3.33	63.00	11.11	1.550	5.00	92.66	28.00	6.850
Unweeded control	-	1.66	54.00	5.80	0.750	2.11	72.00	11.60	2.617
LSD at 5 % level		0.79	2.69	1.08	0.148	0.92	4.23	2.04	0.534

Table 5. Effects of benzoic acid, cinamic acid alone or their mixtures on yield and yield components of wheat cv. Gimmiza 9 (Combined analysis of the two seasons)

Treatments	Concentration (ppm)	Plant height (cm)	No of spikes / plant	Spike length (cm)	No of spiklets/ spike	Weight of grains/spike (g)	Weight of 1000 grains (g)	Grain yield /plant (g)	straw yield /plant (g)
Benzoic acid	250	95.66	4.00	10.32	18.93	1.63	40.00	9.26	2.633
	500	98.66	4.00	11.00	19.46	1.88	40.50	9.53	3.833
Cinnamic acid	250	94.33	3.66	10.12	18.60	1.56	39.00	8.56	2.566
	500	95.66	4.00	10.73	19.34	1.78	40.11	9.23	3.133
	1000	101.66	4.50	11.20	20.70	1.90	40.50	9.55	3.533
Benzoic acid + Cinnamic acid	250+250	101.66	4.66	11.50	21.16	2.00	42.66	10.00	3.953
	250+500	102.66	4.75	11.84	21.66	2.16	43.00	12.33	4.033
	250+1000	105.00	4.90	11.90	21.66	2.20	43.00	13.23	4.133
Benzoic acid + Cinnamic acid	500+250	106.66	5.00	12.00	21.80	2.40	47.50	13.22	4.166
	500+500	108.33	5.16	12.46	22.16	2.56	48.65	13.26	4.500
	500+1000	88.66	3.37	9.12	17.83	0.70	33.08	5.40	1.783
Arelon	3000	111.63	5.16	12.66	23.00	2.78	49.66	14.13	4.700
Hand weeded	-	113.00	5.53	12.76	23.40	2.88	50.66	14.37	5.000
Unweeded Control	-	91.00	3.65	10.10	18.40	1.20	36.33	8.00	2.300
LSD at 5% level		6.75	0.44	0.62	0.99	0.43	2.76	1.05	0.512

The data in **Table (5)** reveal significant increases in weight of grains / spike in response to all treatments used alone or in mixtures as compared to the control. This effect was more detectable with mixtures as well as the herbicide.

Gradual significant increase was obtained in weight of 1000 grains (g) starting from benzoic acid or cinammic acid each alone and their mixtures. This increase reached maximum value at the concentration 500 + 500 ppm of benzoic acid and cinnamic acid mixture (33.91 % increase over the control). In addition, great significant increase was recorded with the herbicide treatment (36.69 %). Moreover, grain yield / plant (g) was more or less similar to this trend as indicated by the results in **Table (5)**.

The increase in grain yield / plant (g) reached pronounced value with the herbicide Arelon (76.63 % increase over the control) followed by the mixture at 500 +500 ppm (65.75 % increase over the control). In general, the greatest significant increase was recorded with free weed control treatment.

Total phenol contents in wheat tissues

Table (6) indicates increase in total phenol contents due to treatments with the two phenolic acids alone or in combination as compared to the untreated control. However, drastic accumulation was accompanied with dual treatments especially at 500 ppm benzoic acid and 1000ppm cinnamic which caused further increase. At contrast to weeds, total phenols resulted from all concentrations of phenolic acids alone or in mixture nearly made weeds under stress. The endogenous phenols resulted from this concentration (500 +1000 ppm), the only one that made wheat plants under stress.

Total content of individual amino acids in wheat grains

Due to the importance of level of individual amino acids and their balance in nutrition, they were estimated as shown in **Table (7)**.

Table 6. Effects of benzoic acid, cinamic acid or their mixtures on endogenous total phenol contents in wheat plants cv. Gimmiza 9 after 50 and 70 days from sowing

Treatments	Concentration (ppm)	Total phenols (mg / g dry weight) in Wheat plants	
		After 50 days	After 70 days
Benzoic acid	250	22.04	23.38
	500	25.42	25.94
Cinnamic acid	250	23.06	24.03
	500	26.51	27.74
	1000	26.56	28.06
Benzoic acid+ Cinnamic acid	250+250	24.13	26.40
	250+500	24.53	25.44
	250+1000	25.24	26.21
Benzoic acid+ Cinnamic acid	500+250	27.49	28.05
	500+500	27.55	29.18
	500+1000	30.12	31.88
Arelon	3000	24.02	23.47
Free weed control	-	21.18	20.29
Unweeded control	-	21.72	21.05
LSD at 5% level		1.53	1.56

Total amino acids increased due to phenolic acid treatments as compared to the control. Pronounced increase in total amino acids was recorded with the mixture of benzoic and cinnamic acids at the concentration of 500 +500 ppm. On the other hand, severe reduction was obtained with the concentration of 500 + 1000 of the same mixture at which there was a great reduction in total amino acids. Individually, there was marked increase by 500 + 500 ppm in aspartic, glutamic acids, valine, phenylealanine, tyrosine and arginine. On the other hand, aspartic and glutamic acids were decreased due to the mixture at 500+ 1000 ppm, valine, phenylealanine and tyrosine as well. It is worthy to mention that the contents of total amino acids estimated in grains yielded from wheat treated with the herbicide was lesser than that of mixture at 500 + 500 ppm.

DISCUSSION

The possible use of secondary metabolites has long been used (Macias, 1993). Although the physiological and ecological actions of

allelochemicals less than the commercial herbicides (Reigosa, *et al* 1999a), They have advantages of great biodegradation (Einhellig, 1993; Macias, 1993). The effects of benzoic and cinnamic acids alone or their mixtures on weed growth fit the pattern of allelochemical effects in that inhibition was concentration dependent (Barkosky and Einhellig, 2003). To what extent does individual phenolic acids under test or their mixture affect the growth of both broad and narrow leaves weeds as well as growth and yield of wheat plants. The effects of these treatments were traced, either alone or combined together.

Evidence is available that both phenolic acids under test inhibit the growth of weeds. The results obtained in (Table 2) reveal that foliar application of phenolic acids especially in dual treatments caused pronounced significant decreases in the fresh and dry weights of both broad weeds (*Beta vulgaris*) and grasses (*Avena fatua*) at the two stages of growth and development particularly at 500 +1000 ppm of benzoic and cinnamic acids, respectively. Inhibition of growth of many weed species in response to application of phenolic

Table 7. Effects of benzoic acid, cinnamic acid alone or their mixture on total amino acid contents in the yielded grains of wheat cv Gimmiza 9

Treatments	Concentration (ppm)	Amino acids (mg / 100g dry weight)																	Total	
		Asp	Theri	Seri	Glut	Prol	Gly	Alan	Cyst.	Val	Meth	Leu	Isoleu	Phen	Tyr	Hist	Lys	Arg		NH ₄
Benzoic acid	250	12.50	7.04	19.15	127.32	55.81	13.68	14.32	0.00	17.72	1.18	14.46	26.84	14.61	19.23	10.20	7.83	120.31	17.37	499.57
	500	21.14	0.61	17.04	163.66	27.93	24.68	14.23	0.00	23.24	1.44	22.6	29.99	11.06	26.07	16.32	15.79	159.19	27.80	602.79
Cinnamic acid	250	12.70	7.08	17.8	104.75	43.44	14.26	16.51	0.00	19.57	0.00	15.32	28.86	14.74	17.59	7.79	8.54	110.33	19.97	459.25
	500	13.35	7.69	18.11	118.43	51.99	16.18	16.63	0.00	17.28	1.62	16.56	31.74	17.84	17.72	10.25	8.70	116.57	20.94	501.60
	1000	23.89	0.29	30.08	126.22	00.00	16.34	18.39	0.00	29.12	2.03	20.37	42.53	11.47	11.12	15.65	16.63	169.42	28.42	561.97
Benzoic + Cinnamic acids	250+250	20.2	10.29	26.7	197.83	00.00	25.95	25.34	0.00	23.55	3.37	25.01	48.68	28.02	30.89	12.08	12.35	147.97	31.68	661.91
	250+500	28.08	15.42	28.71	154.32	28.01	23.11	22.04	0.00	30.52	3.11	26.36	58.33	18.52	14.22	13.08	22.55	193.68	3.73	713.79
	250+1000	22.64	12.03	30.74	198.18	78.42	21.37	23.48	0.00	30.34	3.47	26.66	49.66	22.62	30.25	14.56	14.48	149.25	34.18	762.34
Benzoic + Cinnamic acids	500+250	18.33	1.48	25.95	192.63	81.28	36.41	25.12	0.00	38.31	6.33	30.48	58.32	33.84	31.60	13.76	14.94	231.72	48.88	889.38
	500+500	32.37	9.45	41.16	246.87	77.17	26.42	25.74	0.31	37.75	0.52	24.11	43.92	10.11	32.21	21.69	15.13	291.87	48.45	985.25
	500+1000	4.06	5.05	11.69	43.74	113.28	3.86	11.60	0.00	11.12	0.76	10.51	20.48	9.83	7.12	7.68	5.04	15.33	11.60	292.75
Arelon	3000	35.16	12.44	28.10	156.22	89.26	25.14	28.43	0.00	33.65	8.66	45.21	52.74	17.55	18.46	10.08	18.88	196.11	34.54	809.63
Free weed	-	29.33	13.18	25.39	240.55	85.41	20.23	22.53	0.00	30.25	5.18	44.54	54.28	34.45	33.58	15.46	16.88	244.26	83.24	998.74
unweeded	-	15.29	7.82	18.43	129.57	00.00	6.49	14.62	0.00	16.26	0.003	17.41	32.32	20.57	19.11	10.57	8.41	109.22	19.30	445.393

acids was also recorded by (Rice, 1984; Blum, 1996; Inderjit, 1996; Reigosa *et al* 1999b; Serantes *et al* 2002 and Barkosky and Einhellig, 2003). The results realized with mixture came in agreement with Ben-Hammouda *et al* (1995); Blum (1996) and Macias *et al* (1998) who reported that mixture of phenolic allelochemicals and other organics present in substratum can cause inhibitory effects even though the concentration of individual allelochemical is below required levels to cause allelopathic effects.

On the other hand, the above mentioned conclusion did not agree with that of Inderjit *et al* (2002) who mentioned that no evidence for synergistic activities of phenolic acids in the mixture was noted on perennial ryegrass (*Lolium perenne* L.).

Table (2) also reveals that The degree of inhibition of weed species correlated with the endogenous contents of total phenols in these weeds as indicated in Table (3) which shows that the greatest significant increase in total phenolic content in weeds was accompanied with great inhibitions of weed growth which approximate to 82 and 77 % inhibition in broad and narrow weeds (Table 2). This was obtained with the mixture concentration, 500 +1000 ppm. This may explain that accumulation of phenols is often a characteristic of stress condition. Thus, the elevation of phenolics, indicate a stressful condition (Nemat Alla and Younis, 1995; Ahmed and Rashad, 1996; El-Rokiek, 1996) to weeds under test. This result could be reinforced by that of (Hopkins, 1999 and El-Rokiek, 2002).

Foliar application of the two phenolic acids (benzoic and cinnamic acids) each alone or in mixture as well as the herbicide Arelon on crop plants seemed likely to be promising in increasing growth of wheat.

The results of the present work indicate that phenolic acid mixtures had a greater influence on the extension of shoot growth of wheat than individual ones. The increase in shoot growth of wheat plants treated with phenolic acids was accompanied by greatly significant increases in the fresh and dry weights at the two stages of development (Table 4).

The above mentioned positive effects on the growth of wheat would explain the increased yield of these plants (Table 5). The increase in yield resulted from an increase in the number of spikes / plant, number of spikelets / spike, which was reflected on the weight of grains per plant (grain yield / plant), as well as the weight of 1000 grains.

It should be pointed out, herein, that the increase in wheat growth and development as well as increase in yield of plants treated with phenolic acids or the herbicide Arelon showed alliance with the corresponding decrease in the fresh and dry weights of both broad and narrow weeds. Many workers reported that isoproturon controls weeds and increased grain yields (Metwally *et al* 1999; Metwally and Hassan, 2001; El-Desoki and Metwally, 2001; Walia and Brar, 2003 and Chhokar *et al* 2006). In general, these results coincided with those of other workers who reported that controlling weeds decreased yield loss and increased net return (Singh *et al* 1995; Mathiasen *et al* 2000; Rapparini *et al* 2000; and Tharp *et al* 2004).

We didn't observe inhibition in the amino acid contents of the yielded grains after addition of benzoic acid, cinnamic acids individually or their combination at most concentrations. Stress produced by application of those two allelochemicals on mixture at concentration 500 + 1000ppm. This application involved a great damage to amino acid synthesis at which total amino acid contents diminished severely. It caused a decrease of total amino acid contents with alteration in the levels of individual ones. It should be pointed out, herein, that this treatment resulted in increased level of proline as compared with all other individual amino acids in the same treatment and proline in other treatments, while, control plants had zero proline what may be indicated that plants under stress (Yoshiba, *et al* 1997).

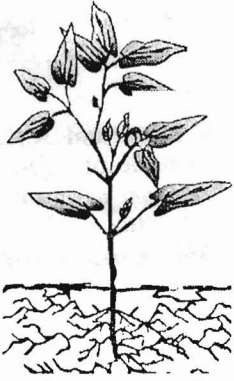
The decrease in total amino acids may be due to decrease in amino acid synthesis (Cooley and Foy, 1992). These results coincided with this author because the decrease in total amino acids correlated with growth inhibition, i.e. the severe reduction in amino acid contents may be attributed to the decrease in amino acid biosynthesis. However, this assumption conflicts with that obtained by Hassan and El-Sherbeny, 1988 and El-Rokiek, 2002 who estimated increase in total amino acids under stress.

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تقييم نشاط كل من حمض البنزويك والسيناميك، منفردة أو في مخاليط على نمو ومحصول نباتات القمح وبعض الحشائش المصاحبة مقارنة بمبيد الحشائش أيزوبروتيرون

[٣]

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١- قسم النبات - المركز القومي للبحوث - الدقى - القاهرة - مصر

تم الرش بمبيد الحشائش أيزوبروتيرون بتركيز (٣٠٠٠ جزء في المليون) وذلك بعد ٣٠ يوم من الزراعة.

وقد أظهرت النتائج بما فيها المبيد تأثير مثبت على كل من الوزن الغض و الوزن الجاف للحشائش المصاحبة وذلك بالمقارنة بالحشائش غير المعاملة. كما أدت معاملات الخلط لكل من حمض البنزويك والسيناميك إلى زيادة في تثبيط نمو الحشائش. أوضحت النتائج أن التثبيط في نمو الحشائش كان مصحوبا بزيادة في المحتوى الداخلى من المواد الفينولية الكلية. وعلى العكس من ذلك قد أظهرت النتائج أن هذا التثبيط في نمو الحشائش كان مصحوبا بزيادة في نمو نباتات القمح متمثلة في زيادة في الطول وكذلك كل من الوزن الغض والوزن الجاف وتبع ذلك زيادة في المحصول. صوحت التأثيرات السابقة بزيادة ملحوظة في الأحماض الأمينية الكلية في الحبوب الناتجة ومن جهة أخرى أوضحت النتائج أن استخدام معاملة الخلط لكل من حمض البنزويك بالتركيز العالى (٥٠٠ جزء في المليون) والتركيز العالى من حمض السيناميك (١٠٠٠ جزء في المليون) إلى تثبيط في نمو نباتات القمح وكذلك المحصول.. صاحب هذا التأثير نقصا ملحوظا في الأحماض لأمينية الكلية في الحبوب الناتجة.

أجريت إختبارات معملية لإختيار التركيزات المناسبة من كلا الحمضين البنزويك والسيناميك وفي تلك الدراسة تم إنبات حبوب القمح وكذلك بذور حشيشتى السلق و الزمير في أطباق بترى محتواة على ورق ترشيع مشبع بالأحماض الفينولية السابقة أو مخلوطهما بعدة تركيزات. وقد أوضحت نتائج هذه الإختبارات أن المعاملة بحمض البنزويك بتركيز ١٠٠٠ جزء في المليون منفردا أو مخلوطا مع حمض السيناميك أدى إلى نقص شديد أو تثبيط كامل في نمو كل من بادرات القمح و الحشائش عند عمر ١٠ أيام. وبذلك تم إختيار التركيزات المناسبة للدراسة فى الصوبة.

أجريت هذه الدراسة بصوبة المركز القومى للبحوث خلال موسمى الشتاء (٢٠٠٤/٢٠٠٥ و ٢٠٠٥/٢٠٠٦) لتقييم نشاط كل من حمض البنزويك و السيناميك، منفردة او مخلوطهما على نمو نباتات القمح و بعض الحشائش المصاحبة (السلق) كمثل للحشائش العريضة و الزمير كمثل للحشائش النجيلية) مقارنة بمبيد الحشائش أيزوبروتيرون. وفى هذه الدراسة تم رش نباتات القمح والحشائش المصاحبة بتركيز ٢٥٠، ٥٠٠ جزء في المليون من حمض البنزويك و تركيز ٢٥٠، ٥٠٠، ١٠٠٠ جزء في المليون من حمض السيناميك أو مخلوطهما وكذلك