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Allelopathic Potential of *Chenopodium ambrosioides* on Germination and Seedling Growth of Some Cultivated and Weed Plants

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Abstract: Phytochemical screening of the study plant powder demonstrated that alkaloids, flavonoides, volatile oils and terpenoids are the principal allelochemicals. Extracts of *C. ambrosioides* were bioassayed on germination and seedling growth inhibition tests of two crops; *Lycopersicon esculentum* (Solanaceae) and *Beta vulgaris var. rapa* (Chenopodiaceae) and two weeds; *Melilotus indicus* (Leguminosae) and *Sonchus oleraceus* (Compositae). The percentage inhibition of water extracts on germination of *L. esculentum* reached 51.4%, of *B. vulgaris* attained 90%, of *M. indicus* reached 81.2% and of *S. oleraceus* reached 83.3% inhibition. Methanolic extract, oil extract and principal allelochemicals of *C. ambrosioides* demonstrated stronger inhibition of germination and seedling growth as compared to that of water extracts. The inhibitory effect of different treatments on both germination and seedling growth increases according to the following order: sterols and terpenes allelochemicals > oil extract > methanol extract > water extract. HPLC of the essential oil showed that the main allelochemicals are α-terpinene, limonene and ascaridole (1-methyl-4(l-methylethyl)-2-3-dioxabicyclo [2.2.2] oct-5-ene). Sterols, terpenes and ascaridole content of the essential oil showed that α-terpinene, limonene and ascaridole amounted to 14.7, 3.6 and 18.6%, respectively.

Key words: Phytochemical screening · α-terpinene · limonene · ascaridole · HPLC.

INTRODUCTION

The genus *Chenopodium* consists of 120 species, nine of which are found in Egypt [1, 2]. The importance of *Chenopodium* species is due to their wide variety of medicinal properties [3]. *Chenopodium ambrosioides* L. (epazote, Mexican tea, wormseed) is widespread species native to tropical America [4]. In Egypt it is known as noxious invasive weed. As pointed out by Hegazy *et al.* [5], the reasons for the success of many invasive weeds are their characteristic biological attributes and their success as "ecological opportunists" due to their high disease resistance, low palatability to herbivores, allelopathic effect on competing species, effectiveness of long distance dispersal, long vegetative life span, wide edaphic, climatic and biotic stress-tolerance and broad cytological and genetic variability. Allelopathic effects of foliage extracts from four Chenopodiaceae species on seed germination of *Lactuca sativa*, showed that allelochemicals play an important role, indirectly, in determining chenopod community structure [6]. There is much evidence that allelochemicals from weeds inhibit crop growth [7-12].

Chenopodium ambrosioides is an annual or short-lived perennial herb that has been used for centuries as condiment, traditional purgative for intestinal worms and many other medicinal purposes [13-15]. The biological activity of *C. ambrosioides* has been shown to affect viruses [16], bacteria [17], fungi [18], nematodes [19] and insects [20]. The broad spectrum biological activity of *C. ambrosioides* suggests that allelopathic interference may play an important role in plant community and vegetation structure [21]. A phytotoxic effect of *C. ambrosioides* is still under worked area [22]. In the present study, the potential allelopathic effects of *C. ambrosioides* on seed germination and seedling growth of two crops; *Lycopersicon esculentum* and *Beta vulgaris var. rapa* and two weeds; *Sonchus oleraceus* and *Melilotus indicus* are evaluated. Phytochemical screening of the study species was investigated.

MATERIALS AND METHODS

Plant material: *Chenopodium ambrosioides* was collected from naturally growing population in Ziaan county, about 5 km south of Gamasa sea-side resort (Egypt). The plant material was air dried and ground into

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powder and used for the purpose of phytochemical screening.

Phytochemical screening: The microsublimation of plant material was carried out following method described by Clause *et al.* [23]. Volatile matters were determined according to Wagner *et al.* [24]. Carbohydrates and glycosides contents were determined by the method described by Molish [25]. Unsaturated sterols and triterpenes were determined according to Liebermann and Burchard [26]. Tannins were determined according to method described by Balbaa *et al.* [27]. Flavonoids were determined according to the method described by Seikel [28]. Saponins were determined according to the method described by Walform *et al.* [29]. Alkaloids and nitrogenous bases were determined according to Fulton [30]. Quantitative determination of alkaloids was carried according to method described by Edeoga *et al.* [31]. Test for free and combined anthraquinones were determined according to the method described by Wallis [32].

These analyses were carried out in the Special Unit of Pharmacognosy and Chemistry of Medicinal Plants, National Research Center (NRC), Giza, Egypt.

Water extract: Extracts were prepared by soaking weighed amounts of air dry plant shoots; 0.125, 0.25, 0.5 and 1 g per 100 ml distilled water at room temperature for 24 h. These concentrations are equivalent to 0.00125, 0.0025, 0.005 and 0.01% (w/v). The extracts were filtered through filter paper (Whatman # 1) and 10 ml of the filtrate were added to every Petri dish (9 cm diameter) containing one layer of filter paper. Distilled water was used as control. Twenty five seed of *Lycopersicon esculentum*, *Beta vulgaris*, *Melilotus indicus* or *Sonchus oleraceus* were placed in each dish. Five replications of each treatment were used and incubated in dark growth chamber at 25°C for 15 days. This temperature was chosen after preliminary seed germination experiments of the test species at different temperature levels. Daily readings of the germinated seeds were recorded during the experimental period and final measurements of the seedling length were recorded. Seedling length was measured as the distance between the shoot and root tips.

Methanol extract: Methanolic extracts of *C. ambrosioides* were prepared from 0.5 g dry plant material soaked for 24 h in 100 ml methanol at 5°C and then filtered. Eight (8) ml of the extracts were poured onto filter paper (9 cm diameter Whatman # 1) and the solvent was allowed to evaporate. The bioassays described above were conducted using the treated filter papers and 10 ml of deionized water in 9 cm petri dishes [22].

Allelopathic effect of the essential oils: Fresh plant material was steam distilled for oil extraction [22]. The allelopathic potential of the essential oil was tested on the germination and seedling growth of the above mentioned test species. Exact 4 µL of the oil (concentration of this oil is equivalent to 0.5 g powder /100 ml distilled water) was placed on each filter paper in addition to 10 ml distilled water for each dish. Five replicate samples were placed at 25°C in a dark growth chamber for 15 days. Daily readings of the germinated seeds were recorded during the experimental period and final measurements of the seedling lengths were recorded.

Extraction of principal allelochemicals: Two hundred grams of air dry shoots of *C. ambrosioides* were ground, extracted with methanol (85%) and concentrated in *vacuo*. Aliquots of 1.7 gram of the crude concentrates were applied to Sephadex LH-20 column and eluted with methanol. Twenty-ml samples were collected every 4.5 min for a total of 30 fractions. The bioactivity was eluted in fractions V-XII (containing principal allelochemicals [22]). These fractions were mixed, the solvent was removed in *vacuo* and an aqueous residue (concentration of this residue is equivalent to 0.5 g powder / 100 ml distilled water) was obtained and bioassayed as described above.

Sterols and terpenes determination: Alpha-terpenes, limonene and ascaridole were extracted from the essential oil according to Gertz Christian [33]. Exact 1 g of oil sample was taken and socked separately in 50% acetonitrile (10 ml). After vortex the samples were filtered in micro filter (45 µm). Terpenes and limonine were examined using a HPLC system (Hp 1050) with a UV detector at 220 nm. The separation was accomplished with a ODS, C18 (5 µm. 4 x 250 mm) column. The mobile phase consisted of eluent acetonitrile / water (70/30 v/v) to (95/5 v/v) through 5 min. The flow rate was 1 ml /min. The column temperature was 27°C with an injection volume of 10 µL. This analysis was carried out in the Biotechnology Unit of Plant Pathology Institute, Agriculture Research Center, Giza, Egypt.

Data were analyzed by ANOVA test to determine the significant differences among the mean values at the $p<0.05$ and $p<0.01$ probability levels using a "general linear model" procedure of the Statistical Analysis System (SAS) program [34].

RESULTS

Phytochemical screening: Phytochemical screening of *Chenopodium ambrosioides* is shown in (Table 1). These data demonstrate that needle crystals are faint present in

Table 1: Phytochemical screening of *Chenopodium ambrosioides*. (+++ = strong presence, ++ = medium presence, + = faint presence and - = absent)

Chemical constituent	Amount
1- Sublimable matter	+ needle crystals
2- Volatile matter	++
3- Carbohydrates and / or glycosides	++
4- Sterol and / or triterpenes	+++
5- Tannins	–
6- Flavonoids:	
a- Free	+++
b- Combined	+++
7- Anthraquinones:	
a- Free	–
b- Combined	–
8- Saponins	–
9- Alkaloids and / or nitrogenous bases	++
10-Quantitative determination of alkaloids	
a-Weight (g)	0.1564
b-(%) alkaloids	1.56

the study species while volatile oil as well as carbohydrates and glycosides are medium. Sterols and terpenoids attained high values. Flavonoides (free and combined) attained high values in the plant powder. Alkaloids, which may represent the principal allelochemicals of the study species, are frequently present in the plant powder, with maximum value reached 1.56 %. Saponins, Tannins and anthraquinones are absent in the plant powder of *C. ambrosioides*.

Water extract: Germination and seedling length of the test species were inhibited under different concentrations of *C. ambrosioides* water extracts (Fig. 1 and photo 1). *Lycopersicon esculentum* recorded significantly ($p < 0.01$) high germination percentage of 94% for control seeds and this was reduced into 66, 50, 48 and 40 % for seeds germinated under water extracts 0.125, 0.25, 0.5 and 1 gram / 100 ml distilled water, respectively. Percentage germination inhibition increased as the concentration of water extract increased, where values increased from

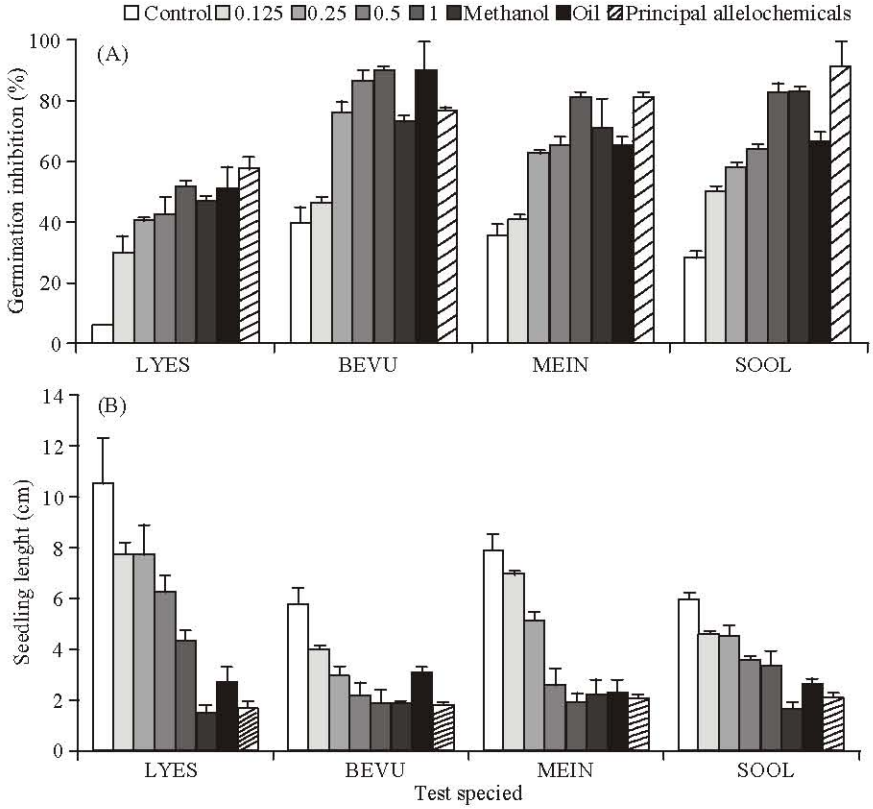

Fig. 1: Allelopathic potential of *Chenopodium ambrosioides* extracts (water, methanol crude extract, oil and principal allelochemicals) on percent germination inhibition (a) and seedling length (b) of the studied test species; *Lycopersicon esculentum* (LYES), *Beta vulgaris* (BEVU), *Melilotus indicus* (MEIN) and *Sonchus oleraceus* (SOOL). Water extract concentrations are 0.125, 0.25, 0.5 and 1 gram per 100 ml distilled water. Vertical bar above the mean is the standard deviation

Appendix Table 1: Allelopathic potential of *Chenopodium ambrosioides* on the percent germination (G %), percent inhibition of germination (GI %), seedling length (S) and seedling growth inhibition (SI %) of the study test species; *Lycopersicon esculentum* (LYES), *Beta vulgaris* (BEVU), *Melilotus indicus* (MEIN) and *Sonchus oleraceus* (SOOL). Mean values are given for all parameters and SD are values between bracts. Asterisks represent significance difference between control and treated seeds: *p < 0.05, **p < 0.01

	LYES				BEVU				MEIN				SOOL			
Treatment	G% (SD)	GI%	S(cm) (SD)	SI%	G% (SD)	GI%	S(cm) (SD)	SI%	G% (SD)	GI%	S(cm) (SD)	SI%	G% (SD)	GI%	S(cm) (SD)	SI%
Control	94a**	6	10.5a*	0	60a**	40	5.8a**	0	64a*	36	7.94a*	0	72a**	28	6a	0
	(4.9)	(0.8)	(1.7)	0	(8.9)	(4.9)	(0.63)	0	(10.2)	(3.5)	(0.7)	0	(7.5)	(2.4)	(0.24)	0
0.125	66ab	29.8	7.74ab	26.5	32ab	46.6	4.02ab	30.6	38ab	40.6	6.94a	12.5	36ab	50	4.54a	24.3
(g/100ml H2O)	(10.2)	(5.7)	(0.5)	(1.68)	(4)	(1.57)	(0.16)	(1.09)	(7.4)	(1.59)	(0.11)	(0.19)	(4.8)	(1.89)	(0.21)	(0.32)
0.25	50ab	40.8	7.74ab	26.5	14b	76.6	3b	48.2	24b	62.5	5.12ab	35.5	30ab	58.3	4.58a	23.6
(g/100ml H2O)	(8.9)	(0.9)	(1.1)	(1.21)	(4.9)	(3.14)	(0.36)	(2.01)	(10.2)	(1.18)	(0.32)	(1.19)	(14.1)	(1.56)	(0.37)	(0.63)
0.5	48b	42.9	6.18ab	41.3	8b	86.6	2.18b	62.4	22b	65.6	2.56b	67.7	26ab	63.8	3.58a	40.3
(g/100ml H2O)	(9.8)	(5.2)	(0.68)	(1.24)	(7.4)	(3.11)	(0.52)	(1.69)	(7.4)	(2.65)	(0.69)	(0.82)	(16.2)	(1.83)	(0.16)	(1.09)
1	40b	51.4	4.3ab	59.2	6b	90	1.94b	66.5	12b	81.2	1.9b	76.1	12b	83.3	3.38a	43.6
(g/100ml H2O)	(8.9)	(2.3)	(0.45)	(1.98)	(4.9)	(1.41)	(0.48)	(3.09)	(7.4)	(1.77)	(0.38)	(0.43)	(7.5)	(2.2)	(0.62)	(3.14)
Methanolic extract	44b	47.2	1.54b	85.3	16b	73.3	2.9b	67.2	18b	71.8	2.26b	71.5	12b	83.3	1.7a	71.6
	(4.9)	(1.32)	(0.31)	(1.23)	(4.9)	(1.57)	(0.06)	(3.41)	(4)	(8.8)	(0.57)	(2.58)	(4)	(1.26)	(0.2)	(1.25)
Oil extract	34b	51.4	2.78b	73.6	14b	90	3.1b	47.2	12b	65.6	2.32b	70.7	6b	66.6	2.66a	55.6
	(5.5)	(6.8)	(0.57)	(1.59)	(10.2)	(9.4)	(0.21)	(1.52)	(7.4)	(2.65)	(0.5)	(2.93)	(4.9)	(3.14)	(0.17)	(0.63)
Principal allelochemicals	40b	57.8	1.75b	83.3	6b	76.6	1.78b	69.3	22b	81.2	2.06b	74.1	24ab	91.6	2.12a	64.6
	(6.3)	(3.68)	(0.21)	(2.21)	(4.9)	(1.1)	(0.14)	(1.57)	(4.5)	(1.77)	(0.18)	(1.38)	(16.2)	(7.9)	(0.15)	(2.04)

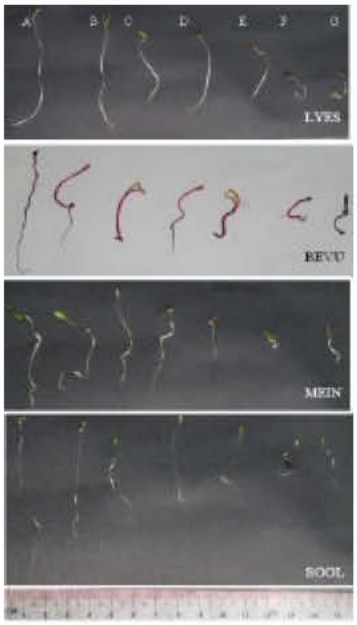

Photo 1: Allelopathic potential of *Chenopodium ambrosioides* on seedling length of *Lycopersicon esculentum* (LYES), *Beta vulgaris* (BEVU), *Melilotus indicus* (MEIN) and *Sonchus oleraceus* (SOOL). A = Control, B = 0.125, C = 0.25, D = 0.5, E = 1 g/ 100 ml water, F = oil extract, G = methanolic extract.

46.6% in *B. vulgaris* seeds under water extract 0.125 g/ 100 ml distilled water to 76.6, 86.6 and 90% on using water extracts of 0.25, 0.5 and 1 g/ 100 ml distilled water, respectively (Fig. 1a).

Seedling length of 7.94 cm was achieved in control treatment of *M. indicus* and this value significantly ($p < 0.05$) reduced to 6.94, 5.12, 2.56 and 1.9 cm in seedlings treated with water extracts of 0.125, 0.25, 0.5 and 1 gram powder / 100 ml distilled water, respectively. Inhibition of seedling lengths in the test species was directly proportional to the increase in concentration of water extract of *C. ambrosioides*. Seedling length inhibition was 12.5 % in *M. indicus* seedlings treated with water extract of 0.125 gram powder / 100 ml distilled water and this value increased to 35.5, 67.7 and 76.1 % in seedlings of the same test plant but treated with water extracts of 0.25, 0.5 and 1 gram powder / 100 ml distilled water, respectively (Fig. 1 b and Appendix Table 1).

Methanol extract: Germination of different test species seeds using methanol extract was greatly inhibited as compared with that of controls. Germination inhibition of *S. oleraceus* seeds (83.3%) was the most affected test species while that of *L. esculentum* seeds (47.2%) represented the least affected test species (Figure 1 a). By comparing seedling lengths of different test species

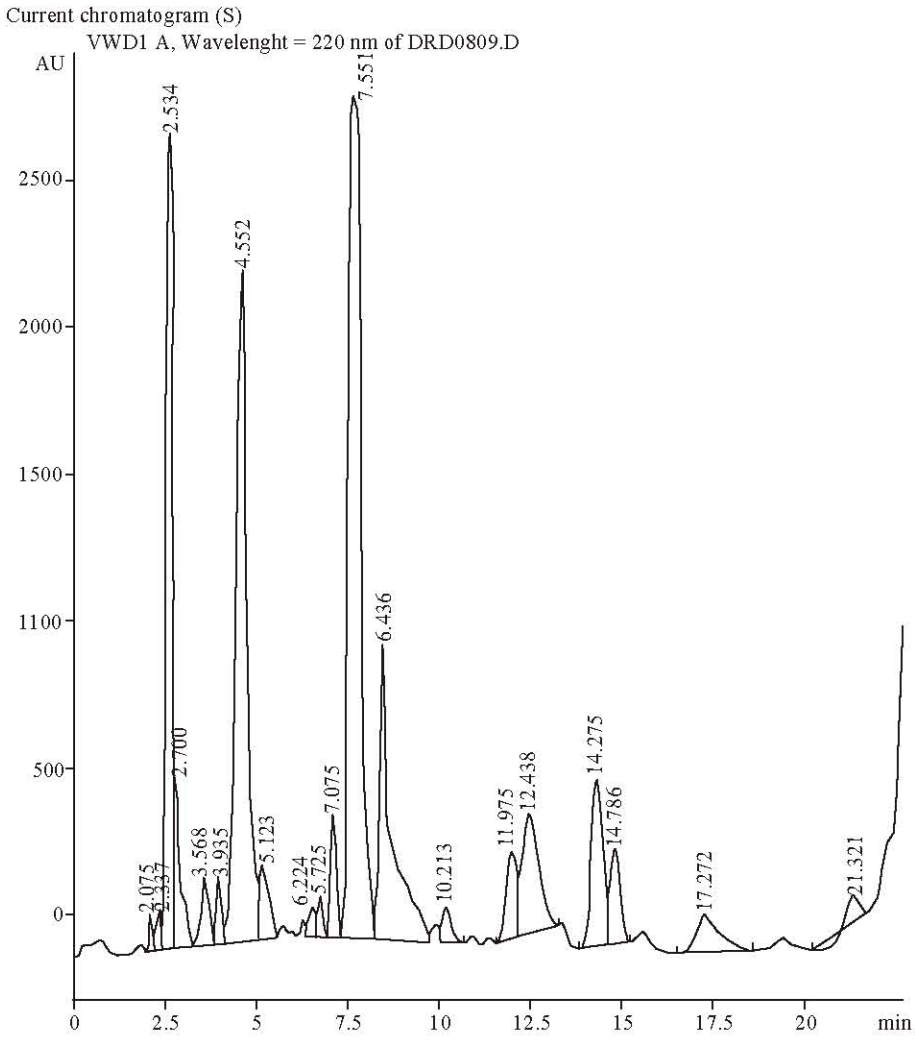

Fig. 2: HPLC profile of *Chenopodium ambrosioides* oil extract

treated with methanol extract of *C. ambrosioides*, maximum value (2.9 cm) was achieved in *B. vulgaris* seedlings, while the minimum value of 1.54 cm was recorded in *L. esculentum* seedlings (Fig. 1 b).

Oil extract: When compared with other test species, oil extract gave maximum germination inhibition of 90% attained in *B. vulgaris* seeds, while this value greatly reduced in *L. esculentum* seeds recording minimum value of 51.4%. Lengths of treated seedlings with oil extract were greatly reduced as compared to that of controls in all test species. Seedling length was 10.5 cm in control treatment of *L. esculentum* and this value significantly ($p<0.05$) reduced into 2.78 cm when seedlings of the same test species treated with oil extract of *C. ambrosioides* (Fig. 1 b).

Principal allelochemicals: The germination inhibition of *S. oleraceus* seeds treated with sterols and terpenes extracted from *C. ambrosioides* attained 91.6%, which represented the maximum germination inhibition value among different test species and treatments (Fig. 1 a). The seedling length of *B. vulgaris* recorded the minimum value of 1.78 cm (Fig. 1 b).

Sterols and terpenes: Qualitative and quantitative determination of sterols and terpenes in *C. ambrosioides* was carried out on the essential oil HPLC profile. HPLC of the essential oil showed that α-terpinene, limonene and ascaridole (1-methyl-4(l-methylethyl)-2-3-dioxabicyclo [2.2.2] oct-5-ene) have three well defined peaks at retention times 2.534, 4.552 and 7.551 min., respectively. Sterols, terpenes and ascaridole quantitative

Table 2: HPLC- Area percent report for *C. ambrosioides* essential oil

Peak #	RT (min)	Type	Width (min)	Area (mAU*sec)	Height (mAU)	Area %
1	2.075	BV	0.087	721.195	125.447	0.303
2	2.337	VV	0.136	1347.100	135.431	0.544
3*	2.534	VV	0.168	33978.400	2771.630	13.738
4	2.76	VV	0.146	6381.240	581.059	2.580
5	3.568	VV	0.221	3481.220	224.339	1.407
6	3.935	VV	0.193	2743.090	224.454	1.109
7**	4.552	VV	0.309	47400.100	2283.240	19.165
8	5.123	VV	0.265	4899.400	252.754	1.981
9	6.524	VV	0.196	1122.430	95.455	0.453
10	6.725	VV	0.161	1476.570	136.205	0.597
11	7.075	VV	0.168	4624.280	412.440	1.869
12***	7.551	VV	0.382	69294.800	2863.680	28.017
13	8.436	VV	0.319	24071.900	1007.370	9.732
14	10.213	VV	0.251	1982.710	117.648	0.801
15	11.975	BV	0.278	5489.660	296.487	2.219
16	12.436	VV	0.526	13807.300	412.268	5.582
17	14.275	BV	0.322	11905.200	571.614	4.813
18	14.796	VV	0.290	6105.270	321.891	2.468
19	17.272	BV	0.580	5417.260	126.211	2.190
20	21.321	BV	0.243	1045.450	90.176	0.422
Totals				247325.000	13049.800	

Sorted by signal, Multiplier: 1.000000, Signal 1: VWD1 A, Wavelength= 220 nm

*= α- terpinene, **=Limonene and ***= Ascaridole

determination showed that α-terpinene, limonene and ascaridole amounted 14.7, 3.6 and 18.6 %, respectively (Fig. 2 and Table 2).

By comparing the obtained results using different treatments and as the concentration of these treatments was the same (equivalent to 0.5 g/ 100 ml), one easily deduced that the inhibitory effect of these treatments on both germination and seedling length increases in the following order: sterols and terpenes allelochemicals > oil extract > methanol extract > water extract. For instance, the germination inhibition was 42.9% for *L. esculentum* seeds treated with water extract (0.5 g / 100 ml) and this value increased in ascending order to 47.2, 51.4 and 57.8% for seeds treated with methanol crude extract, oil extract and finally sterols and terpenes allelochemicals, respectively (Fig. 1a).

DISCUSSION

Several secondary metabolites were isolated from *Chenopodium ambrosioides*, such as monoterpenes [22, 35-37], alkaloids [38], Saponins [39], flavonol glycosides [40]. All these compounds were reported as allelopathic agents [7]. Therefore, the possibility exists that allelopathic affects may arise from this species.

In the bioassays reported here for germination and seedling growth inhibition of *Lycopersicon esculentum*, *Beta vulgaris, Melilotus indicus* and *Sonchus oleraceus* under allelopathic activity of *Chenopodium ambrosioides,* Ascaridole (1-methyl-4(l-methylethyl)-2-3-dioxabicyclo [2.2.2] oct-5-ene) may be the monoterpene responsible for the phytotoxicity of *C. ambrosioides*, that explain the inhibitory effect of the plant on the germination and seedling growth of the test species. This oxygenated monoterpene is the major oil component of *C. ambrosioides*.

The oil of *C. ambrosioides* is stored in bladder trichomes, which occur on the leaf surface and cover the flower ovary [22, 41]. The oil has been found to exhibit biological activities such as molluscicidal activity [42], allelopathy [22], antimalarial agent [43], antibacterial [44], antifungal [45, 46] particularly against skin dermatophytes and antifeedant activity against insects [3]. The insecticidal activity of the volatile oil from *C. ambrosioides* against several grain insects has been reported by different authors [47]. In addition, the oil has been used as a fragrance component in lotions and perfumes [48]. The anthelmintic efficiency of *C. ambrosioides* oil was proved in some studies [49, 50]. Recently, the genotoxic effect and treatment of cancer using *C. ambrosioides* were investigated [13, 14, 51].

In accordance with obtained results of the present work, α-terpinene, limonene and ascaridole were identified as the major constituents in most oil composition extracted by many researchers from *C. ambrosioides* plants grown in United States, Argentina, Slovakia, India, Spain, Brazil, Japan and Mexico [3, 15, 22, 35, 36, 43]. The Egyptian collection of the present work showed that α-terpinene, limonene and ascaridole content amounted to 1.47, 0.36 and 1.86%, respectively.

Ascaridole, an asymmetric monoterpene endoperoxide with anthelmintic properties [52] as shown by [22] who reported that ascaridole (1-methyl-4(l-methylethyl)-2-3-dioxabicyclo [2.2.2] oct-5-ene) is the monoterpene responsible for the phytotoxicity of *C. ambrosioides*, which explain the inhibitory effect of the plant on the germination and seedling growth. In addition, this oxygenated monoterpene is the major component of *C. ambrosioides* oil and it was one of the first naturally occurring characterized endoperoxides [53]. Ascaridole is a potent allelochemical even in lowest concentrations [22]. Research on ascaridole has focused on its pharmaceutical use, first as a nematicide [54] and as an antimalarial agent [43]. The major concentration of ascaridole has been found in the essential oil obtained from seeds [55], therefore it may be liberated during

seed germination. It can also be liberated as a volatile from fresh leaves [22]. As demonstrated by Muller and del Moral [56] that soil colloids adsorb volatile terpenoids from the atmosphere and retain the resulting toxicity for several months. If this happens, ascaridole can be concentrated and germination and growth of contact plants will be inhibited. Under certain conditions, ascaridole is soluble in water. This may explain the strong allelopathic effect of water extract. Ascaridole was also reported to be the pharmacologically active principle in the Peruvian grown *C. ambrosioides* plants which displayed analgesic and sedative properties [57].

Dry leaves and litter of *C. ambrosioides* significantly inhibited seedling growth of tested species. It is also known that the ascaridole content of *C. ambrosioides* plants increases during drying [55]. Therefore, plant establishment and growth would be affected in places where *C. ambrosioides* grows, not only during the life cycle of the species, but also after the plants die due to mulching effect [22].

There is strong evidence that terpenes influence vegetation patterns in natural ecosystems [58]. Therefore, the possibility exists that plants that produce monoterpenes, such as *C. ambrosioides*, have been managed in agricultural systems as a means of pest and weed control and also explain the stronger allelopathic potential of terpenes on the study test species as compared to other treatments and control.

In conclusion, alkaloids, flavonoides, volatile oils and terpenoids seem to be the principal allelochemicals in *C. ambrosioides*. Ascaridole (1-methyl-4(l-methylethyl)-2-3-dioxabicyclo [2.2.2] oct-5-ene) may be the monoterpene responsible for the phytotoxicity of *C. ambrosioides* on the germination and seedling growth of the study test species. The inhibitory effect of different potential allelochemicals in the present work on both germination and seedling growth increases in the following order: sterols and terpenes allelochemicals > oil extract > methanol extract > water extract.

REFERENCES

1. Täckholm, V., 1974. Students ? Flora of Egypt. Beirut, Publ. Cairo, University.
2. Boulos, L., 1999-2005. Flora of Egypt, four vol. Al-Hadara Publishing, Cairo, Egypt.
3. Tapondjou, L.A., C. Adler, H. Bouda and D.A. Fontem, 2002. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. J. Stored Prod. Res., 38: 395-402.
4. Munz, P.A., 1975. A California Flora and Supplement. University of California Press, pp: 369.
5. Hegazy, A.K., M. Diekmann and G. Ayad, 1999. Impact of plant invasions on ecosystems and native gene pools. In: Environment 2000 and Beyond, pp: 275-310.
6. Jefferson, L.V. and M. Pennacchio, 2003. Allelopathic effects of foliage extracts from four Chenopodiaceae species on seed germination. J. Arid Environ., 55: 275-285.
7. Rice, E.L., 1984. Allelopathy. Academic Press, Orlando.
8. Hegazy, A.K. and M.F. Fadl-Allah, 1995. Inhibition of seed germination and seedling growth by *Cleome droseifolia* and allelopathic effect on rhizosphere fungi in Egypt. J. Arid Environ., 29: 3-13.
9. Ridenour, W.M. and R.M. Callaway, 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. Oecologia, 126: 444-450.
10. El-Khatib, A.A., A.K. Hegazy and H.K. Galal, 2004. Allelopathy in the rhizosphere and amended soil of *Chenopodium murale* L. Weed Biol. Manag., 4: 35-42.
11. Florentine, S.K. and M.E. Westbrooke, 2005. Invasion of the noxious weed *Nicotiana glauca* R. Graham after an episodic flooding event in the arid zone of Australia. J. Arid Environ., 60: 531-545.
12. Florentine, S.K., M.E. Westbrooke, K. Gosney, G. Ambrose and M. O'Keefe, 2006. The arid land invasive weed *Nicotiana glauca* R. Graham (Solanaceae): Population and soil seed bank dynamics, seed germination patterns and seedling response to flood and drought. J. Arid Environ., 66: 218-230.
13. Ruffa, M.J., G. Ferraro, M.L. Wagner, M.L. Calcagno, R.H. Campos and L. Cavallaro, 2002. Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. J. Ethnopharm., 79: 335-339.
14. Flávia, R.F., V.B. Nascimento, S.P. Gustavo, Paulo Vitor, C.G.M. Márcia, A.S. Lucilene, S.A. Ana Paula, S.B.B. Elizabeth and N.M.G. Rosane, 2005. Ascitic and solid Ehrlich tumor inhibition by *Chenopodium ambrosioides* L. treatment. Published by Elsevier Inc.
15. Jamali, A., M. Kouhila, L. Ait Mohamed, J.T. Jaouhari, A. Idlimam and N. Abdenouri, 2006. Sorption isotherms of *Chenopodium ambrosioides* leaves at three temperatures. J. Food Engin., 72: 77-84.

16. Verma, H.N. and V.K. Baranwal, 1983. Antiviral activity and the physical properties of the leaf extract of *Chenopodium ambrosioides* L. Proc. Indian Acad. Sci., 92: 461-465.
17. Begum, J., M. Yusuf, J.J. Chowdhury and M.A. Wahab, 1993. Studies of essential oils for their antibacterial and antifungal properties. Part I. Preliminary screening of 35 essential oils, Bangladesh. J. Sc. Ind. Res., 28: 15-24.
18. Dubey, N.K. and N. Kishore, 1987. Fungitoxicity of some higher plants and synergistic activity of their oils. Trop. Sci., 27: 23-27.
19. Garcia-Espinosa, R., 1980. *Chenopodium ambrosioides* L. planta con uso potencial en el combate de nematodos fitoparasitos. Agrociencia, pp: 21-24.
20. Peterson, G.S., M.A. Kandil, M.D. Abdallah and A.A. Farag, 1989. Isolation and characterisation of biologically-active compounds from some plant extracts. Pest. Sci., 25: 343-353.
21. Jiménez-Osornio, J.J., 1991. Ecologicl basis of the management of non-crop species in the chinampa agroecosystem. Ph.D. Thesis. University of California, Riverside.
22. Jiménez-Osornio, J.J., J. Kumamoto and C. Wasser, 1996. Allelopathic activity of *Chenopodium ambrosioides* L., Biochem. Syst. Ecol., 24: 195-205.
23. Clause, E.P., V.E. Tyler and L.R. Brady, 1971. "Pharmacognosy", 6th ed., Lea and Febiger, Philadelaphia, pp: 20.
24. Wagner, H., S. Bladt and E.M. Zgainsk, 1983. "Drogen Analyse", Springer Verlag, Berlin, pp: 6-22.
25. Molish, H., 1963. Monatsh. Chem., 7(1886); through Stank, J., Gerny, M., Koccourek, J. and Pacak, J., ''The Monosaccharides", Publishing House of Czechoslovak Academy of Science, Prague, pp: 872.
26. Liebermann, C. and H. Burchard, 1985. Chem. Zent., 16, 1 (1890); through El Deeb, K.S., "Chromatographic and Thermal Analysis of Certain Volatile Oil Containing Drugs" Ph.D. Thesis, Cairo University, Cairo, pp: 47.
27. Balbaa, S.I., S.H. Hilal and Zaki, 1981. "Medicinal Plants Constituents", Dar El-Shaab Printing House, Cairo, 3rd ed., pp: 366.
28. Seikel, M.K., 1962. "Chromatographic Methods of Separation, Isolation and Identification of Flavonoid Compounds", In: "The Chemistry of Flavonoid Compounds", ed. Geissman, T.A., MacMillan Co., New York, pp: 34.
29. Walform, M.L., P.W. Morgan and F.L. Benton, 1940. J. Amer. Chem. Soc., 62, pp: 1481.
30. Fulton, C.C., 1932. American Journal of Pharmacology, 104: 244.
31. Edeoga, H.O., D.E. Okwu and B.O. Mbaebie, 2005. African Journal of Biotechnology. 4: 685-688.
32. Wallis, T.E., 1967. "Text Book of Pharmacognosy", J. and A. Churchill LTD, London, 5th ed., pp: 449.
33. Gertz Christian, 1990. HPLC tips and tricks. Great Britain at the Iden Press, Oxford, pp: 608.
34. SAS, 1985. SAS/STAT guide for personal computers, version 6 edition. SAS Institute, Cary, NC.
35. Sagrero-Nieves, L. and J.P. Bartley, 1995. Volatile Constituents from the Leaves of *Chenopodium ambrosioides* L. J. Essen. Oil Res., 7: 221-223.
36. Patricia, A.O., E. Olusegun, E. Tinna and L. Into, 1999. Essential Oil Constituents of *Chenopodium ambrosioides* L. Leaves from Nigeria. J. Essen. Oil Res., 11: 220-222.
37. Ahmed, A.A., 2000. Highly oxygenated Monoterpenes from *Chenopodium ambrosioides*, J. Natur. Prod., 63: 989-991.
38. Haseeb, A., B. Singh, A.M.O. Khan and S.K. Saxena, 1978. Evaluation of nematicidal property in certain alkaloid bearing plants. Geobios, 5: 116-118.
39. Gupta, G.S. and M. Behari, 1972. Chemical investigation of *Chenopodium ambrosioides*. J. Indian Chem. Soc., 49: 317-319.
40. Jain, N., M.S. Alam, M. Kamil, M. Ilyas, M. Niwa and A. Sakae, 1990. Two flavonol glycosides from *Chenopodium ambrosioides*. Phytochemistry, 29: 3988-3991.
41. Bonzani, N.E., G.E. Barboza, M.A. Bugatti and L. Ariza Espinar, 2003. Morpho-histological studies in the aromatic species of *Chenopodium* from Argentina. Fitoterapia, 74: 207-225.
42. Hmamouchi, M., M. Lahlou and A. Agoumi, 2000. Molluscicidal activity of some Moroccan medicinal plants. Fitoterapia, 71: 308-314.
43. Pollack, Y., R. Segal and J. Golenser, 1990. The effects of ascaridole on the in-vitro development of *Plasmodium falciparum*. Parasit. Res., 76: 570-572.
44. Lall, N. and J.J.M. Meyer, 1999. *In vitro* inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. J. Ethnopharm., 66: 347-354.
45. Kishore, N., A.K. Mishra and J.P.N. Chansouria, 1993. Fungitoxicity of essential oils against dermatophytes. Mycoses, 36: 211-215.

46. Bourrel, C., R. Dargent, G. Vilrem and A. Gaset, 1995. Chemical analysis and fungistatic properties of some essential oils in a liquid medium. Effects on hyphal morphogenesis. Riv. Ita. EPPOS, 6: 31-42.
47. Su, H.C.F., 1991. Toxicity and repellency of *Chenopodium* oil to four species of stored-product insects. J. Entomol. Sci., 26: 178-182.
48. Pare, P.W., J. Zajicek, V.L. Ferracini and I.S. Melo, 1993. Antifungal terpenoids from *Chenopodium ambrosioides*. Biochem. System. Ecol., 21: 649-653.
49. Ketzis, J.K., A. Taylor, D.D. Bowman, D.L. Brown, L.D. Warnick and H.N. Erb, 2002. *Chenopodium ambrosioides* and its essential oil as treatments for *Haemonchus contortus* and mixed adult-nematode infections in goats. Small Rumin. Res., 44: 193-200.
50. Marsha, B.Q., J.Q. Robert and M.N. Justin, 2002. Ethnophysiology and herbal treatments of intestinal worms in Dominica, West Indies. J. Ethnopharm., 80: 75-83.
51. Gadano, A.B., A.A. Gurni and M.A. Carballo, 2006. Genotoxic effects of *Chenopodiaceae* family. J. Ethnopharm., 103: 246-251.
52. Mark, A.J. and C. Rodney, 1984. Biosynthesis of ascaridole: Iodide peroxidase-catalysed synthesis of a monoterpene endoperoxide in soluble extracts of *Chenopodium ambrosioides* fruit. Arch. Biochem. Biophys., 235: 254-266.
53. Mageli, O.L. and C.S. Sheppard, 1972. Organic peroxides and peroxy compounds, general description. In Organic Peroxides (P. Swern, ed.), 1: 3.
54. Mac Donald, D., K. VanCrey, P. Harrison, P.K. Rangachari, J. Rosenfeld, C. Warren and G. Sorger, 2004. Ascaridole-less infusions of *Chenopodium ambrosioides* contain a nematocide(s) that is(are) not toxic to mammalian smooth muscle. J. Ethnopharmacol., 92: 215-221.
55. Weiland, G.S., L.B. Broughton and J.E. Metzger, 1935. Wormseed oil production. Univ. Maryland Agric. Station Bull., 384: 315-335.
56. Muller, C.H. and R. del Moral, 1966. Soil toxicity induced by terpenes from *Salvia leucophylla*. Torrey Bot. Club. Bull., 93: 130-137.
57. Okuyama, E., K. Umeyama, Y. Saito, M. Yamazaki and M. Satake, 1993. Ascaridole as a pharmacologically active principle of Paico, a medicinal Peruvian plant. Chem. Pharm. Bull., 41: 1309-1311.
58. Muller, W.H., 1986. Allelochemical mechanisms in the inhibition of herbs by chaparral shrubs. In: The Science of Allelopathy (A. R. Putnam and C. S. Tang, eds), John Wiley and Sons, pp: 189-199.