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Allelopathic management of the noxious weed; *Phalaris minor* Retz. growing in *Triticum aestivum* L. fields

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ABSTRACT

The present investigation aims to study the biological activity of different concentrations (10, 20 and 40%) of *Deverra tortuosa* (DTSCP) and *Haplophyllum tuberculatum* (HTSCP) shoot crude powder as well as a mixture (w/w) of both donor species on some growth parameters, some nutrients (N, P, K, Ca, Na, Mg, Fe, Mn and Zn), photosynthetic pigments, and protein profile of *Triticum aestivum* (crop species) and *Phalaris minor* (weed species) in pure and mixed cultures. In general the measured growth parameters (seedling fresh and dry weight, seedling shoot and root length and leaf area) were more affected in *P. minor* compared to *T. aestivum.* There was a significant decrease in the concentration of micronutrients as well as the total photosynthetic pigment contents of *P. minor* seedlings grown in both DTSCP and HTSCP. Furthermore, treatments with both donor species differentially affected protein expression of the two recipient species. New proteins have been expressed in the treated test plants as compared to controls. The expression of these new proteins could be explained on the basis that to neutralize the effect of allelochemicals produced by invasive plant powders on the treated test plants.

Key words: *Deverra tortuosa*, *Haplophyllum tuberculatum*, Growth parameters, Nutrient content, Photosynthetic pigments, SDS-Page.

INTRODUCTION

Allelopathy is a physiological process with ecological implications (Reigosa *et al*., 2006). An organism produces one or more biochemical (allelochemicals) that influence the growth, survival and reproduction of other organisms. It also involves chemical interactions at all levels of complexity, from microorganisms to higher plants (Rice, 1974). When plants are exposed to allelochemicals, their growth and development are affected (Putnam and Duke, 1978; Niakan *et al*., 2008). Weeds are known to cause enormous losses for a large number of field crop species due to their interference in agroecosystems (Mohler, 2001). They have a significant economic impact on agricultural production (Buhler, 1999) as evidenced by the efforts spent on their management. The reduction in crop yield may also be attributed to the allelopathic property exhibited by a number of weeds, especially, the aggressive ones. Such species of weeds, because of their growth habit, make agricultural operations more difficult. Furthermore, the crop contaminated with the weed seeds, is considered to be of poor quality such as seeds of *Avena fatua* and *Phalaris minor* in wheat and barley seeds (Abd El-Hamid and Hassanien. 1998).

The biological solution to minimize the perceived hazardous impacts from herbicides and insecticides in agriculture production lies in the field of allelopathy. The harmful impact of allelopathy can be exploited for pest and weed control (Narwal, 1994; Kohli *et al*., 1998). Much research has documented the potential of allelopathic plants to affect weed emergence. The question of what allelopathic plants should be selected, how they are applied, and their benefits should be seen as a requisite before introducing them to the farmers for field usage (Xuan *et al*., 2005). In this regard, the use of crops having allelopathic properties can reduce the dependency on synthetic herbicides and increase crop yields (Khanh *et al*., 2005). Wheat (*Triticum aestivum* L., Poaceae) is considered the main cereal crop in Egypt. Management must be designed to find a long-term method of control of canary grass (*Phalaris minor* Retz. Poaceae); annual croplands weed in Nile Delta. It is found predominantly in fields cultivated for wheat. It is indigenous to the Mediterranean region and was introduced to Australia, Africa, Hawaii, India, Pakistan and since then to many countries of the world (Kaushik *et al*., 2005). Therefore, the purpose of the present study was to carry out an evaluation on the biological activity of *Deverra tortuosa* (Desf.) DC. (Apiaceae) and *Haplophyllum tuberculatum* (Forssk.) A. Juss. (Rutaceae) crude powders of their aerial shoot on some growth and physiological parameters of the most problematic weed in *T. aestivum* L. fields; *P. minor*. We hope that the study will provide information about the possibilities of using the two donor species as bioherbicides.

MATERIALS AND METHODS

Plant materials and experimental design

Shoots of the two donor species (*Deverra tortusa* and *Haplophyllum tuberculatum*) have been collected from Matruh (260 km west of Alexandria city) during the vegetative stage**.** The plant materials were dried in shade then ground in a Wiley Mill to coarse uniform texture and stored in glass jars until use. Seeds of the weed (*Phalaris minor*) and crop species (*Triticum aestivum* cv. Gemmiza 10) were purchased from the Agricultural Research Center, El-Dokki, Giza, Egypt.

The soil samples were finally sterilized at (90ºC for 48 h) to remove any microorganisms and weed seeds. Twenty seeds of each of the weed and crop species were sown in plastic pots (16 cm in diameter) in pure and mixed culture practices with about 1500 g of sandy loam soil thoroughly mixed (w/w) with 10,20 and 40 % of electrically crushed crude powder of the shoots of the target species. One treatment was run as control with

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zero percent of crude powder. Treatments were arranged in a completely randomized block design with three replicates. The plants were watered every two days on the average with normal tap water. The amount of water corresponding to average soil–plant evapotranspiration calculated from weight loss over a 24 –hour interval. The experiment was performed under normal laboratory conditions (20±2°C temperature, 75±2% relative humidity, and 14/10 h light/dark photoperiod).

After 21 days, the homogenous seedling were carefully collected from each treatment, washed with tap water to remove the adhering soil particles, and then, by distilled water, gently blotted with filter paper. The seedlings were separated into shoots and roots for the determination of seedling fresh weight as well as seedling length. Additionally, leaf area of the two recipient species was also evaluated. Other samples were dried at 65^{o}C till constant weight to determine the seedling dry weight.

Photosynthetic pigments

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined spectrophotometrically according to Metzner *et al.* (1965).

Determination of minerals

Seedlings were carefully and thoroughly cleaned, blotted dry between absorbing paper and their dry weights were measured after oven drying at 70°C for 72h.Oven dry samples of seedlings were finely ground and assayed for-mineral ion content by the wet digestion method (Humphries, 1956). Minerals (N, P, K, Ca, Na, Mg, Fe, Mn, and Zn) were determined using an atomic absorption spectrophotometer (Perkin-Elmer, 2380) and expressed on the basis of dry weight. The extract obtained was subjected for assaying K and Ca concentrations using flame photometer (CORNNG 400).

SDS-PAGE

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to distinguish and fragment total soluble protein for treated *T. aestivum and P. minor* samples according to the method of Laemmli (1970).The total proteins content were determined by method described by Lowry *et al.* (1951).

Statistical Analysis

All the data of the present study were subjected where appropriate; to standard one-way analysis of variance (ANOVA) and student's t-test (Zar, 1984) using the COSTAT 2.00 statistical analysis software manufactured by CoHort Software Company**.** Where a significant difference was detected by ANOVA test, pair-wise comparisons of means were performed using Least Significant Differences (LSD) at 0.05 probability level.

RESULTS

Growth parameters

The allelopathic effects of *Deverra tortuosa* (DTSCP) and *Haplophyllum tuberculatum* (HTSCP) shoot crude powder as well as a mixture (w/w) of both on fresh and dry weight of *Triticum aestivum* and *Phalaris minor* seedlings are illustrated in Tables 1.

There was a slight decrease in the fresh weight of *T. aestivum* seedlings in both pure and mixed cultures with increasing the concentrations of DTSCP while HTSCP had a relatively more negative effect (Table 1). The percent decrease in fresh weight was about 83% in seedlings treated with 40% HTSCP grown in pure culture and 84% in those seedlings grown in mixed culture. At concentration 40%, a fresh weight of about 0.114 g was achieved in *P. minor* seedlings in pure culture. This accounts for a decrease of about 80% in the fresh weight compared to the corresponding control. Similarly, in mixed culture the percent decrease in *P. minor* seedlings fresh weight was 80.6%. Dry weight of the control *Phalaris minor* seedlings differs between seedlings grown in pure and mixed cultures .It was 0.178 g in pure culture seedlings and 0.14 g in seedlings grown in mixed culture. It decreased gradually with increasing concentration of both DTSCP and HTSCP till it reached 0.044 g at 40% DTSCP and HTSCP in pure culture, with a percent decrease of 74.7%. Similarly; in mixed culture the percent decrease reached 74.6% at the same concentration. Treatment with different concentrations of HTSCP achieved a significant reduction in total seedling length as well as shoot and root lengths of both treated plants and the reduction was much dramatic in *P. minor* **(**Table 2). In pure culture a decrease of 60% in seedling length was exhibited at 20% concentration and reached 87% at 40% concentration. On the other hand, in mixed culture it reached 92.9% at 40% HTSCP concentration. Upon applying 20% DTSCP the percent decrease in *P. minor* seedling length reached 45% in pure culture which increased to 77% at 40% concentration. The different concentrations of a mixture of both DTSCP and HTSCP dramatically reduced the length of *P. minor* seedlings. In pure culture the percent decrease in seedling length was 50% at concentration of 20 % which reached 78.5% at concentration of 40%. Likewise, in mixed culture, the percent decrease was 49.5% and reached 80 % at 20 and 40% concentrations respectively.

Table 1: Variation in seedling fresh and dry weight (g plant^{-1}) of *Triticum aestivum* and *Phalaris minor* in pure and mixed cultures as affected by different concentrations of *Deverra tortuosa* shoots crude powder (DTSCP), *Haplophyllum tuberculatum* shoot crude powder (HTSCP) and both (w/w), twenty one days after sowing. Data are means of three replicates.

Concentration (%)	Fresh weight (g plant^{-1})						Dry weight (g plant^{-1})					
	DTSCP		HTSCP		DTSCP + HTSCP		DTSCP		HTSCP		DTSCP + HTSCP	
	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed
	Triticum aestivum											
0	0.784^{c}	0.702^{c}	0.743^{d}	0.740^{c}	0.775^{d}	0.766^{d}	0.300^{d}	0.297^{d}	0.309^{d}	0.276^{d}	0.296^{d}	0.322^{d}
10	0.745^{c}	0.684^{c}	0.711^{c}	0.724^{c}	0.699^{c}	0.687^{c}	0.285^{c}	0.290^{c}	0.296^{c}	0.270^{c}	0.267^{c}	0.289^{c}
20	0.542^{b}	0.502^{b}	0.447^{b}	0.428^{b}	0.527^{b}	0.489^{b}	0.208^{b}	0.213^{b}	0.186^{b}	0.160^{b}	0.201^{b}	0.205^{b}
40	0.285^{a}	0.254^{a}	0.124^{a}	0.115^{a}	0.214^{a}	0.185^{a}	0.104^{a}	0.102^{a}	0.042^{a}	0.043^{a}	0.072^{a}	0.068^{a}
P. value*	0.009		0.275		0.025		0.303		0.034		0.096	
	Phalaris minor											
0	0.575^{d}	0.447^{d}	0.528^{d}	0.475^{d}	0.556^{d}	0.438^{d}	0.182^{d}	0.147^{d}	0.178^{d}	0.140^{d}	0.174^{d}	0.142^{d}
10	0.453^{c}	0.387^{c}	0.343^{c}	0.312^{c}	0.401^{c}	0.324^{c}	0.135^{c}	0.122^{c}	0.101^{c}	0.079^{c}	0.115^{c}	0.094^{c}
20	0.242^{b}	0.185^{b}	0.173^{b}	0.157^{b}	0.272^{b}	0.195^{b}	0.093^{b}	0.078^{b}	0.072^{b}	0.059^{b}	0.104^{b}	0.072^{b}
40	0.148^{a}	0.102^{a}	0.084^{a}	0.027^{a}	0.114^{a}	0.085^{a}	0.057^{a}	0.043^{a}	0.025^{a}	0.008^{a}	0.044^{a}	0.036^{a}
P. value*	0.014		0.013		0.013		0.018		0.013		0.013	

Different letters within each column indicate a significant difference at probability level ≤ 0.05 according to ONE WAY ANOVA.

*P-value was considered significant at ≤ 0.05 probability level according to paired t-test.

Table 2: Variation in seedling, shoot and root length (cm) of *Triticum aestivum* and *Phalaris minor* in pure and mixed cultures as affected by different concentrations of *Deverra tortuosa* shoot crude powder (DTSCP), *Haplophyllum tuberculatum* shoot crude powder (HTSCP) and both (w/w), twenty one days after sowing. Data are means of three replicates.

Concentration (%)	Seedling length (cm)						Shoot length (cm)						Root length (cm)					
	DTSCP		HTSCP		DTSCP + HTSCP		DTSCP		HTSCP		DTSCP + HTSCP		DTSCP		HTSCP		DTSCP + HTSCP	
	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed
Triticum aestivum																		
0	85.76^{d}	85.23^{d}	85.92^{d}	84.12^{d}	85.96^{d}	84.85^{d}	41.25^{d}	40.21^{d}	41.84^{d}	39.85^{d}	41.85^{d}	40.07^{d}	44.51^{d}	45.02^{d}	44.08^{d}	44.27^{d}	44.11^{d}	44.78^{d}
10	78.05^{c}	77.45^{c}	70.89^{c}	68.69^{c}	75.73^{c}	74.56^{c}	37.54^{c}	36.54^{c}	34.52^{c}	32.54^{c}	36.87^{c}	35.21^{c}	40.51^{c}	40.91^{c}	36.37^{c}	36.15^{c}	38.86^{c}	39.35^{c}
20	59.34^{b}	54.01^{b}	52.04^{b}	45.47^{b}	60.68^{b}	56.01^{b}	28.54^{b}	25.48^{b}	25.34^{b}	21.54^{b}	29.54^{b}	26.45^{b}	30.80^{b}	28.53^{b}	26.70^{b}	23.93^{b}	31.14^{b}	29.56^{b}
40	22.66^{a}	19.7^{a}	15.54^{a}	11.92^{a}	16.92^{a}	15.57^{a}	12.34^{a}	10.24^{a}	8.54^{a}	6.12^{a}	9.21^{a}	8.77^{a}	10.32^{a}	9.46^{a}	7.00^{a}	5.80^{a}	7.71^{a}	6.80^{a}
P. value*	0.066		0.023		0.048		0.018		0.005		0.024		0.228		0.113		0.293	
Phalaris minor																		
0	68.12^{d}	55.96^{d}	67.15^{d}	57.06^{d}	67.69^{d}	56.62^{d}	35.71^{d}	28.42^{d}	34.28^{d}	29.02^{d}	35.22^{d}	28.74^{d}	32.41^{d}	27.54^{d}	32.87^{d}	28.04^{d}	32.47^{d}	27.88^{d}
10	57.69^{c}	44.38^{c}	48.07^{c}	36.12^{c}	52.93^{c}	39.7^{c}	30.24^{c}	22.54^{c}	24.54^{c}	18.37^{c}	27.54^{c}	20.15^{c}	27.45^{c}	21.84^{c}	23.53^{c}	17.75^{c}	25.39^{c}	19.55^{c}
20	37.27^{b}	30.09^{b}	26.52^{b}	20.8^{b}	33.71^{b}	28.59^{b}	19.54^{b}	15.28^{b}	13.54^{b}	10.58^{b}	17.54^{b}	14.51^{b}	17.73^{b}	14.81^{b}	12.98^{b}	10.22^{b}	16.17^{b}	14.08^{b}
40	15.72^{a}	12.88^{a}	8.89^{a}	4.03^{a}	14.49^{a}	11.54^{a}	8.24^{a}	6.54^{a}	4.54^{a}	2.05^{a}	7.54^{a}	5.86^{a}	7.48^{a}	6.34^{a}	4.35^{a}	1.98^{a}	6.95^{a}	5.68^{a}
P. value*	0.017		0.009		0.022		0.017		0.009		0.021		0.018		0.009		0.024	

Different letters within each column indicate a significant difference at probability level ≤ 0.05 according to ONE WAY ANOVA.

*P-value was considered significant at ≤ 0.05 probability level according to paired t-test

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Increasing the concentration of HTSCP in the soil caused a gradual decrease in a single leaf area of *T. aestivum* seedlings grown in pure culture (Table 3). The percent decrease was 3.3, 20.4 and 40.3% in concentrations 10, 20, and 40 % respectively. However, a slight increase in leaf area was recorded in seedlings grown in mixed culture with 10% HTSCP. At concentrations 20 and 40%, the percent decrease in leaf area was 14 and 29.3% respectively. Applying the mixture of DTSCP and HTSCP in the soil also caused a gradual decrease in leaf area of *T. aestivum* seedlings grown in pure culture. The percent decrease was 5.3, 31.1 and 47.7% in concentrations 10, 20, and 40 %.respectively. The most pronounced inhibitory effect of applying 40% HTSCP on area of a single leaf was observed in *P. minor* seedlings grown in pure culture. This treatment reduced the leaf area to about 70%. The same concentration, however, reduced the leaf area of *T. aestivum* to only 14%.

Table 3: Variation in the area of a single leaf (cm^2) of *Triticum aestivum* and *Phalaris minor* seedlings in pure and mixed cultures as affected by different concentrations of *Deverra tortuosa* shoot crude powder (DTSCP), *Haplophyllum tuberculatum* shoot crude powder (HTSCP) and both (w/w), twenty one days after sowing. Data are means of three replicates.

concentration (%)	DTSCP		HTSCP		DTSCP + HTSCP	
	Pure	Mixed	Pure	Mixed	Pure	Mixed
	Triticum aestivum					
0	9.34^{c}	8.18^{d}	8.13^{d}	8.45^{c}	9.22^{d}	8.02^{d}
10	9.02^{c}	7.84^{c}	7.86^{c}	8.47^{c}	8.73^{c}	7.14^{c}
20	7.16^{b}	6.03^{b}	6.47^{b}	7.26^{b}	6.35^{b}	5.87^{b}
40	5.27^{a}	4.77^{a}	4.85^{a}	5.97^{a}	4.82^{a}	4.08^{a}
P. value*	0.005		0.012		0.013	
	Phalaris minor					
0	7.58^{c}	5.84^{c}	7.24^{d}	5.42^{c}	8.04^{c}	5.27^{d}
10	7.04^{c}	5.11^{c}	6.85^{c}	5.17^{c}	7.42^{b}	4.77^{c}
20	5.21^{b}	4.17^{b}	4.28^{b}	3.39^{b}	4.89^{a}	3.25^{b}
40	3.58^{a}	2.18^{a}	2.14^{a}	1.85^{a}	4.24^{a}	2.78^{a}
P. value*	0.002		0.023		0.004	

Different letters within each column indicate a significant difference at probability level ≤ 0.05 according to ONE WAY ANOVA. *P-value was considered significant at ≤ 0.05 probability level according to paired t-test.

Nutrient contents

Data recorded in Table 4 showed that the most pronounced effect of applying HTSCP was the decrease in N content of both treated plants. The percent decrease was much higher in seedlings grown in mixed culture (75%) compared with those grown in pure culture (46 %). Similarly, P, K and Mg demonstrated the same trend.

The percent decrease in Ca content of *T. aestivum* seedlings was 19.35% in pure culture. However the content increased in seedlings grown in mixed culture and the percent increase was 37%. Na content increased upon treatment with DTSCP and the increase however, was recorded only in seedlings grown in pure culture (9.77%). In mixed culture, the Na content decreased markedly with a percent of 23.24%. As well, Mg content decreased with a percent of 24.57% and 48.38% in pure and mixed cultures respectively. To go through with this and except for Ca, the content of all the elements studied was decreased upon treatment with

DTSCP, and the percent decrease was higher in seedlings grown in mixed culture compared to those grown in pure one.

Photosynthetic pigments

The total content of the photosynthetic pigments of the recipient species upon applying HTSCP, DTSCP and a Mixture of both was presented in Table 5. The content in *T. aestivum* was reduced to 79% in pure culture and 72.7% in mixed culture with respect to HTSCP which may be ascribed to the decrease in both Chl.a and Chl.b. The reduction percentage in Chl.b, however was higher than that of Chl.a, it accounted to 95.4% in pure culture and 90.4% in mixed culture compared to the corresponding control values. The reduction in Chl.a was almost similar in pure and mixed cultures. It reached 78.6% in pure and 73.6% in mixed culture, respectively. The percent reduction in carotenes, however, was less than that of chlorophylls a and b, it accounted to 58.5% and 44.7% in pure and mixed cultures, respectively. In the same way, The pigment content of seedlings grown in DTSCP and a mixture of both was markedly reduced, and the percent reduction accounted to 72% in pure culture and 67% in mixed culture.

Table 4: Variation in the concentrations of some nutrient elements of *Triticum aestivum* and *Phalaris minor* seedlings in pure and mixed cultures as affected by *Deverra tortuosa* shoot crude powder (DTSCP), *Haplophyllum tuberculatum* shoot crude powder (HTSCP) and a mixture of both (w/w), twenty one days after sowing and at maximum crude powder concentration (40%). Data are means of three replicates.

Parameter		Control		DTSCP		HTSCP		DTSCP + HTSCP	
		Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed
		Triticum aestivum							
Macronutrient Element (mg g^{-1})	**N**	6.44^{d}	5.23^{d}	5.88^{d}	3.46^{c}	4.85^{d}	1.31^{b}	5.69^{d}	2.84^{c}
	P	4.23^{c}	3.19^{c}	2.86^{c}	2.03^{b}	2.05^{c}	0.49^{a}	2.22^{c}	1.02^{b}
	K	3.27^{c}	3.03^{c}	2.34^{c}	1.26^{b}	1.60^{b}	0.14^{a}	1.81^{b}	0.85^{a}
	Ca	0.31^{a}	0.34^{a}	0.25^{a}	0.54^{a}	0.52^{a}	0.69^{a}	0.38^{a}	0.55^{a}
	Na	7.48^{e}	6.11^{e}	8.29^{e}	4.69^{d}	9.48^{e}	2.70^{c}	8.29^{e}	0.54^{a}
	Mg	2.36^{b}	2.15^{b}	1.78^{b}	1.10^{b}	1.16^{b}	0.43^{a}	1.61^{b}	0.80^{a}
Micronutrient Element (ppm)	**Fe**	55.62^{g}	40.23^{g}	44.93^{g}	37.89^{g}	36.85^{h}	23.37^{e}	43.21^{g}	32.46^{e}
	Mn	27.23^{f}	26.34^{f}	17.47^{f}	11.95^{e}	11.69^{g}	7.31^{d}	17.47^{f}	9.73^{d}
	Zn	64.33^{g}	57.48^{h}	60.95^{h}	46.42^{h}	56.55^{i}	64.12^{f}	46.33^{h}	59.14^{f}
P. value*		0.056		0.056		0.056		0.056	
		Phalaris minor							
Macronutrient Element (mg g^{-1})	**N**	2.31^{b}	2.02^{b}	2.11^{b}	1.24^{b}	1.74^{b}	0.47^{a}	2.04^{c}	1.02^{b}
	P	1.54^{a}	1.23^{a}	1.04^{a}	0.74^{a}	0.73^{a}	0.18^{a}	0.82^{a}	0.37^{a}
	K	5.62^{d}	4.11^{d}	4.02^{c}	2.17^{c}	2.75^{c}	0.24^{a}	3.11^{d}	1.46^{b}
	Ca	1.41^{a}	1.23^{a}	1.12^{a}	2.47^{c}	2.45^{c}	3.12^{c}	1.75^{b}	2.48^{c}
	Na	3.41^{c}	3.54^{c}	3.78^{c}	2.14^{c}	4.32^{d}	1.23^{b}	3.78^{d}	2.07^{c}
	Mg	2.31^{b}	2.06^{b}	1.74^{a}	1.08^{b}	1.14^{b}	0.42^{a}	1.58^{b}	0.78^{a}
Micronutrient Element (ppm)	**Fe**	20.31^{g}	18.42^{h}	16.41^{f}	13.84^{f}	13.46^{g}	8.54^{e}	15.78^{f}	11.84^{e}
	Mn	16.41^{f}	14.20^{g}	10.54^{e}	7.21^{d}	7.05^{e}	4.41^{d}	10.54^{d}	5.87^{d}
	Zn	14.67^{e}	11.28^{e}	13.84^{d}	10.54^{e}	12.84^{f}	14.56^{f}	5.87^{e}	13.04^{f}
P. value*		0.0146		0.015		0.014		0.013	

Different letters within each column indicate a significant difference at probability level ≤ 0.05 according to ONE WAY ANOVA.
*P-value was considered significant at ≤ 0.05 probability level according to paired t-test.

Table 5: Variation in the mean concentration of different pigment fractions (mg g fresh weight^{-1}) of *Triticum aestivum* and *Phalaris minor* seedlings in pure and mixed cultures as affected by *Deverra tortuosa* shoot crude powder (DTSCP), *Haplophyllum tuberculatum* shoot crude powder (HTSCP) and both (w/w), twenty one days after sowing and at maximum crude powder concentration (40%). Data are means of three replicates.

Parameters	Control		DTSCP		HTSCP		DTSCP + HTSCP	
	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed
	Triticumaestivum							
Chl. "a"	4.72^{c}	2.69^{b}	1.36^{b}	1.21^{b}	0.74^{a}	0.54^{a}	0.99^{b}	0.68^{a}
Chl. "b"	2.80^{b}	2.09^{b}	0.40^{a}	0.07^{a}	0.13^{a}	0.20^{a}	0.03^{a}	0.15^{a}
Carotenoids	1.95^{a}	1.41^{a}	0.64^{a}	0.40^{a}	0.81^{a}	0.78^{a}	0.90^{b}	0.86^{a}
Chl. a/b ratio	1.69^{a}	1.29^{a}	3.40^{d}	17.94^{c}	5.84^{c}	2.62^{c}	28.75^{d}	4.52^{c}
Total pigments	9.46^{d}	6.18^{c}	2.40^{c}	1.68^{b}	1.68^{b}	1.53^{b}	1.92^{c}	1.69^{b}
P. value*	0.033		0.047		0.047		0.182	
	Phalaris minor							
Chl. "a"	2.25^{b}	1.48^{b}	0.55^{a}	0.52^{a}	0.75^{a}	0.14^{a}	0.54^{a}	0.23^{a}
Chl. "b"	3.26^{c}	2.67^{c}	0.26^{a}	0.27^{a}	0.16^{a}	0.20^{a}	0.28^{a}	0.40^{a}
Carotenoids	0.52^{a}	0.25^{a}	0.19^{a}	0.57^{a}	1.02^{b}	0.86^{a}	0.10^{a}	0.06^{a}
Chl. a/b ratio	0.69^{a}	0.56^{a}	2.17^{c}	1.96^{b}	4.61^{c}	0.72^{a}	1.94^{b}	0.59^{a}
Total pigments	6.02^{d}	4.41^{d}	1.00^{b}	1.37^{b}	1.94^{b}	1.20^{b}	0.92^{a}	0.69^{a}
P. value*	0.030		0.212		0.024		0.117	

Different letters within each column indicate a significant difference at probability level ≤ 0.05 according to ONE WAY ANOVA.
*P-value was considered significant at ≤ 0.05 probability level according to paired t-test.

Protein profiles of the recipient species

Figures (1) A & B represents the electrophoretic pattern of *Triticum aestivum* and *Phalaris minor* when seedlings were treated with DTSCP, HTSCP or a mixture of both at maximum crude powder concentration (40%). It revealed the presence of several peptides, which differed in migration position and band intensity. The figure shows that ,6, 6, 7, 11, 7, 9, 8, 6, 8, 4,4, 6, 11, 4, 5 and 7 protein bands were excised in *Triticum aestivum* pure control, *Triticum aestivum* pure + DTSCP, *Triticum aestivum* pure +HTSCP, *Triticum aestivum* pure + both DTSCP and HTSCP, *Phalaris minor* pure control, *Phalaris minor* pure +DTSCP, *Phalaris minor* pure +HTSCP, *Phalaris minor* pure + both DTSCP and HTSCP, *Triticum aestivum* mixed *control*, *Triticum aestivum* mixed + DTSCP, *Triticum aestivum* mixed + HTSCP, *Triticum aestivum* mixed + both DTSCP and HTSCP, *Phalaris minor* mixed control, *Phalaris minor* mixed + DTSCP, *Phalaris minor* mixed + HTSCP *and Phalaris minor* mixed + both DTSCP and HTSCP respectively.

The control seedlings of *Triticum aestivum* grown in pure culture revealed the presence of six peptides with molecular masses ranging from 185 to 10 kDa. Treatment with a mixture of both DTSCP and HTSCP however induced the formation of 11 protein bands in *T. aestivum* seedlings (APP.VII, lane 5).In these seedlings high and low molecular masses proteins of 200,199,198,189,169,162,137,126,125,79 and 1 kDa were synthesized in response to this treatment.

Application of HTSCP to *P. minor* grown in pure culture revealed the presence of six bands with molecular masses 172,163,126,100,77 and 25kDa,with corresponding band intensities 22,19,20,13,15 and 7 respectively. Meanwhile, only 4 peptides were synth-

esized in *T. aestivum* upon treatment with HTSCP. Their molecular masses were 182,162,130 and 74 kDa and corresponding band intensities 21, 20, 46 and 10 respectively.

On the other hand, several peptides (11) with different band intensities were present in *Phalaris minor* control seedlings grown in mixed culture, with molecular masses ranging between 208 to 60 kDa. Treatment with DTSCP revealed the presence of only 4 protein bands with molecular masses of 193,169,100 and 80 kDa with band intensities 33, 39, 6 and 19 respectively. Similarly, treatment with HTSCP revealed the presence of 5 protein bands with molecular masses ranging between 197 and 91 kDa.

For *Triticum aestivum,* protein band with 200 KDa was common between *Triticum aestivum* seedlings grown in pure culture +HTSCP *and Triticum aestivum* grown in pure culture + both DTSCP and HTSCP. Moreover, 165 KDa protein band was existed in all of *Triticum aestivum* pure + HTSCP *Triticum aestivum* pure +both DTSCP and HTSCP. Nevertheless, 134 KDa protein band was existed in all of *Triticum aestivum* seedlings grown in pure culture + DTSCP and *Triticum aestivum* grown in pure culture + the mixture of both DTSCP and HTSCP. Only protein band with 125 KDa was common among *Triticum aestivum* mixed control, *Triticum aestivum* mixed + DTSCP.

126 KDa protein band was common between *Phalaris minor* pure control and *Phalaris minor* pure+ the mixture of both DTSCP and HTSCP. Also, common protein band with 100 KDa between *Phalaris minor* pure + HTSCP *and Phalaris minor* pure + the mixture of both DTSCP and HTSCPwas regarded. Finally, protein band of 39 KDa was common between *Phalaris minor* pure+DTSCP and *Phalaris minor* pure +HTSCP.

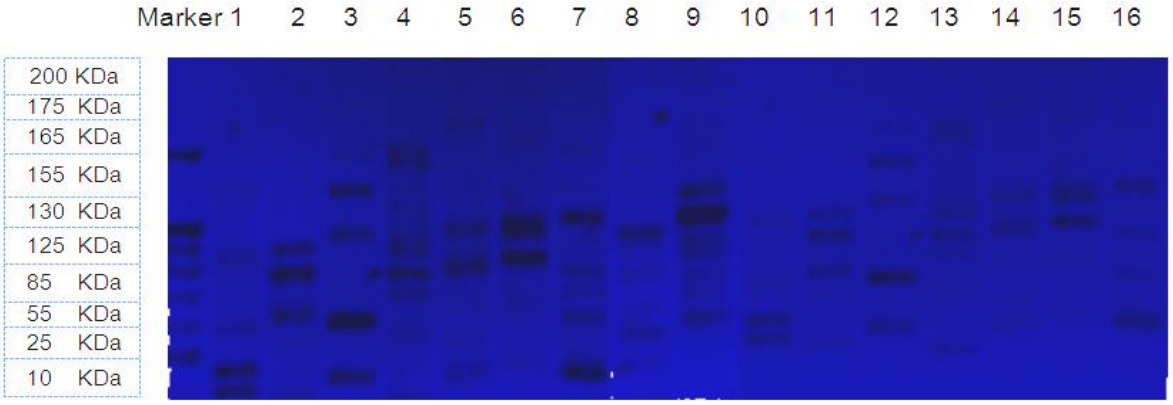

Figure (1): A: SDS-PSGE analysis of leaves separated by gel electrophoreses as resolved by comasie brilliant blue for scanning the up-down regulated proteins of 21 day old seedlings of *Triticum aestivum* and *Phalaris minor* at maximum crude powder concentration (40%). The lanes:

1- Protein marker
2- *Triticumaestivum* pure control
3- *Triticumaestivum* pure *Deverratortuosa*
4- *Triticumaestivum*pure*Haplophyllumtuberculatum*
5- *Triticumaestivum* pure *Deverratortuosa* + *Haplophyllumtuberculatum*
6- *Phalaris minor* pure control
7- *Phalaris minor* pure *Deverratortuosa*
8- *Phalaris minor* pure *Haplophyllumtuberculatum*
9- Phalaris minor pure *Deverratortuosa* + *Haplophyllumtuberculatum*
10- *Triticumaestivum* mixed control
11- *Triticum aestivum* mixed *Deverra tortuosa*
12- *Triticumaestivum* mixed *Haplophyllumtuberculatum*
13- *Triticumaestivum* mixed *Deverratortuosa* + *Haplophyllumtuberculatum*
14- *Phalaris minor* mixed control
15- *Phalaris minor* mixed *Deverratortuosa*
16- *Phalaris minor* mixed *Haplophyllumtuberculatum*
17- *Phalaris minor* mixed *Deverratortuosa* + *Haplophyllumtuberculatum*

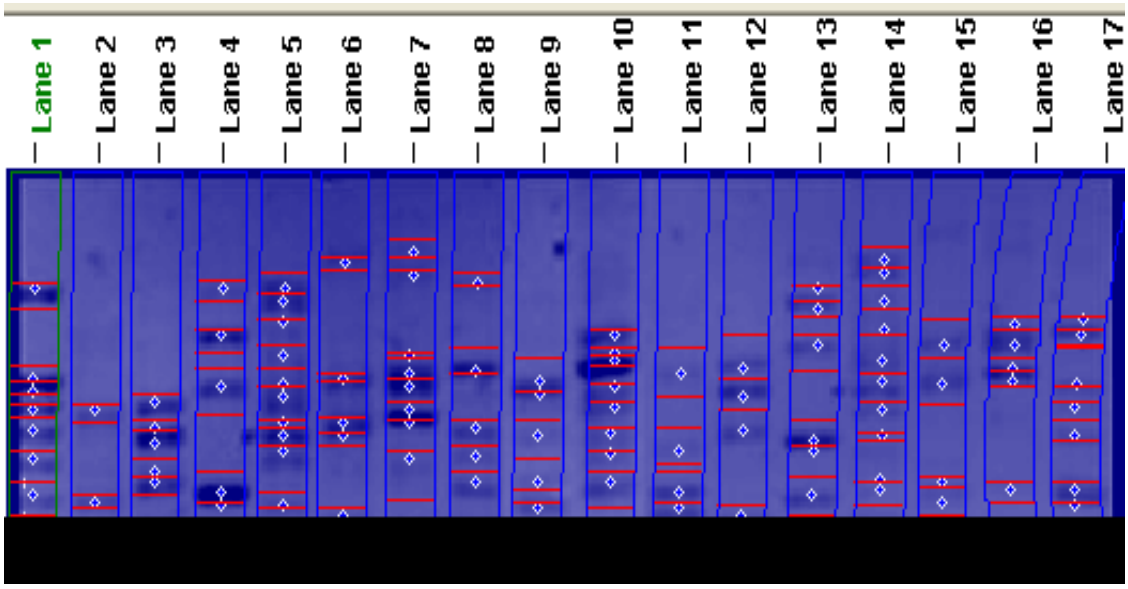

Figure (1): B: SDS-PSGE computerized analysis of leaves separated by gel electrophoreses as resolved by comasie brilliant blue for scanning the up-down regulated proteins of 21 day old seedlings of *Triticumaestivum* and *Phalaris* minor at maximum crude powder concentration (40%). The lanes:

1- Protein marker

2- *Triticumaestivum* pure control

3- *Triticumaestivum* pure *Deverratortuosa*

4- *Triticumaestivum*pure*Haplophyllumtuberculatum*

5- *Triticumaestivum* pure *Deverratortuosa* + *Haplophyllumtuberculatum*

6- *Phalaris minor* pure control

7- *Phalaris minor* pure *Deverratortuosa*

8- *Phalaris minor* pure *Haplophyllumtuberculatum*

9- Phalaris minor pure *Deverratortuosa* + *Haplophyllumtuberculatum*

10- *Triticumaestivum* mixed control

11- *Triticumaestivum* mixed *Deverratortuosa*

12- *Triticumaestivum* mixed *Haplophyllumtuberculatum*

13- *Triticumaestivum* mixed *Deverratortuosa* + *Haplophyllumtuberculatum*

14- *Phalaris minor* mixed control

15- *Phalaris minor* mixed *Deverratortuosa*

16- *Phalaris minor* mixed *Haplophyllumtuberculatum*

17- *Phalaris minor* mixed *Deverratortuosa* + *Haplophyllumtuberculatum*

The protein band 197 KDa was common between *Phalaris minor* mixed control and *Phalaris minor* mixed +HTSCP. Furthermore, 172 KDa protein band was common between *Phalaris minor* mixed control and *Phalaris minor* mixed+HTSCP. Moreover, 126 KDa protein band was common between *Phalaris minor* mixed control and *Phalaris minor* mixed + the mixture of both DTSCP and HTSCP, protein band with 104 KDa was common between *Phalaris minor* mixed control and *Phalaris minor* mixed +DTSCP. Also, 91 KDa protein band was common among *Phalaris minor* mixed control, *Phalaris minor* mixed + HTSCP and *Phalaris minor* mixed + a mixture of both DTSCP and HTSCP. Finally, 80 KDa protein band was common between *Phalaris minor* mixed + DTSCP and *Phalaris minor* mixed+ th*e* mixture of both DTSCP and HTSCP.

DISCUSSION

Take advantage of allelopathic plant extracts in combination with another plant extracts or herbicide for weed control in crop fields is well recognized (Dilipkumar and Chuah, 2013). However, these types of studies are still scanty. Addressing this need, the present study investigates the action of shoots crude powder of *Haplophyllum tuberculatum* (HTSCP) and *Deverra tortuosa* (DTSCP),each applied alone or in 50% mixture, on some growth parameters and physiological aspects of *Phalaris minor* (weed species) and *Triticum aestivum* (crop species) in pure and mixed cultures under laboratory conditions.

The significant reduction in seedling length of *P. minor* in the present study may be attributed to the reduced rate of cell division and cell elongation due to the presence of allelochemicals in the donor species crude powder (Javaid and Anjum, 2006).Several studies had shown that compounds of plant origin affect mitotic activity of growing roots (Rizvi *et al*., 1992; Einhellig, 1996). Such an inhibitory effect on mitosis may directly decrease plant growth, and so mitotic activity can be used to evaluate root growth resulting from cell division of meristematic cells and cell expansion in the elongation zone of roots (Dayan *et al*., 2000; June, 2006). El-Kenany and El-Darier (2013) indicated that cold and hot aqueous extracts of *Lantana camara* (donor species) exhibit strong inhibitory allelopathic effect on the germination efficiency of *Phalaris minor* and *Sorghum bicolor* (recipient species). The germination of *P. minor* was highly sensitive to the donor hot extract in comparison with *S. bicolor* and finally the hot extract had greater inhibitory effect on the germination of *P. minor* compared to the cold.

The water extracts of leaves and bark of *Eucalyptus tereticornis* were tested for seed germination and primary root and shoot development of *Phaseolus vulgaris* seedlings. The extract was found to be most inhibitory in primary root development. The affected *P. vulgaris* seedlings were attributed to an unknown water soluble substance(s) present in leachate. It is hypothesized that the allelopathic substance(s) present in the litter of *eucalyptus* interfere(s) with the growth of the mycorrhizal fungi present in the root system and this in turn affects the nutrient uptake and growth of seedlings (Sale, 2013).

Recently, *Hypophyllum tuberculatum* aqueous extracts (HTAE) were tested on germination efficiency and growth parameters of *Lepidium sativum* and *Raphanus sativus* seeds. At the full-strength concentration (100%), the hypocotyl length (HL) was more sensitive than radicle length under HTAE. It was obvious that the allelopathic effect was prominent in *L. sativum* compared with *R. sativus* indicating the resistance of the latter to the allelochemicals extracted from HTAE (El-Darier *et al.*, 2014; Hemada and El-Darier, 2015).

Allelopathic inhibition of mineral uptake was a domino effect results from alteration of cellular membrane functions in plant roots. Conclusive experiments have shown that specific allelochemicals (e.g. phenolic acids and flavonoids) inhibit mineral absorption by plant roots. The physiological mechanism of action of these allelochemicals involves the disruption of normal membrane functions in plant cells. These allelochemicals can depolarize the electrical potential difference across membranes, a primary driving force for active absorption of mineral ions (Balke, 1985, Rice, 1984). In the present study, the concentration of almost studied nutrients in the two examined plant tissues was highly reduced. It could be concluded that the allelopathic compounds released from DTSCP and HTSCP significantly suppressed the uptake of the elements studied. Furthermore, El-Darier (2002) reported that the *Eucalyptus rostrata* water extract inhibits the uptake of N, P and K in *Vicia faba* and *Zea mays* seedlings.

In the present study, significant reduction in the amount of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were recorded in response to the allelochemical stress of DTSCP, HTSCP and the mixture of both. Reduction in Chl a, Chl b and total Chl were previously reported as a result of allelochemical stress (Ervin and Wetzel, 2000; Moradshahi *et al*., 2003; Singh *et al*., 2009) which could be attributed to the inhibition of chlorophyll biosynthesis (inhibition of supply orientation) and/or the stimulation of chlorophyll degradation (stimulation of consumption orientation) (Yang *et al*., 2002 and 2004). Siddiqui (2007) reported reduction in chlorophyll content of *Vigna mungo* due to the allelochemicals present in leachate of black pepper which possibly target enzymes responsible for the conversion of porphyrin precursors. Abu-Romman (2011) reported that the photosynthetic pigments of pepper seedlings were reduced by allelochemical stress. To go through with this, pepper-seedlings content of carotenoids was also decreased in response to

allelochemicals. Carotenoids are pivotal accessory pigments playing major roles in photosynthesis by collecting light and transferring the excitation energy to the chlorophyll and by stabilizing proteins of the light-harvesting complex (Plumley and Schmidt, 1987; Demmig-Adams, 1990)

Application of HTSCP to *P. minor* grown in pure culture revealed the presence of six bands with molecular masses 172, 163, 126, 100, 77 and 25kDa,with corresponding band intensities 22, 19, 20, 13, 15 and 7 respectively. Meanwhile, only 4 peptides were synthesized in *T. aestivum* upon treatment with HTSCP. Their molecular masses were 182, 162, 130 and 74 kDa and corresponding band intensities 21, 20, 46 and 10 respectively. Protein expression in *P. minor* of the present study may have reduced especially upon treatment with the mixture of HTSCP and DTSCP as compared to controls. This reduction might be a manifestation of cell damaged caused by allelochemical stress (Cruz-Ortega *et al.*, 2002; Rehman *et al.*, 2005). In addition, the present work demonstrated that these allelochemicals significantly interfered with the protein expression of both *T. aestivum* and *P. minor*. This interference took place either by induction or repression of the protein expression (Hegazy *et al.*, 2007). The induction or repression of protein expression could take place either on transcriptional or translational level. These allelochemicals could play an important role in inhibiting enzymes involved in these two processes. This is in accordance with findings of Baziramakeng *et al.* (1997) who pointed out that the methionine incorporation into proteins was reduced by allelochemicals, and findings of Romero *et al.* (2002) who recorded that the protein pattern of *L. esculentum*was severely inhibited by all allelopathic plants, and in accordance with findings of El-Khatib*et al.* (2004) who demonstrated that allelochemicals produced by *Chenopodiu mmurale* decreased the protein contents of *L. esculentum* and other test plants. The control seedlings of *T. aestivum* grown in pure culture revealed the presence of six peptides with molecular masses ranging from 185 to 10 kDa. Treatment with a mixture of both DTSCP and HTSCP however induced the formation of 11 protein bands in *T. aestivum* seedlings. In these seedlings high and low molecular masses proteins of 200, 199, 198, 189, 169, 162, 137, 126, 125, 79 and 1 kDa were synthesized in response to this treatment. These results indicate that in *T. aestivum*, certain proteins may be involved in tolerance to allelochemical stress and are formed due to the interaction between DTSCP and HTSCP.

Treatments with HTSCP and DTSCP or both differentially affected protein expression of *T. aestivum* and *P. minor*. New proteins have been expressed in the treated test plants as compared to controls. The expression of these new proteins could be explained on the basis that to neutralize the effect of allelochemicals produced by invasive plant powders on the treated test plants. This is in accordance with Cruz-Ortega *et al*., (2002) who mentioned that plants appear to respond to allelochemical stress by increasing the expression of specific proteins. Moreover, some environmental stresses induce expression of proteins not specially related to a particular stress, but as a reaction to cell damage. These include some classes of heat shock proteins (Heikkila *et al*,. 1984), thiol proteases (Williams *et al.* 1994), proteinase inhibitors (Reviron *et al.*, 1992), polyamine (Turano and Kramer 1993; Botella *et al.*, 2000) and anti-oxidative enzymes (Freitas *et al*., 2007).

Hussein *et al*., (2013) compared the allelopathic potentiality of two invasive species; *Heliotropium curassavicum* and *H. Bacciferum* on germination, seedling growth and protein expression of *Calotropis procera, Faba sativa* and *Lycopersicon esculentum*. They found that Expression of proteins in treated plants was significantly increased or decreased at the level of number and intensity of protein bands as compared to control plants, depending on the type and concentration of extract treatment.

CONCLUSION

Based on the results of this study, the species with the strongest allelopathic potential, *Haplophyllum tuberculatum* must be examined for its selective action on other field conditions. Analysis of possible allelochemicals is also required. The isolation and characterization of growth inhibitors, which might be responsible for the strong allelopathic potential, are needed. There is possibility of using these allelochemicals for the discovery and development of environmental friendly herbicides to control weeds.

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