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The Probable Allelopathic Interference of *Nigella sativa* L. Seed Extracts with *Lupinus termis* L.

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ABSTRACT

The main objective of the present study was to assess the effect of cold and hot *Nigella sativa* seeds aqueous extracts (NSSCAE and NSSHAE, respectively) and *Nigella sativa* seeds crude powder (NSSCP) on germination, some growth parameters and chemical constituents of *Lupinus termis* L. in mixed cropping system. Proximate analysis of *Nigella sativa* seeds showed that it contains oil, crude protein and ash beside significant amounts of vital mineral elements. The germination percentage was notably decreasing with increasing the concentrations of NSSCAE and NSSHAE. The effect on hypocotyl (HL) and radicle lengths (RL) of *L. termis* seeds was highly recognized. The applied concentrations of NSSCP caused a significant decline in growth parameters of *L. termis*. In addition, there was an inverse proportional relationship between increasing NSSCP concentrations and chlorophyll a and b, carotenoids and total pigment content of leaves. The variation in some biochemical constituents of *L. termis* seeds as affected by different concentrations of NSSCP was documented. The study concluded that seeds of *Nigella sativa* adversely affect seed germination and seedling growth of *L. termis*.
Key words: Allelopathy, *Lupinus termis*, *Nigella sativa*.

INTRODUCTION

Biologically active molecules (allelochemicals) produced by different plants and their residues may convert to other forms and affect growth of similar or non-similar plants (Seigler, 1996). Allelopathy is a physiological process with ecological implications (Reigosa *et al*., 2006). It is a complex phenomenon in which secondary metabolites produced by plants, micro-organisms, viruses and fungi, affect growth and development of other biological systems (Saffari and Torabi-Sirchi, 2011). These effects have been demonstrated in both greenhouse and field experiments and in mono and mixed cultures (Reigosa *et al*., 2006). Recently weed scientists are more interested in weed management by allelopathy (Hesammi, 2013). Meanwhile, El-Kenany and El-Darier (2013) suggested that *Lantana camara* aqueous extract could be used as a potential allelopathic substance for weed control.

Allelopathic potentiality under field conditions can be utilized in different ways. For example, surface mulch (Cheema and Khaliq, 2000), incorporation into the soil (Sati *et al*., 2004), aqueous extracts (Iqbal and Cheema, 2007a), rotation (Narwal, 2000), smothering (Singh *et al*., 2003) or mix cropping/intercropping (Iqbal and Cheema, 2007b). Crop residues is the name given to plant materials left in the field for decomposition after the harvesting/thrashing of a crop is over (Kumar and Goh, 2000). These residues can pose a chemical as well as a physical effect on the growth and development of subsequent crops and weeds (Mason-Sedun *et al.*, 1986). The decomposing crop residues release a variety of allelochemicals, particularly the phenolics, in the soil causing adverse effects on the other plants (Nelson, 1996). The most commonly found allelochemicals, cinnamic and benzoic acids, flavonoids, and various terpenes (Singh *et al*., 2003); these compounds are known to be phytotoxic (Einhellig, 2002).

Multiple cropping is common practice in subtropical and tropical regions (Young *et al.,* 1989). Because there is a large move from mono-cropping to multiple cropping practices, one should be aware by the chemical interfering between the mixed crops in order to avoid undesirable potential effects of some crop on the others. A little is known about the allelopathic interaction of intercropped plants in mixed farming systems, consequently the main objective of the present study was to assess the probable allelopathic effects of *Nigella sativa* L. seeds on germination and growth of *Lupinus termis* L.

MATERIALS AND METHODS

The Study Species

Nigella sativa L. (Black cumin, Ranunculaceae, donor) and *Lupinus termis* L. var. balady (Lupin, Fabaceae, recipient) seeds were purchased from the National Research Center (NRC), Dokki, Giza. Both of them were newly harvested. The seeds of *N. sativa* were kept in glass jars at 5^{o}C, the extract was freshly prepared.

Germination Bioassay

For the preparation of cold and hot aqueous extracts, the method described by Al-Charchafchi *et al.* (2007) was used. Dried powder of *N. sativa* seeds (75 g) were extracted with 1000 ml autoclaved distilled water (for hot extract, the mixture was boiled for 5 minutes), magnetically stirred for 4 hours and allowed to stand 4 days under laboratory conditions. The supernatant was taken and centrifuged at 3000 rpm for 15 min; this would be the full strength concentration (100%). Then it was kept in a refrigerator at 5^{o}C until used. Series of dilutions (treatment) were prepared from the stock solution (5, 10, 20 and 40%) beside the control (distilled water). Petri-dish experiment was carried out to investigate the bioactivity of the different concentrations (treatments) of donor species (*N. sativa*) seed cold and hot aqueous extract (NSSCAE and NSSHAE) on germination percentage (GP), hypocotyl length (HL) and radicle length (RL) of the recipient species (*L. termis*). For each treatment, ten seeds of the recipient

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species were arranged in 18 cm diameter Petri-dishes on two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from 20-23°C and night temperature from 14-16°C.

Ten ml of each concentration level of the donor species extracts or distilled water (control) were added. Before sowing, the seeds of *L. termis* were surface sterilized by soaking for two minutes in 4% sodium hypochlorite, then, rinsed four times with distilled water. Treatments were arranged in a complete randomized block design with three replicates. Measurements of GP, HL and RL were recorded daily (Table 2).

Growth Experiment

Pot experiment was carried out in three replicates to test the effect of different concentrations of *N. sativa* seeds crude powder (NSSCP) on germination efficiency, seedling growth, pigment contents, nutrient concentrations and ash beside some biochemical constituents of *L. termis*. Ten seeds from the recipient species (*L. termis*) were sown in plastic pots 30 cm diameter filled with 2.2 kg sandy clay loam soil (soil samples from natural sites, where the alleged allelopathic materials are not present, were used to undergo the pot experiment). NSSCP/soil (w/w) prepared in concentration levels of 5, 10, 20 and 40% were thoroughly mixed with the soil before sowing. The experiment was performed under normal laboratory conditions with day temperature ranging from 19 -22°C. The plants were watered every two days on the average with normal tap water. Mature plants of the recipient species were harvested three months after planting. One treatment was run as control without NSSCP. Soil analyses were performed according to Allen *et al*. (1984).

Plant height, root length, number of leaves and total leaf area were determined. The samples were weighted fresh then dried at 40°C till constant weight to determine the dry weight of plant.

Protein contents was estimated according to Al-Gaby (1998), mineral constituents (Ca, Na, K, Mg, Fe, Zn, Cu and Mn) were determined according to Larrauri *et al*. (1996), for phosphorus content, the phosphomoly-bdovanadate method was used (AOAC, 1990). Fatty acid composition was analyzed by gas-liquid chromatography after derivatization to fatty methyl esters with 2 M KOH in methanol at room temperature (IUPAC, 1992). The photosynthetic pigments; chlorophyll a, b and carotenoids were extracted and determined using the spectrophotometric method recommended by Metzner *et al*. (1965). Total N was determined colorimetrically by Nessler's method according to Chapman and Pratt (1987). The percentage of total alkaloids was calculated as described by Kam *et al*. (1999).

A total phenol in different solvent extracts was determined spectrophotometrically following Folin-Ciocalteu method (Iqbal *et al*., 2005). Determination of free proline was carried out in aqueous extract using the acid ninhydrin method described by Bates *et al.* (1973), while total carbohydrate contents was estimated by the procedure described by Murata *et al.* (1986). Data were subjected to standard analysis of variance (ANOVA) (Zar, 1984).

RESULTS

Proximate analysis of *N. sativa* seeds showed that oil, crude protein and ash content attained values of about 24.55, 23.43 and 3.12% respectively (Table 1). Potassium is the most abundant element followed by calcium, magnesium and phosphorus.

The other elements, in descending order by quantity, were sodium, iron, zinc, manganese and copper. Data also showed that linoleic, oleic, palmatic and stearic acids account for about 43.8, 21.9, 17.8 and 3.7% of total fatty acid pool size respectively. The ratio of linoleic acid to oleic acid was about 2:1. Mono and polyunsaturated fatty acids attained values of about 28.3 and 54.2% respectively.

Germination percentage (GP) was notably decreasing with increasing the concentrations of NSSCAE and NSSHAE (Figure 1a & b respectively). The percentage decreased from 100% for control (0%) to about 33% and 20% for 40% NSSCAE and NSSHAE concentration levels respectively after nine days from sowing.

Highly significant correlations were calculated from simple linear regression obtained by plotting GP of the recipient species versus the different concentrations of NSSCAE and NSSHAE as evidenced by the high values of the coefficient of determination (R^2).

HL was significantly reduced upon applying different levels of NSSCAE and NSSHAE (Figure 2 a & b). The length was reduced from 2.7 cm and 2.5 cm for control to 1.1 cm and 0.9 cm at 40% NSSCAE and NSSHAE respectively after nine days from the beginning of the experiment. The regressions lines between HL and the different concentrations of NSSCAE and NSSHAE confirmed that the different effects were concentration dependent. Similarly, RL decreased with increasing NSSCAE and NSSHAE concentrations (Figure 3 a & b). At 40 % of the two extracts, RL was 0.96 cm and 0.6 cm as compared to 3.57 cm and 3.4 cm for control level respectively recorded at nine days after sowing. Highly significant correlations were proved by the very high values of the coefficient of determination (R2) for the two types of extracts. The t-value for the effect of applied NSSCAE and NSSHAE respectively on the GP, HL and RL of L. termis seedlings was listed in Table (2). The variation between the cold and hot extract was highly significant (P-value = 0.048, 0.003 and 0.0004 respectively) for the three mentioned parameters.

Table (1): Some chemical constituents (dry basis) and fatty acid composition of the Egyptian *Nigella sativa* L. seeds. Values are the means of three replicates.

Chemical characteristics			
Component	**Value (±SD)**	**Fatty acid compositions**	**(mg/100 g of total fatty acids)**
Oil[a]	24.55±0.7	Palmitic	17.8±0.15
Crude protein[a]	23.43±0.14	Palmitoleic	0.83±0.05
Ash[a]	3.12±0.02	Stearic	3.7±0.08
Potassium[b]	734.7±5.02	Oleic	21.9±0.21
Magnesium[b]	223.5±9.67	Linoleic	43.8±0.35
Calcium[b]	432.12±25.69	Saturated fatty acids (SAFA)	27.1
Phosphorus[b]	60.12±1.2	Monounsaturated fatty acids	28.3±0.28
Sodium[b]	17.56±1.87	Polyunsaturated fatty acids	54.2±0.61
Iron[b]	12.02±0.66		
Copper[b]	1.21± 0.06		
Zinc[b]	11.0±0.44		
Manganese[b]	2.21±0.12		

a: In % dry matter basis, b: In mg kg^{-1}of dry matter

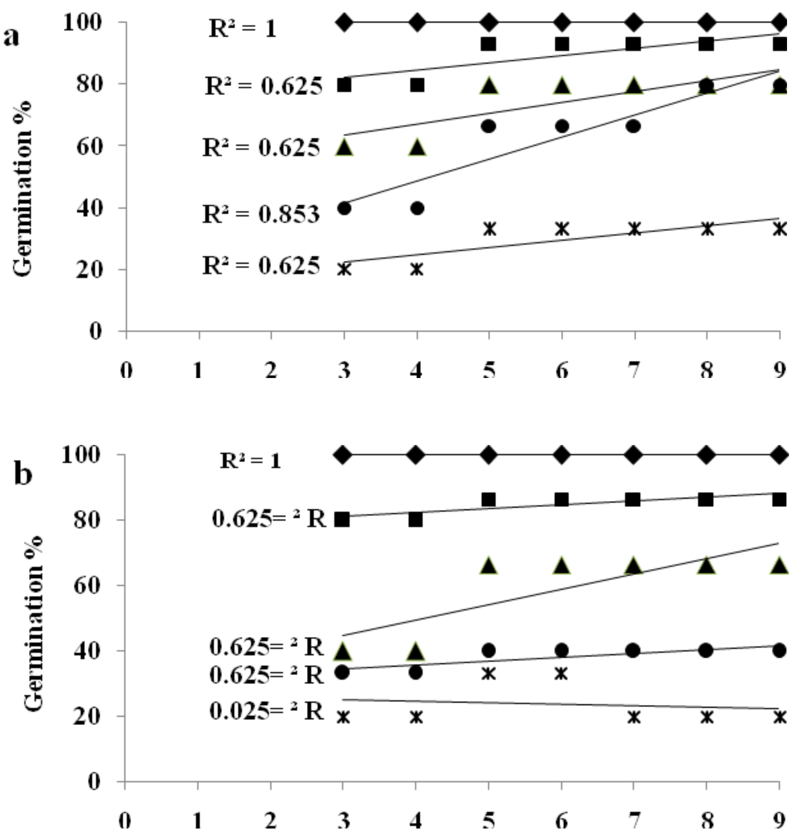

Figure (1): Regression analysis between germinationpercentagesof*Lupinustermis*seedsand the different concentrations of *Nigella sativa*seeds cold (a) and hot (b) aqueous extracts.

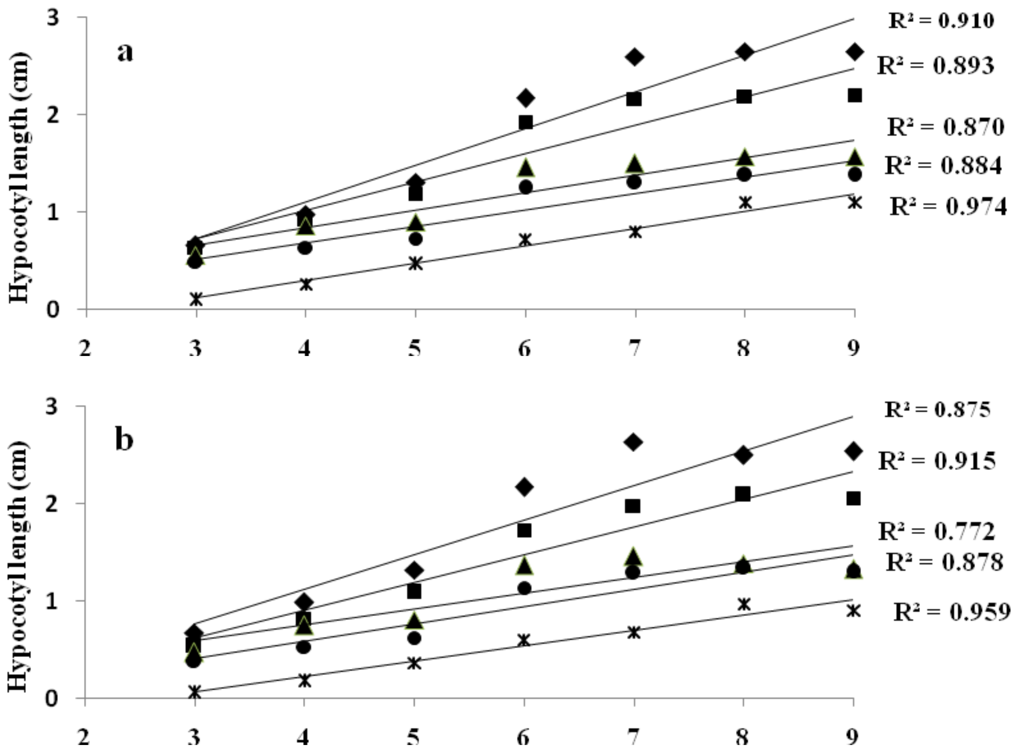

Figure (2): Regression analysis between hypocotyl lengthsof*Lupinustermis*and the different concentrations of *Nigella sativa*seedscold (a) and hot (b)aqueous extracts.

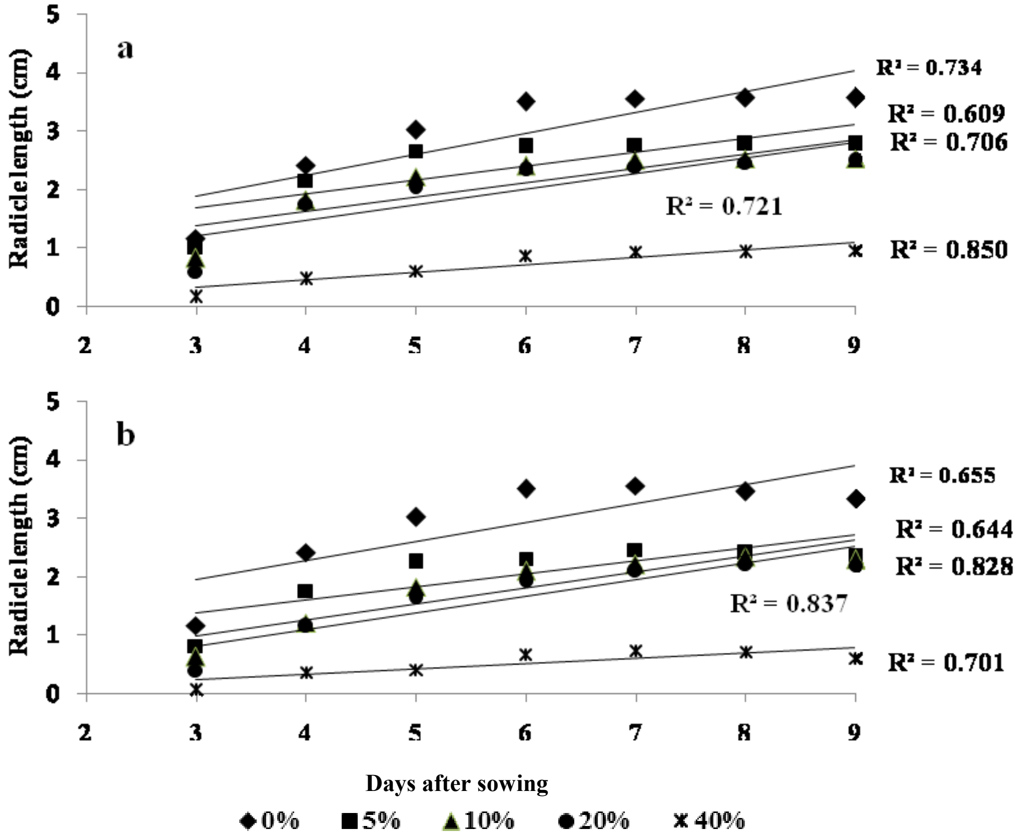

Figure (3): Regression analysis between radicle lengthsof*Lupinustermis*and the different concentrations of *Nigella sativa*seedscold (a) and hot (b) aqueous extracts.

Table (2): Effect of cold and hot *Nigella sativa* L. seeds aqueous extract (NSSCAE and NSSHAE respectively) on germination percentages, hypocotyl (HL) and radicle lengths (RL) of 9-days-old *Lupinus termis* L. seedlings.

Treat.(%)	Germination percentage (GP)		Hypocotyl length (HL)		Radicle length (RL)	
	Cold	Hot	Cold	Hot	Cold	Hot
0	100	100	2.65	2.54	3.57	3.34
5	93.3	86.6	2.2	2.06	2.78	2.38
10	80.0	66.6	1.57	1.33	2.53	2.30
20	80.0	40.0	1.38	1.30	2.50	2.20
40	33.3	20.0	1.1	0.91	0.96	0.61
P- Value	0.048		0.003		0.0004	

T- value was highly significant at $P<0.05$

Some physical and chemical characteristics of the soil used in the pot experiment are presented in Table (3). On the other hand, data presented in Table (4) demonstrate the effect of different concentrations of NSSCP on some growth parameters of *L. termis* plants.

The data showed that increasing in NSSCP concentration from control to 40% caused a significant decline in plant height, number of leaves, root length, fresh and dry weight and the total leaf area per plant. The highest means for the above mentioned growth criteria was observed in plants grown under the control conditions.

On the other hand, the lowest values were demonstrated with the presence of concentration 40% giving rise to a reduction percentage of about 74, 78, 88, 75, 95 and 90%, respectively relative to the control. The results presented in Table (5) showed that there was an inverse proportional relationship between increasing the severity of different percentages of NSSCP on one hand, and leaves content of chlorophyll a and b, carotenoids and total pigment content on the other hand.

The maximum means were attained under the control level. Minimum records of the above mentioned parameters were attained at the highest NSSCP concentration level (40%) with a significant difference between the maximum and minimum records. Reduction percentages of about 75, 94, 78 and 81% were calculated for the pigment fractions respectively. Conversely, Chl a/b increased to about four folds from control to the maximum NSSCP concentration. The variation in some biochemical constituents of *L. termis* seeds as affected by different concentrations of NSSCP is demonstrated in Table (6). Generally, total nitrogen, proteins (two-folds) and proline (three-folds) increased as the applied concentration of NSSCP increased. On the contrarily, total phenols, total alkaloids and total carbohydrates decreased markedly proportionally to the increase of NSSCP. The reduction percentages were about 63, 33 and 44% respectively.

The potassium concentration was suppressed under NSSCP application, with more than 3 fold decrease for 40% NSSCP compared to the control.

Table (3): Some physical and chemical characteristics of the experimental soil.

Character	Value ± SD	Soil Texture (sandy clay loam)	
EC [a]	2.71 ± 0.01	Sand [d]	56.20 ± 4.50
pH	7.70 ± 0.4	Clay [d]	32.20 ± 3.06
Ca [b]	12.00 ±1.3	Silt [d]	12.80 ± 1.70
Mg [b]	18.00 ± 2.34	**Available Nutrients (mgg^{-1})**	
Cl [b]	17.00 ± 2.8	N	1.10 ± 0.001
CO_3 [b]	34.00 ± 3.4	P	0.50 ± 0.001
SO_4 [c]	2.06 ± 0.03	K	3.50 ± 0.12
OM [d]	8.68 ± 1.02		

a: In ds/m b: In mg/kg c: In ppm d: In %

Table (4): Growth criteria of *Lupinustermis* L. seedlings as affected by different concentrations of *Nigella sativa* L. seeds crude powder (NSSCP) (w/w). Values are the means of three replicates.

Treat. (%)	Plant height (cm)	No. of leaves/ plant	Root length (cm)	Fresh wt./plant (g)	Dry wt./plant (g)	Total leaf area/plant (cm^2)
0	38.08^a	9.17^e	18.83^b	3.98^a	1.12^c	63.56^a
5	32.50^b	7.83^a	13.96^c	3.01^b	1.34^a	48.98^b
10	24.45^c	5.89^b	9.45^d	2.40^c	0.85^e	35.65^c
20	18.32^d	3.00^c	4.90^a	1.65^d	0.30^d	18.5^d
40	10.00^e	2.00^d	2.20^e	1.00^e	0.06^b	6.38^e

Table (5): Chlorophyll content (mg/g f. wt.) of *Lupinustermis* L. leaves as affected by different concentrations of *Nigella sativa* L. seeds crude powder (w/w). Values are the means of three replicates.

Treat. (%)	Chlorophyll a	Chlorophyll b	Chlorophyll a/b	Carotenoids	Total pigment content
0	3.64^a	1.65^a	2.20^a	0.422^a	5.71^c
5	2.54^b	0.90^b	2.82^c	0.390^c	3.83^d
10	1.89^c	0.43^d	4.39^b	0.300^d	2.62^e
20	1.23^d	0.21^e	5.86^d	0.154^f	1.59^f
40	0.90^e	0.10^c	9.00^e	0.091^b	1.09^a

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

Table (6): Variation in some biochemical constituents of *Lupinustermis* L. seeds as affected by different concentrations of *Nigella sativa* L. seeds crude powder (NSSCP) (w/w). Values are the means of three replicates.

Treat. (%)	Total Nitrogen (%)	Total Protein (%)	Total Phenols (%)	Total Alkaloids (%)	Proline (mg/100g)	Total Carbohydrates (%)
0	3.04^a	19.00^c	30.89^c	0.15^a	0.63^a	60.79^a
5	3.90^b	24.37^d	24.00^d	0.15^b	0.75^b	52.79^b
10	5.00^c	31.25^e	17.00^e	0.10^c	1.06^c	42.52^c
20	6.09^d	38.06^a	11.44^a	0.10^d	2.02^d	34.34^d
40	No seeds were produced at this concentration level					

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

Commonly, there was a general trend of decrease in the estimated mineral elements with the increase in treatment concentrations of NSSCP (Table 7). The application of NSSCP resulted in a significant decrease in the concentration of total nitrogen from 26 mg/g d.wt. at control to a minimum of about 9.45 mg/g d.wt. for 40% NSSCP concentration with a reduction percentage of about 64% relative to control. Similarly, phosphorus concentration attained a reduction percentage of about 93% relative to control.

Table (7): Allelopathic effect of different concentrations of *Nigella sativa* L. seeds crud powder on the concentration (mg g^{-1} d.wt) of some mineral elements in *Lupinus termis* L. plants at vegetative stage. Values are the mean of three replicates.

Treat. (%)	N	P	K
0	26.04^a	2.95^c	30.89^c
5	20.59^b	2.01^d	24.00^d
10	15.22^c	1.35^e	17.00^e
20	13.09^d	0.86^a	11.44^a
40	9.45^e	0.21^b	9.21^b

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

DISCUSSION

The use of other crops in mixed cultures may alleviate the problem of autotoxicity caused by repeatedly planting crop monocultures (Han *et al*., 2008). Nowadays, there is a large move from monocropping to polycropping practices, in almost of the agricultural ecosystems along the world. But, one should be aware by the chemical interfering between the mixed crops in order to avoid undesirable potential effects of one crop on the other. For instance, Ahangar *et al.* (2014) suggested that neem and eucalyptus shouldn't be grown near agricultural fields in order to alleviate their effects on crop productivity. Not using these plants species as a part of agroforestry would substantially avoid the possible allelopathic effect of these plants on various agricultural crops.

Data concerned with the allelopathic effects of NSSCAE and NSSHAE on the different germination and growth parameters of *L. termis* in the present study was highly differential. The germination percentage was notably decreasing with increasing the concentrations of NSSCAE and NSSHAE. The decrease in seed germination percentages of the recipient species significantly correlated with the concentrations of NSSCAE and NSSHAE This finding is congruent with the results of Chung and Miller (1995a). These results indicate that *N. sativa* seeds residues release allelopathic substances which accumulate in bioactive concentrations and adversely affect seed germination, seedling growth, and nutrient and metabolite contents of *L. termis*.

A number of studies have suggested that plant residues (seeds, leaves, roots) affect the growth and development of other plants including crops by releasing allelochemicals into the immediate soil environment (Batish *et al*., 2007; Han *et al.,* 2008). The considerable inhibition of seed germination may be due to the inhibitory effect of allelochemicals such as water soluble saponins, hormones, enzymes and polyphenols found in *N. sativa* seeds which could affect growth directly or by altering the mobilization of storage compounds during germination (Cheikh-Rouhou *et al*., 2007). These results coincide with that of Farrag *et al.* (2013) who concluded that allelochemicals produced by *Heliotropium curassavicum* and *H. bacciferum* extracts significantly caused inhibition in germination and seedling growth of *Calotropis procera, Faba sativa* and *Lycopersicon esculentum*.

The allelopathic potential of NSSCAE and NSSHAE concentrations on HL and RL of *L. termis* seeds was highly recognized. Generally, all concentrations significantly reduced the two growth parameters after nine days from sowing. The magnitude of reduction was concentration dependent. The reduction of HL and RL may be due to phytotoxic activity of phytochemicals present in aqueous extracts of *N. sativa* seeds. El-Darier *et al*. (2013) attained similar results on the effect of *Haplophyllum tuberculatum* aqueous extract on *Lepidium sativum* and *Raphanus sativus* seeds. Some allelochemicals like lignans (Sheriha *et al.,* 1987), alkaloids (Al-Rehaily *et al*., 2001), and monoterpenes and sesquiterpenes in the essential oil of *Haplophyllum tuberculatum* (Yari *et al*., 2000) may be responsible for their inhibitory effects. Fag and Stewart (1994) suggested that the inhibitory effect of *Acacia nilotica* was related to the presence of allelochemical including tannins, wax, flavonoids and phenolic acids.

However, the significant inhibition of plumule and radicle lengths of *Chenopodium album* and the reduction in their growth rate may be due to the pronounced amounts of phenols such as caffiec acid, coumaric acid, vanelic acid, benzoic acid, chlorogenic acid, ferulic acid and other phenolic acids present in the extract of *Mangifera indica* (El-gandaby *et al.*, 2014). On contrary, El-Darier and Zein El-Dien (2011) reported that water extracts of allelopathic plants had more pronounced effects on radicle than on hypocotyl growth.

Meanwhile, Salhi *et al.* (2012) reported that there is significant phototoxic effect of *Zygophyllum album* on germination of hypocotyl and radicle lengths of *Bromus tectorum.* In the same context, Mubeen *et al.* (2012) concluded that the combined application of *sorghum* and *sunflower* water extracts has overall more inhibitory effects on the germination of *Oryza sativa*, *Trianthema portulacastrum, Dactyloctenium aegyptium* and *Eleusine indica* when compared to their sole application, and the inhibitory substances in crop water extracts of sorghum and sunflower could be used as a potent bio herbicide.

The applied concentration of NSSCP caused significant decline in plant height, number of leaves, root length, fresh and dry weight and the total leaf area per plant at full vegetative stage. The mixed cropping is a common practice in farmer fields.

There was an inverse proportional relationship between increasing the severity of different percentages of NSSCP on one hand and content of leaves of chlorophyll a and b, carotenoids and total pigment content on the other hand. Conversely, Chl a/b increased to about four folds from control to the maximum NSSCP concentration. The results of the present study are matched with that obtained by khalil and Ismael (2010) on the effect of water stress on pigment content of *L. termis* leaves. Our results were explained on the concept of the effect of NSSCP on the water uptake by the recipient species which may be the essential factor for tissue drought. The results were fortified by many authors such as Sanchez-Blanco *et al.* (2006), Zhang *et al.* (2006) and Abdalla and El-khoshiban (2007).

In conclusion, seeds of *N. sativ*a adversely affect seed germination and seedling growth of *L. termis* that are commonly intercropped with it. Meanwhile, the poor quality of *L. termis* individuals resulted from the harmful effect of NSSCP may be one of the drawbacks of the chemical stress occurs in the mixed cropping systems.

Therefore, *N. Sativa* seeds must be considered as an allelopathic species posing risk in a rotation or an intercropping of mixed cropping system. With a view to alleviate its adverse effects on intercropping or subsequent crops, farmers should be conscious of the seeds librated from the mature legumes and mixed with soil.

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التداخل المحتمل للإبعاد التضادي الكيميائي بين نبات الحبة السوداء ونبات الترمس في نظام زراعى مختلط

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الملخص العربى

تهدف الدراسة الحالية إلي تقييم أثر المستخلصات المائية للحبة السوداء علي الطريقة الباردة والساخنة وكذلك المسحوق الخام على إنبات الترمس وكذلك على بعض معايير النمو و المكونات الكيميائية للنبات في نظام الزراعة المختلط. وقد أظهر التحليل التقريبي لبذور حبة البركة أنه يحتوي على زيت وبروتين خام ورماد بجانب كميات كبيرة من العناصر المعدنية الحيوية. وقد أظهرت الدراسة الحالية تبايناً واضحاً لكلا المستخلصين علي العوامل المختارة لنبات الترمس. فقد تأثرت نسبة الإنبات، طول السويقة السفلي وطول الجذير بالسلب بتزايد تركيز المستخلصات وكذلك طول السويقة السفلي وطول الجذير. وبصفة عامة فإن تركيزات المستخلصات قد أدت إلي تناقص كلا المعيارين حيث تبين أن النسبة العالية في التناقص والتي سجلت بعد تسعة أيام من الزراعة كانت عند التركيزات العليا من كلا المستخلصين . وبالإضافة إلي ذلك فقد أظهرت النتائج أنه كلما ذادت تركيزات المسحوق الخام لبذورنبات الحبة السوداء كلما تناقصت معايير النمو المختلفة لنبات الترمس.ومن خلال هذه الدراسة فقد تبين أن هناك إرتباطاً عكسياً بين زيادة نسب إضافة المسحوق الخام من ناحية ومحتوي الأوراق من كلورفيل أ و ب والكاروتينات ومحتوي الأصباغ الكلية من ناحية أخري. وعلي العكس من ذلك فقد زادت نسبة كلورفيل أ:ب أربعة أضعاف بالمقارنة بالعينة الضابطة عند التركيز الأعلي للمسحوق الخام. وفي النهاية فقد خلصت الدراسة إلي أن بذور نبات الحبة السوداء قد أظهرت بعض التأثيرات السلبية علي الإنبات ونمو البادرات لنبات الترمس. لذلك يمكن أن نعتبر أن نبات الحبة السوداء قد يكون مصدر خطورة وسمية علي الأنواع الحقلية الأخري اللاحقة أو المنزرعة معه في نظام زراعي مختلط. لذلك ينبغي علي المزارعين أن يكونوا علي دراية كافية بذلك أثناء جمع المحصول لتجنب أو التقليل من بذور الحبة السوداء المنفرطة في أرض المزرعة.