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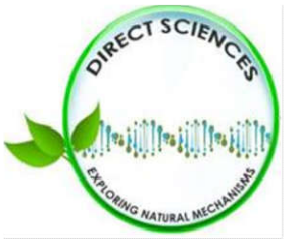

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Effect of Casuarina Allelochemicals on Growth of Cultivated and Weed Plants

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Abstract: The effect of allelochemicals from *Casuarina cunninghamiana* branchlets was assessed on the growth and metabolomic content of three recipient plants: *Cucumis sativus* L. (cucumber), *Lycopersicon esculentum* Mill. (tomato) and *Brassica nigra* L. (black mustard). The aqueous extract delayed the germination rates of the three species with pronounced effect on *B. nigra* and *L. esculentum.* The aqueous extract significantly reduced the seeds germination percentage of *B. nigra*. The shoot and root lengths were reduced significantly under these concentrations and the germination index (GI) registered lower value due to the effect of the extract on root length. Under these concentrations (1- 4 %) the seeds germination percentage of *L. esculentum* was not significantly affected but the root length was dramatically decreased. No significant change either in seeds germination percentage or in the root length up to the concentration of 4 % was recognized in *C. sativus*.

Application of allelochemicals from *C. cunninghamiana* branchlets not only affected the germination and growth criteria but also affected the biochemical and metabolomic content in the recipient crop species. A significant increase in carbohydrates, proteins and phenolics content was also observed in *L. esculentum* and *C. sativus*, meanwhile a significant decrease in favonoids and proline content was observed in both plants. Stress on germinated crop seeds resulted in a significant decrease in photosynthetic pigments content under lower concentrations from *C. cunninghamiana* branchets aqueous extract in both plants but the content was increased significantly under the highest concentration in tomato. Sensitivity of the weed *(B. nigra*) to the allelochemicals from the donner plant may open the door to exploit *C. cunninghamiana* to overcome the growth of weeds species associated to crop plants through using this plant as natural herbicides.

Key words: Allelopathy, *Casuarina cunninghamiana*, *Cucumis sativus,* Germination percentage, *Lycopersicon esculentum*, Root length, Shoot length, PCA

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Introduction

Tree allelopathy is defined as the capability of certain trees to improve the growth of certain plants on other associated plants in an environmental system (Nandal et al., 1994). This ability of a tree to stimulate or stop the growth of some plants in a system is attributed to the presence of some secondary metabolites or allelochemicals in its tissues (Nandal et al., 1994). Many definitions of the term allelopathy were established and among the most important one was that of Rice, 1984 " The direct or indirect inhibitory and stimulatory effects of one plant on another through the secretion of chemical compounds escaping into the environment". The International Allelopathy Society (IAS) defined allelopathy as "Any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects". The secondary metabolites or allelochemicals secreted by donor plants and that may have an allelopathic potential are water soluble metabolites belonging to different chemical classes such as phenolics, hydroxamic acids, terpenoids, alkaloids (Bargali and Bargali, 2009, Kruse et al., 2000), saponins (Oleszek and Jurzysta, 1987; Edewor et al., 2009) and isothiocyanates (glucosinolates byproduct) (Bialy et al., 1990). Secondary metabolites from the donor plant reached the recipient plants through different ways including leaching, root exudation, volatilization, and decomposition of plant residues in soil (Kobayashi, 2004).

The success of allelopathic research in crop protection may depend on the degree of resistance or susceptibility of crops and weed plants to allelochemicals (Abdel-Farid et al., 2013; Gomaa et al., 2014; Mohamed, 2015). In Egypt, vegetables represent the most important crops of economic significance to Egyptian people. The most important factor that may negatively affect the vegetable crop productivity is the competition between these vegetables and associated weed species. Allelopathic research may be used to search for some allelochemicals that suppress the growth of weed species associated with these economic crops (Kohli et al., 1998).

The present study was designed to investigate the effect of allelochemicals of *C. cunninghamiana* on seeds germination and seedlings growth of cultivated crops (cucumber and tomato) and their associated weed species (*Brassica nigra*). The objectives will also be extended to assess the change in metabolomic and physiological characteristics of recipient plants under allelochemical stress.

Materials and Methods

Plant collection

The branchlets of *Casuarina cunninghamiana* were collected from Aswan University campus, Aswan, Egypt.

Preparation of aqueous extract

The branchlets of *C. cunninghamiana* were dried at room temperature, then ground to fine powder using an electrical mixer. The stock solution (12.5 %) was prepared by soaking the powder in sterilized distilled water for 24 h at room temperature. The extract was filtrated and series of concentrations (1, 2, and 4 %) were prepared using dilution method with sterilized distilled water.

In vitro germination experiment

The same size seeds of *Cucumis sativus* (cucumber), *Lycopersicon esculentum* (tomato) and *Brassica nigra* (black mustard) were soaked in distill

water for 30 min, then surface sterilized using 70 % ethanol for 30 sec, shaked in 5 % sodium hypochlorite for 10 min and finally washed four times with sterilized distilled water. After sterilization, 10 seeds of each plant under investigation were transferred into 12 cm sterile Petri dish with filter paper and then wetted with 8 ml distilled water (control) and different treatment solutions of 1, 2 and 4 % of the aqueous extract. All these steps were carried out under sterilized conditions (safety cabinet). Distilled water was added when necessary. Each treatment was replicated four times. Petri dishes were labeled and incubated in the growth chamber at the Unit of Environmental Studies and Development (UESD) maintained at 23 ºC ± 2 and 14/10 h light/dark illumination. Germinated seeds were counted when a 2 mm radicle has emerged from seed coat and seen by naked eye (El-khatib and Abd-Ellah, 1998; Grange et al., 2000). Counting process was recorded daily at the same time. After 8 days, seeds germination percentage was recorded, shoot and root lengths were measured. Data representing seedlings growth (root and shoot lengths) were based on the randomly selected number of seedlings from replicates of each treatment. Germination index were calculated using the length of radicle and seeds germination percentage in control and treated plants according to Zucconi et al. (1981) based on the following equation:

$GI = [(G/G_0) X (L/L_0)] X 100$

Where: GI = the germination index, G = germination percentage in treated seeds, G_0 = germination percentage in control, L = length of radicle in treated seeds with the extract, L_0 = radicle length in control.

Percentage germination inhibition (PGI) was calculated according to Hegazy and Fadl-Allah (1995) from the following equation:

Percentage germination inhibition (PGI) = [100 – (% germination of treated seeds/ % germination of control seeds) X 100].

Pots experiment

The same size seeds of *C. sativus* and *L. esculentum* were surface sterilized as mentioned above. Five seeds of cucumber and 8 seeds of tomato were sowed in a plastic pot (15 cm height and 10 cm diameter) contains 400 g of peat moss, sand and clay in ratio (1.5:1:1.5). Pots were transferred to the growth chamber under conditions of 23º C and 14/10 h light/dark illumination. Every two days, pots were watered. After 24 days, pots were irrigated with nutrient solution (Hoagland's solutions) for two times. Thinning of seedlings was carried out 28 days after planting leaving three cucumber plants and five tomato plants in each pot. Non-treated plants were kept as control and stressed plants were subjected to the concentrations (1, 2, 4 %) from branchlets of *C. cunninghamiana* aqueous extract. Plants were harvested seven days post treatment. Harvested plants were washed in distilled water to remove salts and soil remains from the surface tissues. Fresh samples were used for the determination of chlorophyll a, chlorophyll b, carotene and proline contents. The rest of plant samples were dried at room temperature, then grounded using electrical mixer to fine powder which used in estimation of carbohydrates, proteins, saponins, flavonoids, total phenolics and total antioxidant capacity (Mohamed, 2015).

Plant analysis

Determination of chlorophyll

Fifty mg of fresh samples was extracted using 5 ml of 100 % methanol overnight then homogenized and centrifuged for 10 min at 1000 rpm. The supernatant was separated and the absorbance was read at 666, 653 and 470 nm. Chlorophyll A, Chlorophyll B, total carotene and total chlorophyll were calculated according to Dere et al. 1998. The pigment level was expressed as µg/g FW.

Determination of free proline

Proline content was determined using ninhydrin method according to Bates et al., 1973. The absorbance was read at 520 nm using toluene as a blank and the free proline content was determined from a curve constructed with proline standard. Proline concentration was calculated as a fresh weight basis (mg/g FW).

Determination of carbohydrates and proteins

Carbohydrates were determined using anthrone reagent (Morris, 1948). The regression equation was extracted from the concentrations of glucose standards and their absorbance. The concentrations of carbohydrates in plant samples were calculated and expressed as mg/g DW. Proteins were determined using Folin Ciocalteau reagent by Lowry et al., 1951. The content of total protein was expressed as mg/g dry weight (DW).

Determination of phenolics, flavonoids, saponins and total antioxidant capacity

The total phenolics content in the extract was determined according to the Folin-Ciocalteau method (Singelton et al., 1999) with gallic acid as a standard and expressed (mg) as gallic acid equivalents per gram of extract. The absorbance of the samples was measured at 695 nm with spectrophotometer (Thermo Spectronic Genesys 5). Total flavonoids content was determined using the aluminum chloride colorimetric method according to Zhishen et al., 1999. Quercetin was used to make the calibration curve and the results were expressed as mg quercetin equivalents per gram of extract. Saponins content was determined using vanillin solution according to Ebrahimzadeh and Niknam, 1998. The absorbance of the samples was measured at 473 nm with spectrophotometer and saponins content in samples was calculated from a standard curve constructed with purified saponins. Measurment of total antioxidant capacity was determined spectrophotometrically according to Prieto et al., 1999. The total antioxidant activity was expressed as (mg) ascorbic acid/ g of sample.

Statistical analysis

One way analysis of variance (ANOVA) (F-test) from Minitab version 12.21 was used to assess the significant difference of all the data recorded in the studies including difference between the percentages of germination, shoot and root lengths. ANOVA also was used to test the significant difference in secondary metabolites and photosynthetic pigments content in control and treated plants. The data presented in the form of mean with standard deviation. p values < 0.05 considered significant, $p < 0.01$ considered highly significant and $p < 0.001$ considered very highly significant. Principal component analysis (PCA) was performed with the SIMCA-P software (v. 12.0, Umetrics, Umeå, Sweden) for primary and secondary metabolites, photosynthetic data and also morphological parameters.

Results

Effect of aqueous extract of *C. cunninghamiana* on seeds germination and seedlings growth of some cultivated and weed plants

The results indicated that allelochemicals from *C. cunninghamiana* delayed the seeds germination of the tested species (Table 1). The seeds germination percentage and seedlings growth of *C. sativus* were not affected meanwhile the shoot length was significantly increased under the allelochemical stress of *C. cunninghamiana* (Fig. 1A, D and G). Even the lower concentrations had not affected the percentage of seeds germination in *L. esculentum*, the root length was significantly reduced (Fig. 1B and E). Both seeds germination percentage and seedlings growth of *B. nigra* was significantly reduced (Table 1 and Fig. 1C, F and I).

The results of percentage of germination inhibition (PGI) and seedlings growth index (GI) referred to the prominent negative effect of *C. cunninghamiana* aqueous extract on the growth of *L. esculentum* and *B.nigra*. In *B. nigra*, PGI is very high and GI is very low due to the effect of extract on both percentage of germination and root length. No effect of the aqueous extract of *C. cunninghamiana* either on the PGI or on GI in *C. sativus* (Table 1).

Table 1. Effect of *C. cunninghamiana* aqueous extract on seeds germination and seedlings growth of cultivated and weed species

Treatment	Germination Percentage (%) (Days after germination)								
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	**PGI**	GI
C. sativus									
Control	40 ± 8.2	95 ± 5.7	98 ± 5.0	98 ± 5.0	100 ± 0.0	100 ± 0.0	100 ± 0.0		
1%	90 ± 9.2	95 ± 5.7	95 ± 5.7	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	0.0	**120**
2%	5 ± 5.7	85 ± 5.7	90 ± 8.2	98 ± 5.0	98 ± 5.0	98 ± 5.0	98 ± 5.0	2.0	**86.7**
4%	0 ± 0.0	30 ± 8.2	78 ± 5.0	78 ± 5.0	88 ± 5.0	90 ± 8.2	90 ± 8.2	10.0	**77.2**
L. esculentum									
Control	2.5 ± 5.0	2.5 ± 5.0	10.0 ± 8.2	43 ± 9.5	58 ± 20.6	68 ± 26.3	93 ± 9.5		
1%	0 ± 0.0	5.0 ± 5.7	5.0 ± 5.7	15 ± 5.7	30 ± 8.2	53 ± 22.2	65 ± 17.3	17.0	**10.7**
2%	0 ± 0.0	0 ± 0.0	0 ± 0.0	2.5 ± 5.0	15 ± 12.9	48 ± 17.0	80 ± 16.3	9.0	**6.8**

4%	0.0 ± 0.0	2.5 ± 5.0	2.5 ± 5.0	2.5 ± 5.0	18 ± 15.0	55 ± 20.8	75 ± 17.3	14.0 **14.3**
B. nigra								
Control	5.0 ± 5.7	13 ±12.5	30 ± 8.2	33 ± 9.5	35 ± 5.7	53 ± 9.5	58 ± 5.0	
1%	0 ± 0.0	0 ± 0.0	10 ± 8.2	10 ± 8.2	10 ± 8.2	20 ±11.5	30 ± 8.2	53.0 **26.5**
2%	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	8 ± 9.5	89.0 **0.38**
4%	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	8 ± 5.0	89.0 **0.18**

PGI = Percentage of germination inhibition, GI = Germination index

Effect of *C. cunninghamiana* aqueous extract on primary and secondary metabolites and photosynthetic pigments content in *C. sativus* and *L. esculentum*

Secondary metabolites and photosynthetic pigments content was profiled in *C. sativus* and *L. esculentum* after plants subjection to concentrations of *C. cunninghamiana* aqueous extract (Table 2). Both *C. sativus* and *L. esculentum* showed more or less the same pattern of variation in primary and secondary metabolites profiling and photosynthetic pigments content. The content of phenolics was significantly increased with increasing the concentrations of the aqueous extract of *C. cunninghamiana* in both plants particularly in *L. esculentum*.

Secondary metabolites such as flavonoid and saponins in addition to the amino acid proline content has showed significant decrease in cucumber and tomato (Table 2). TAC was increased significantly under the lowest concentration, whereas it decreased significantly under the moderate concentration in cucumber. In tomato the TAC was significantly decreased with increasing the concentrations (Table 2). Carbohydrates was increased significantly ($p < 0.001$) in *C. sativus* under 1 and 2 % from *C. cunninghamiana* allelochemicals. In *L. esculentum*, carbohydrates increased significantly under 1 % of *C. cunninghamiana* allelochemicals ($p < 0.05$) and increased non significantly under 4 % from *C. cunninghamiana* allelochemicals (Table 2). Proteins was increased significantly at the highest concentrations of allelochemicals in *C. sativus* ($p < 0.001$) and increased significantly under any concentrations in *L. esculentum* ($p < 0.01$, 0.001 and 0.001), respectively (Table 2).

Photosynthetic pigments represented by chlorophyll a, b, total chlorophyll (a + b) and carotenoids showed different pattern of variation in both plants. In *C. sativus*, chlorophyll a, b and total chlorophyll content was significantly decreased with increasing the concentrations of the aqueous extract of *C. cunninghamiana*, whereas, carotenoids content was decreased at the highest concentration used (4 %) (Fig. 2A). In *L. esculentum*, total chlorophyll, chlorophyll a and b content was decreased with lower concentrations but increased significantly with the highest concentration used (4 %). Carotenoids content in *L. esculentum* control was under the detectable level, and then the content was increased with increasing the concentrations of *C. cunninghamiana* aqueous extract (Fig. 2B).

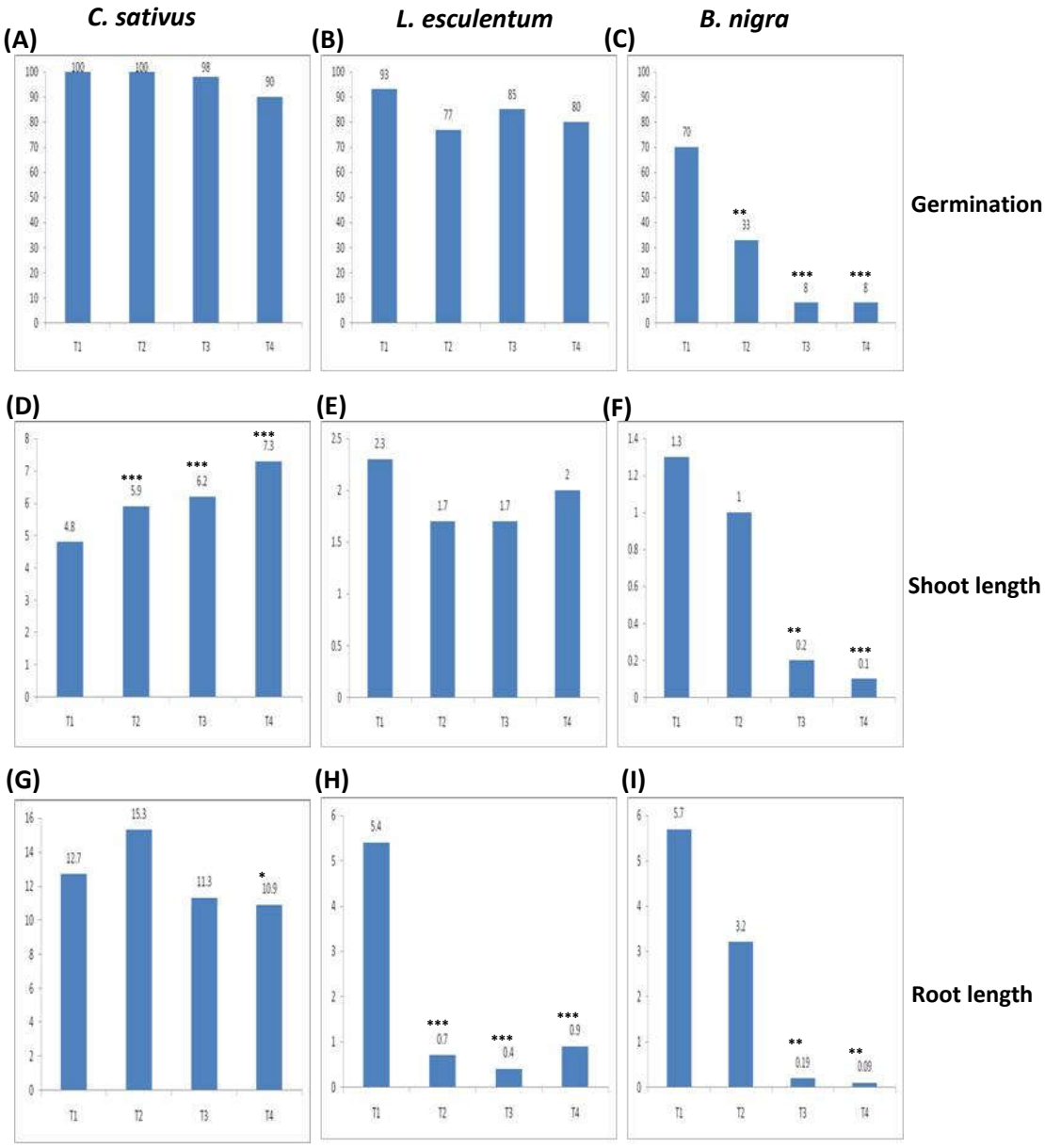

Fig. 1. Effect of aqueous extract of *C. cunninghamiana* on seeds germination and seedlings growth of crop and weed species. A-C: Seeds germination, D-F: Shoot length and G-I: Root length of the three species. T1 = Control, T2 = 1 %, T3 = 2 % and T4 = 4 %.

Table 2. Metabolomic profiling of *C. sativus* and *L. esculentum* after subjection to *C. cunninghamiana* aqueous extract.

Plant	Conc.	Flavonoid (mg/g)	Saponins (mg/g)	Pheonlics (mg/g)	TAC (µg/g)	Proline (mg/g)	Carbohydrates (mg/g)	Proteins (mg/g)
C. sativus	**Control**	1.39 ± 0.04	20.81 ± 0.30	0.43 ± 0.01	1690 ± 3	0.06 ± 0.001	9.73 ± 0.20	26.17 ± 0.09
	1%	1.06 ± 0.03***	13.95 ± 0.02***	0.32 ± 0.0***	2113 ± 4***	0.05 ± 0.001**	17.01 ± 0.32**	28.6 ± 2.08
	2%	1.19 ± 0.03**	12.70 ± 0.01***	0.68 ± 0.02***	1220 ± 4***	0.035 ± 0.001***	14.74 ± 0.31**	26.89 ± 0.63
	4%	1.36 ± 0.03*	15.94 ± 0.04***	0.44 ± 0.0	1686 ± 7	0.05 ± 0.005**	10.04 ± 0.10	28.10 ± 0.12***
L. esculentum	**Control**	2.74 ± 0.09	31.86 ± 0.93	0.30 ± 0.02	1717 ± 8	0.051 ± 0.001	11.48 ± 0.29	53.25 ± 0.82
	1%	2.59 ± 0.03*	32.18 ± 0.28	0.36 ± 0.02*	1707 ± 28	0.041 ± 0.003*	12.06 ± 0.05*	56.33 ± 0.69**
	2%	2.43 ± 0.11*	32.41 ± 0.27	0.35 ± 0.01*	1605 ± 28**	0.045 ± 0.004	9.8 ± 0.91*	66.30 ± 0.93***
	4%	2.22 ± 0.01***	30.05 ± 0.27*	0.35 ± 0.02*	1583 ± 15***	0.032 ± 0.008*	12.02 ± 0.75	59.51 ± 0.51***

Data considered significant at $p < 0.05$, TAC = Total antioxidant capacity

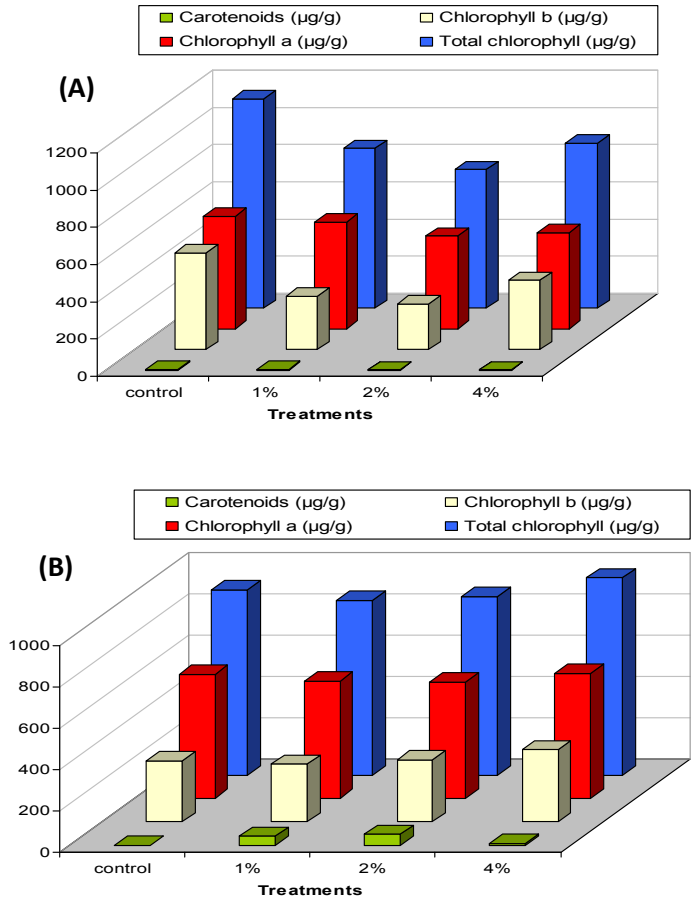

Fig. 2. Photosynthetic pigments of *C. sativus* (A) and *L. esculentum* (B) after subjection to *C. cunninghamiana* extract.

Study of the allelochemicals effect on seeds germination, seedlings growth and metabolites content in tomato using metabolomic combined with multivariate data analysis

Data obtained from seeds germination and seedlings growth together with the metabolomic contents after allelochemicals stress in tomato was analyzed using multivariate data analysis. Multivariate data analysis reduced the dimensionality of the data into only one score scatter plot which discriminated control and treatments into groups (Fig. 3A). Score loading plot distributed the determined parameters including germination percentage, shoot and root lengths in addition to the metabolites and photosynthetic pigment content of tomato on the discriminated groups (Fig. 3B). Score biplot (Fig. 3C) which includes both groups and their characterized parameters in the same figure (score biplot gives data of score and loading plot in one figure).

PC1 and PC2 explain about 67.9 % from the variation among data. Score scatter plot showed three groups including first one that was the control, second one includes low concentrations (1 and 2 %) while the third includes high concentration (4 %). Score loading plot showed that the percentage of germination, root and shoot lengths in addition to the content of saponins, proline, flavonoids, carotenoid

and TAC were reduced in tomato with increase in the concentration of the aqueous extract of *C. cunninghamiana*, where as chlorophyll a and b, proteins, carbohydrates and phenolics were increased with increase in the concentration of the aqueous extract of *C. cunninghamiana* (Fig. 3 A-C).

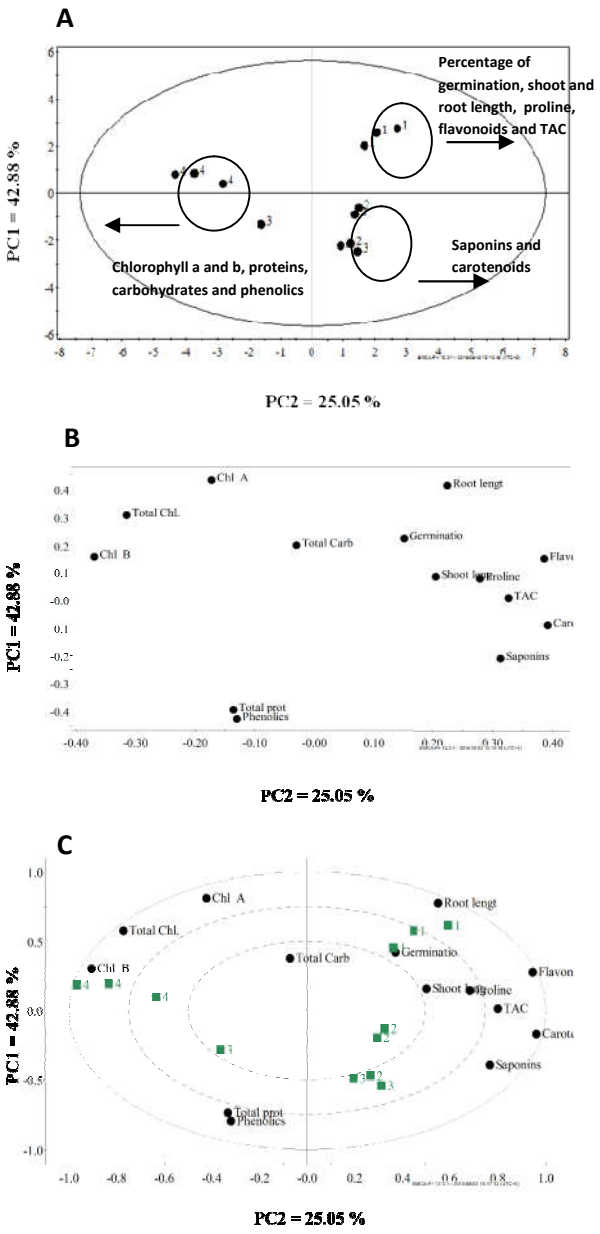

Fig. 3. Principal component analysis (PCA) of data obtained from tomato under allelochemical stress **(A)** score scatter plot of PC1 vs. PC2, **(B)** score loading plot of PC1 vs. PC2 and **(C)** score biplot of PC1 vs. PC2. 1 = control, 2 = 1 % of *C. cunninghamiana*, 3 = 2 % of *C. cunninghamiana* and 4 = 4 % of *C. cunninghamiana*.

Discussion

The interference of allelochemicals from the donor species with the enzymes controlling seeds germination and seedlings growth may be the reason of delaying the seeds germination and the reduction of the percentage of seeds germination and seedlings growth in sensitive species particularly with the highest concentrations used (Kamal, 2010). *Casuarina cunninghamiana* branchlets have important growth inhibitor allelochemicals such as saponins, tannins and phenolics (unpublished data). Some of these secondary metabolites if present in higher amounts may affect negatively the seeds germinations and seedlings growth (Mohamed, 2015). The accumulation of secondary metabolites due to allelochemical stress in the recipient species beyond the proper level may also cause self-toxification in the recipient seedlings. The negative effect of concentrated extracts on seedlings growth may be due to the effect of tannins, saponins and phenolics present in *C. cunninghamiana* extract and membrane permeability (Politycka, 1996; Reigosa et al., 2001) and on cell division (Callaway and Aschhoug, 2000; Al-Wakeel et al., 2007). In the current study, the magnitude of the reduction of seeds germination or seedlings growth is concentration dependent. Recently, the germination percentage inhibition (GPI) and also the reduction of root length of wheat and black mustard were increased with increasing the concentrations of the extract of different parts of *Acacia* species (Mohamed, 2015). The aqueous extract of *C. cunninghamiana* below the concentration of 4 % had no negative effect on either percentage of seeds germination or seedlings growth of *C. sativus*. Moreover the

shoot length of *C. sativus* was stimulated under these concentrations. Meanwhile these low concentrations were significantly reduced the percentage of seeds germination and seedlings growth (shoot and root lengths) in *B. nigra* and affected negatively root length in *L. esculentum*. These findings are in agreement with many previous reports regarding the selectivity effect of the allelochemicals on growth of different species (Abdel-Farid et al., 2013; Leela et al., 2014; Gomaa et al., 2014; Mohamed, 2015). *Acacia* species affected negatively *B. nigra* seeds germination and seedlings growth and had no effect on *Triticum aestivum* grains germination or seedlings growth at lower concentrations (Mohamed, 2015). *Casuarina equisetifolia* extract stimulated the growth of wheat growth, while negatively affected the growth of *Phalaris minor* the wheat associated weed (Hozayn et al., 2015).

Allelochemicals stress may affect negatively on the photosynthetic pigments. This may interpret the reduction of photosynthetic pigments in both cultivated plants after allelochemical stress from *C. cunninghamiana*. The aqueous extracts containing allelochemicals from *A. nilotica* and *A. seyal* reduced the photosynthetic pigments in *T. aestivum* (Mohamed, 2015). With increasing the concentrations of *A. nilotica* leaf residue, a decrease in photosynthetic pigments was recognized in *P. sativus* (Al-Wakeel et al., 2007). Kamal, 2010 reported a significant decrease in chlorophyll content in two varieties of *T. aestivum* under allelochemical stress from *H. annus*.

Increasing the content of phenolics compounds in *L. esculentum* is a result of stress caused by *C. cunninghamiana* allelochemicals. *Cicer arientinum* showed higher content of phenolics after exposure to high concentrations from the aqueous leaf leachate of different tree species under laboratory conditions (Das et al., 2012). The same results were reported in case of *T. aestivum* and *P. sativum* subjected to *A. nilotica* allelochemicals (Al-Wakeel et al., 2007; Mohamed, 2015). The reduction of saponins content in cultivated crops is not consistent with previous reports such as Mohamed, 2015 who reported higher content of saponins in *T. aestivum* under *Accaia* allelochemical stress and this may be attributed to the synthesis of other compounds from the same pathway on expenses of saponins precursors. Proline was reduced after allelochemicals application and this is contradictory with previous reports (Abdel-Farid et al., 2013). Proline is an osmotic regulator amino acid which shows significant increase in plants subjected to different types of stresses particularly drought stress (Djanaguiraman et al., 2005; Das et al., 2012). The reduction of proline in this study particularly under high concentrations of extract may be attributed to the consumption of proline to build up other metabolites that regulate the osmosis in stressed plants.

According to Das et al., 2012 and Gomaa et al., 2014, each plant species responds in a different way to the same allelochemicals. This is one of the positive sides of allelopathy to use allelochemicals of one plant such as *C. cunninghamiana* to control the growth of weed species (*B. nigra*) associated to economic cultivated crops such as *C. sativus*. Subjection of *L. esculentum Casuarina* leachate affects negatively on the plant growth by reducing their root length even under very low concentrations of *C. cunninghamiana* extract.

Metabolomic combined with multivariate data analysis provides valuable information regarding the effect of allelochemicals on tomato seeds germination and seedlings growth and also on metabolic alteration in plant after allelochemical stress.

This study showed the importance of multivariate data analysis (MVDA) in the evaluating the correlation between the morphological changes and metabolomic alteration in plants under allelochemical stress, which is clarified in their scores and biplot loadings. Metabolomic combined with MVDA will not only predict the effect of allelochemical stress on plant growth but also will enable the researchers to breed new cultivars that will be tolerant to allelochemical stress.

Conclusion

C. cunninghamiana aqueous extract reduced significantly percentage of seeds germination and seedlings growth of weed species and root length of *L. esculentum* at low concentrations, where no significant effect was noticed in growth parameters in *C. sativus* under the same concentrations. The magnitude of reduction of seed germination, shoot and root length after treatment with *C. cunninghamiana* extract followed the order of: *B. nigra* > *L. essculentum* > *C. sativus*. The allelopathic potential of *C. cunninghamiana* may be exploited for preparing natural bioherbicides controlling weeds species associated to economic crops. Farmers should clarify their fields from *C. cunninghamiana* litter or residues before *L. esculentum* is sown in such fields due to the negative impact of the aqueous extract of *C. cunninghamiana* even at very low concentrations on the root length and consequently on *L. esculentum* growth. Metabolomic combined with MVDA is a promising tool in studying the effect of allelochemical stress on crop plants.

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