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Phytotoxic activity of foliar applications of *Melia azedarach* L. extracts on growth and yield of *Cicer arietinum* L. in open field condition

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Abstract: The present study was carried out, 2013-2014,in Department of Plant Protection-Damascus University, Syria, to assess of phytotoxic activity of the dried leaves and seeds organic extracts of *Melia azedarach* L. *on* growth parameters of *Cicer arietinum* L. (chickpea). Extracts of the plant materials were used in open field condition. The plant materials were extracted in organic solvents (petroleum ether and ethanol) and were applied on foliage of *C. arietinum* at 5, 10 and 20%. The extracts of *M. azedarach* increased chlorophylls A, chlorophylls B, shoot length, shoot & root dry weight, 100 grains weight and yield when used at 5% concentrate. While no significant phytotoxic activity of organic solvents extracts of the leaves and seeds of *M. azedarach* when used at 10% concentrate on parameters growth were found. In the contrary, in case 20% concentrate of organic solvents extracts had significant phytotoxic activity on parameters growth. In general, the tested organic extracts could be arranged according to their phytotoxic activity in the following descending order, *M. azedarach* Seeds Ethanol > *M. azedarach* Leaves Ethanol > *M. azedarach* Leaves Petroleum ether > *M. azedarach* Seeds Petroleum ether. However, organic extracts of the dried leaves and seeds of *M. azedarach* at 5 and 10% concentrations had no phytotoxic activity on growth and development of *C. arietinum* plants and increase of seed yield in chickpea in open field condition.
Key words: Phytotoxic, Chickpea (*Cicer arietinum* L.),. Organic extracts, *Melia azedarach.*

Introduction

Melia azedarach L. is a botanical species belonging to the family Meliaceae also known as Ku-lian, China tree or Chinaberry tree. *M. azedarach* L. is native to tropical Asia .it is wide spread and naturalized in most of the tropics and subtropical countries. it was introduced and naturalized in Philippines, United States of America. Brazil, argentine, many African and Arab countries[1,2]. *M. azedarach* has also showed antifungal[3], antibacterial[4], cytotoxic[5], antimalarial[6], anthelmintic[7], antilithic[8], antifertility activity[9] and insecticidal properties[10]. The pesticide activity of *M. azedarach* is due to biologically active triterpenoids with an antialimentary effect, i.e., they inhibit the feeding of phytophage insects producing death and malformations of subsequent generations[11]. Our previous study demonstrated that the oil extract of chinaberry seed, can be used as an environmental friendly insecticide for *Myzus persicae* Sulzer control[12].

Chickpea (*Cicer arietinum* L.) family Leguminaceae is one of the most important pulse crops. Seed of chickpea contains 17.1% protein, 5.3% fats, 61.2% carbohydrates, 3.9% fibers and 2.7% minerals[13]. *Cicer arietinum* was one of the first legume crops domesticated. Today it is a key component of cropping systems in many parts of Asia and Africa, providing families of resource-poor farmers with a valuable source of dietary protein. *C. arietinum* was one of the first legume crops domesticated is also becoming established in some agriculturally advanced nations in response to a growing world demand[14]. Yet chickpea yields (1729kg/ha) in Syria[15] have fallen below expectation. Low yield of chickpea attributed to its susceptibility to several fungal, bacterial, and viral diseases. Among the diseases affecting chickpea, Damping- off, wilting root- rot and Ascochyta blight diseases[16].

Synthetic pesticides are important tools in pest control although they have been used excessively with negative consequences such as toxicity towards farmers, consumers, and wild animals, interruption of natural control and pollination, water pollution, and the evolution of resistance pests have acquired to these products[17,18]. According World Health Organization (WHO) annually 200,000 peoples are killed worldwide by synthetic chemical pesticides poisoning[19] Due to its carcinogenicity, teratogenicity, high and acute residual toxicity, ability to create hormonal imbalance, sperma totoxicity and a lot of side effects, so its use has been restricted[20]. In general as the population increases, the agriculture productivity cannot meet the demand of food, to overcome from this problems we should improve the productivity of cropping by following the methods such as improving irrigation methods, control pests and insects by using chemicals or biological methods, using fertilizers for more production[21]. Botanical insecticides have been used in agriculture for at least two thousand years in Asia and the Middle East[22]. The main reason for the scarce development of *M. azedarach* as a commercial insecticide in comparison to *Azadirachta indica* lies in that the fruits of the former contain meliatoxin, a triterpenoid that is toxic for mammals[23]. However, the chemical composition of *M. azedarach* varies notably between its wild and cultivated state. The fruits developed in Argentina have triterpenoids instead of meliatoxin, mainly meliartenin which is a strong insect antialimentary that could be useful for pest and disease management[11]. Phytotoxicity is a term used to describe the toxic effect of a compound on plant growth. Such damage may be caused by a wide variety of compounds, including trace metals, pesticides, salinity or allelopathy, which is the process used by a plant to release toxic chemicals into the ground to kill neighboring plants[24].

Aim of the work

The aim of the study was to estimation phytotoxicity of seeds and leaves organic extracts (Petroleum ether and Ethanol) of *Melia azedarach* L., on growth parameter and yield of *Cicer arietinum* plants in open field conditions.

Materials and Methods

Collection of plant material:

Fresh and healthy leaves and seeds (mature) of *Melia azedarach* L. were collected from the garden in Damascus, Syria. The samples (1 kg each) of seeds and leaves (mature) were washed thoroughly in tap water and sterile distilled water, air-dried at 27°C for about 14 days.

Seed samples:

Healthy and clean seeds of the Chickpea (*Cicer arietinum*) cultivar Ghab3 were obtained from the local market in Damascus, Syria.

Extraction of Chinaberry (*Melia azedarach* L.) Plant Parts:

The dried each 500 g leaves and seeds materials were grounded separately in a mechanical mill and sieved through 2 mm sieve and soaked 24 h in 1000ml organic solvents (Petroleum ether 98% or Ethanol 98%) in 2000 ml beaker, then stirred vigorously for 15 minutes and allow to stand for 1 hour and then filtered through four folds of sterile cheese cloth to obtain organic extracts.

The solvents (Petroleum ether or Ethanol) was evaporated at lower temperature under reduced pressure in rotary flash evaporator to get the 100 ml extracts. The organic extracts stored at 4^{o}C in dark bottles to reduce the allelochemicals degradation until further use. The concentrated organic extracts were diluted with distilled water to obtain 5, 10 and 20 % concentrations. Tween-20 was added as an adjuvant to the *Melia* extracts at a rate of 0.1%. Emulsions of *Melia azedarach* based organic extracts named MLP (*Melia azedarach* leaves

Petroleum ether),MLE (*Melia azedarach* Leaves Ethanol), MSP (*Melia azedarach* Seeds Petroleum ether) and MSE (*Melia azedarach* Seeds Ethanol) respectively.

Testing of *Melia azedarach* extracts for phytotoxicity of *Cicer arietinum* in open field conditions:

The field trials were conducted at the Experimental Farm of Faculty of Agriculture, Damascus University, Damascus, Syria in 2013 and 2014 growing seasons. The sowing was done on 25/12/2013. Field plots (3.5 × 3.5 m) comprised three rows and 20 plants per row arranged in a completely randomized block design. Three plots were used as replications for each treatment as well as for the untreated control treatment. Field trials were repeated twice. The soil was silty loamy in texture with pH 8.5, low in organic carbon 0.54% and available nitrogen (N) 0.66 kg/ha but medium in available phosphorus (P) 19.7 kg/ha.

Thirteen treatments were applied, viz control and *M. azedarach* extracts (MLP, MLE, MSP and MSE) used at 5 ,10 and 20% concentrations, respectively. Treatments with *M. azedarach* extracts at 5% , 10 % and 20% concentrations were performed as foliar application, till runoff. Plant extracts were applied with knapsack hand sprayer, after six weeks of sowing seed. The crop was raised following the recommended agronomic practices. Chlorophyll A and chlorophyll B content in *Cicer arietinum* plants leaves were recorded at 25days after application of organic extracts were determined according to[25,26].

At harvest time (25/5/2014), ten plants from each plot were randomly uprooted along with the soil core. Roots were washed off to remove the adhering soil and then data on root and shoot length (cm) and dry (g) weight. the average accumulated yield was calculated for each treatment including the untreated control. grains weight and grain yield were recorded from each treatment.

Statistical Analysis.

All experiments were performed twice. Analyses showed no significant interaction between the two tests run for any of the treatments. Therefore, results from duplicate tests were combined for the final analysis. The obtained data were subjected to statistical analysis using SPSS package version 20. The mean values were compared by Tukey's L.S.D (P = 0.05).

Results and Discussion

The present study was undertaken to determine the effect of *M. azedarach* L. extracts namely: MLP (*M. azedarach* Leaves Petroleum ether),MLE (*M. azedarach* Leaves Ethanol), MSP (*M. azedarach* Seeds Petroleum ether) and MSE (*M. azedarach* Seeds Ethanol) on growth parameters of *C. arietinum* in open field conditions.

Photosynthetic pigments:

Data in Table (1) indicate that the application of *M. azedarach* extracts (MSP, MLP, MLE and MSE) during the vegetative growth of *Cicer arietinum* leaves gave different changes in the concentrations of the considered photosynthetic pigments, i.e. chlorophylls A and B as compared with the untreated plants (Control). The extract types proved influential in this respect. Data indicate the significantly increasing effects of the two tested *M. azedarach* extracts (MSP & MLP) when used at 5 and 10 % concentrations on photosynthetic pigments contents in treated leaves than the untreated ones. MSP treatments achieved the highest stimulation on pigment concentration compared with MLP. The percent increase at 5% concentration reached 28.12 &17.35 % (MSP) and 20.97 & 14.19 % (MLP) in case of chl. A and chl. B, respectively. On the contrary, the ethanolic extracts of *M. azedarch* (MSE & MLE) when used at 5 and 10 % concentration had no significant influence on photosynthetic pigments contents and showed slight increasing effect on the studied biochemical aspects in *C. arietinum* leaves. The percent increase at 5% concentration reached 11.18 &1.76 % (MLE) and 8.78 & 1.02 % (MSE) in case of chl. A and chl. B , respectively. Such stimulation effect on photosynthetic pigments of extracts treated leaves may be attributed to the increase of the essential nutrients N, P, and K amounts in treated leaves[27]. The growth stimulating effect is not exclusively by its adverse effect on pathogen or by an increase in nutrient uptake. Also substances with hormone like properties can stimulate of effect biomass allocation in plants. In addition to hormones, medicinal plant extracts contain saponins and polyphenols which could be the active compounds causing the effect on growth and yield of the plant[28]. As for 20% concentration application of the tested organic extracts, data showed significant reduced in the considered photosynthetic pigments of *C. arietinum* leaves than untreated plants. This trend of reduce was pronounced with all analyzed samples of plants treated with both organic extracts . The percent decrease in pigments by petroleum ether extracts treated leaves of *C.arietinum* reached -3.41 & -3.62% (MSP) and -5.28 &-5.10 % (MLP) in case of chl. A and chl. B, respectively. The corresponding values with ethanol extracts reached -10.51 & -5.66 % (MLE) and -11.85 & -6.22 % (MSE) with the same mentioned pigments, respectively. Chlorophylls are biomolecules which act as component of pigment protein complexes embedded in the photosynthetic membranes and play a major role in photosynthesis process[29]. Several researchers have mentioned that chlorophyll content and ion uptake reduced significantly by allelochemicals[30]. It has been reported that the allelochemicals produced by invasive species affect the photosynthesis and plant growth by destroying the chlorophyll[31]. The action of allelochemicals affects large number of biochemical reactions of target species resulting in alteration of different physiological functions[32]. Our results are also in agreement with the findings of[33], which reported similar results regarding the effects of allelochemicals on chlorophyll content and photosynthesis process in plants.

Table 1. Effect of *M. azedarach* organic solvent on chlorophylls A and chlorophylls B (mg/g leaf) *C. arietinum* plants after foliar treatment in field conditions .

Chlorophylls B (mg/g leaf)			Chlorophylls A (mg/g leaf)			Treatments
Concentration			Concentration			
20%	10%	5%	20%	10%	5%	
1.039 (-3.62)	1.214 (12.62)	1.265 (17.35)	2.013 (-3.41)	2.474 (18.71)	2.670 (28.12)*	MSP
1.023 (-5.10)	1.201 (11.41)	1.231 (14.19)	1.974 (-5.28)	2.432 (16.70)	2.521 (20.97)	MLP
1.017 (-5.66)	1.083 (0.46)	1.097 (1.76)	1.865 (-10.51)	2.293 (10.03)	2.317 (11.18)	MLE
1.011 (-6.22)	1.079 (0.09)	1.089 (1.02)	1.837 (-11.85)	2.225 (6.77)	2.267 (8.78)	MSE
1.078			2.084			Control
0.11			0.28			L. S.D 5%

MSP (*M. azedarach* Seeds Petroleum ether), MLP (*M. azedarach* Leaves Petroleum ether), MLE (*M. azedarach* leaves Ethanol) and MSE (*M. azedarach* Seeds Ethanol) .
(*) The percent % of pigments contents = (pigments contents of tested plant/ pigments contents of control) x 100;

Phytotoxic of *M. azedarach* extracts on shoot and root length of *C. arietinum* .

MSP, MLP ,and MLE when used at 5% concentration gave significantly highest shoot length (56, 54and 53 cm), respectively compared by the untreated control (49cm), while MSE gave no significant increased in shoot length (50 cm) compared to untreated plants (control) table (2). In case 10% concentrate the seeds and leaves petroleum ether extracts of *M. azedarach* gave significant increase in shoot length (53 and 52 cm), respectively. While, the leaves and seeds ethanol extracts gave no significant increase in shoot length (51 and 50 cm), respectively. 0n the other hand, obtained results (in the same table) showed that the 20 % concentration of organic extracts significant reduce the shoot length in all treatments compared to untreated plants (control). The results indicated that the organic extracts of *M. azedarach* did not affect the root length of chickpea plants (Tables 1). However, the maximum mean value of root length was found to be at 5% concentration of MSP (26.10 cm). The minimum mean value of root length was found to be at 20% concentration of MSE (24.10 cm). Moreover, our results showed more inhibitory effects on shoot than root length. This might be due to direct contact of shoot with the extracts containing inhibitory allelochemicals. Several reports have shown that the amount of allelochemicals varied in plant organs, growth stages and plant variety[34]. In addition, hormonal balance destruction can be one of the most important reasons for reducing growth and seedlings length. Some of the mechanisms of allelopatic activity are like plant hormones[35]. For example, phenol acids and poly phenols reduce growth by the auxin to stop oxidative decarboxylation. Decrease in the growth of plant in the tomato, cucumber and cress seeds[36]by plant extracts have been reported. Similar findings were reported by[37] who investigated the allelopathic effect of three *Amaranthus* spp. (Pigweeds) on wheat (*Triticum durum*). These findings supports the results of[38], percentage of germination, plumule and radicle length of rice and cowpea decreases with the increasing concentration of *acacia auriculiformis* leaf leachates. In the same time[39] reported that extracts of black pepper (*Pipper nigrum*) seeds and Cigar flower leaves had moderate phytotoxic effects on the root system of cotton seedlings. Our results similar to[40] assessed the effect of seed treatment and fumigation of artificially infested cowpea with volatile oil of air dried leaves of *Ageratum conyzoides* (Asteraceae) at concentrations of 2.5 to 10 Ml / 9.5 g bean and they found that no adverse physical effects on the bean at these concentrations. Moreover,[41] mentioned that maize germination was significantly reduced by leaf extracts of *Gliricidia sepium*, *Tetrapleura tetraptera*, *Lonchocarpus sireceus*, *Senna siamea* and *Leucaena leucocephala. Terminalia superba, Tetrapleura tetraptera, Pithecellobium dulce, Gliricidia sepium and Senna siamea* significantly reduced maize root growth at the lowest extract concentration, while shoot length was most significantly reduced by *Giricidia sepium. Leucaena leucocephala, Alchronea coordifolia, Pithecellobium dulce, Terminalia superba* and *Tetrapleura tetraptera* at all concentrations.

Tables 2. Effect of *M. azedarach* organic solvent on shoot and root length (cm) of *C. arietinum* plants after foliar treatment in field conditions .

Root Length (cm)			Shoot Length (cm)			Treatments
Concentration			Concentration			
20%	10%	5%	20%	10%	5%	
25.16	25.13	26.10	46	53	56	**MSP**
25.14	25.14	25.12	44	52	54	**MLP**
24.12	24.26	24.17	35	51	53	**MLE**
24.10	24.12	24.51	30	50	50	**MSE**
25.14			49			**Control**
1.09			**2.11**			**L. S.D 5%**

MSP (*M. azedarach* Seeds Petroleum ether), MLP (*M. azedarach* Leaves Petroleum ether), MLE (*M. azedarach* leaves Ethanol) and MSE (*M. azedarach* Seeds Ethanol) .

Phytotoxic of *M. azedarach* extracts on shoot and root dry weight of *C. arietinum* .

In the present study the shoot and root dry weight were significantly affected by different concentration of *M. azedarach* organic extracts when compared to untreated plants. Table (3) showed that application of MSP and MLP when used at 5 and 10% concentrations significantly increased the shoot and root dry weight of *C. arietinum* plants more than that occurred with MLE, MSE and untreated plants. In the contrary, MLE and MSE when used at 20% concentration significantly reduced the shoot and root dry weight of *C. arietinum* plants as compared with MSP, MLP and untreated plants at harvest time. Data in table (3) show that MSP when used at 5%, 10 and 20 % concentrations gave the highest dry weight of shoot (9.83, 9.78 & 6.98 g) and root (1.14, 1.10 and 0.67 g), respectively, compared by the other treatments. While MSE gave the lowest dry weight of shoot (8.14, 8.52 & 4.14 g) and root (0.82,0.65 & 0.21g) respectively, at the same concentrations. MSP and MLP showed more stimulation in these parameters at harvest time. As the concentration increases the dry weight also decreases. The increase in root and shoot dry weight of *C. arietinum* plants could be attributed to an increase in nutrient levels in *C. arietinum* leaves[42]. Also, the reduction in growth of root and shoot probably had effects on both physiological and biological functions of a plant such as anchoring, absorption of water and other essential nutrients required by plant for its survival. This might have contributed to the decrease in both length and dry weight of the *C. arietinum* plants. On the other hand our results correlate and similar observation was established by[43] who reported the reduction in dry weight of chilli, soybean, maize and rice at higher concentrations of aqueous leaf extract from *Mangifera indica* L. On the other hand the dry weight of the Mung bean was found highest in control and it decreased significantly when treated with different concentration of root extract of *Moringa oleifera* which was revealed by[44]. The essential oils isolated from sweet fennel and sweet basil were the most phytotoxic on barnyard grass, whereas those isolated from lacy phacelia and anise were the least phytotoxic[45]. The essential oils extracted from *Acorus calamus, Curcuma longa, Pimpinella anisum* and *Vetiveria zizanioides* plants have antifungal activity *in vitro* against the soil-borne pathogens. These oils did not show any phytotoxic effect on the germination of chickpea seedlings[46].

Table 3. Effect of *M. azedarach* organic solvent on shoot and root dry weight (g) *C. arietinum* plants after foliar treatment in field conditions.

Dry weight (g) of root			Dry weight (g) of shoot			Treatments
Concentration			Concentration			
20%	10%	5%	20%	10%	5%	
0.67	1.10	1.14	6.98	9.78	9.83	MSP
0.63	0.98	1.01	6.14	9.01	9.13	MLP
0.24	0.77	0.86	4.43	8.97	8.17	MLE
0.21	0.65	0.82	4.14	8.52	8.14	MSE
0.74			8.12			Control
0.12			**0.28**			L. S.D 5%

MSP (*M. azedarach* Seeds Petroleum ether), MLP (*M. azedarach* Leaves Petroleum ether), MLE (*M. azedarach* leaves Ethanol) and MSE (*M. azedarach* Seeds Ethanol) .

Phytotoxic of *M. azedarach* extracts on yield and 100 grains weight (g) of *C. arietinum*.

Data in Table (4) indicate that MSP, MLP, MLE and MSE when used at the 5% and 10% concentrates, generally, elevated the 100 grains weight (g) and yield of treated *C. arietinum* foliage . Moreover, MSP and MLP when used at the 5% and 10% concentrates significantly increased the 100 grains weight (g) and yield of *C. arietinum* plants compared with MLE , MSE and untreated plants (control). Whereas, MSP when used at 5% concentrate gave the highest increasing effect in the 100 grains weight (26.70 g) and yield (1840 kg/h) of *C. arietinum* plants. On the other hand, the tested organic extracts when used at 20% spraying caused in general, significantly decreased in the 100 grains weight (g) and yield of *C. arietinum* plants compared with untreated plants. Also, by the end of experiment MSE gave the lowest 100 grains weight (17.70g) and yield (1200kg/h) of *C. arietinum* plants compared with untreated plants (control) and all treatments. In general, application of the two organic solvents when used at the 5% and 10% caused no deleterious effects on the 100 grains weight (g) and yield of *C. arietinum* plants. The same findings were reported by[47] found that plant extracts of *Vernonia amaygdalina, Bryophyllum. pinnatus, Ocimum gratissimum* and *Eucalyptna globules* under field conditions increased significantly the plant height, shelf life, relative water content and chlorophyll contents of the cowpea seedlings. Furthermore, application of these extracts on the cowpea plants significantly enhanced the Leaf Area Index, number of branches and ponds per plant, total dry matter per plant, weight per pod, 100 grains weight and grain yield. At the same time vital seeds of *Cicer arietinum* germinate and terminate the growth in two weeks from the germination. In case of mint oil the suspension of Tween 80 and the oil dehydrate the seeds of *Cicer arietinum* at concentrations (10, 25 and 50 ppm). Components of mint oil inhibit cell division at the apical meristems in the seedlings, toxify the embryo, inhibit the formation of spindle fibers during mitotic division and consequently inhibit cell division. Constitutes of mint oil inhibit cell elongation and lead to plant stunting. 0.1 ppm concentration of mint oil has a little effect on the growth of *Cicer arietinum* and has no phytotoxic effect on the seedlings. The concentrations (5, 10, 25 and 50 ppm) of clove oil inhibit the growth of the seedlings gradually compared with the control. While 0.1 and 1.0 ppm concentrations of clove oil have no phytotoxic effect on *Cicer arietinum* seedlings[24].

Table 4.Effect of *M. azedarach* organic solvent on yield (kg/h) and 100 grains weight (g) of *C. arietinum* plants after foliar treatment in field conditions.

100 seed weight (g)			Yield (Kg /h)			Treatments
Concentration			Concentration			
20%	10%	5%	20%	10%	5%	
20.90	25.10	26.70	1520	1750	1840	MSP
19.70	24.40	26.60	1430	1678	1810	MLP
18.60	23.90	25.30	1356	1667	1690	MLE
17.70	23.50	24.10	1200	1640	1670	MSE
23.15			1600			Control
0.23			68.14			L. S.D 5%

MSP (*M. azedarach* Seeds Petroleum ether), MLP (*M. azedarach* Leaves Petroleum ether), MLE (*M. azedarach* leaves Ethanol) and MSE (*M. azedarach* Seeds Ethanol) .

Reviewing the above-mentioned results all growth parameters increased in all treatments at 5% concentration compared with control. The tested organic extracts could be arranged according to their phytotoxic activity in the following descending order, MSE > MLE > MLP >MSP. At Our results are also in garment with the another experiment ethanolic extracts of *Melia azedarch*, *Eucalyptus robosat* and *Sapium sebiferum* had no significant influence on growth and development of Soybean seedlings[48]. Effect of tea seed extracts on growth of beet, mustard, oat and barley were studied. Different concentrations of these extracts increased the growth, yield and biomass of the crops. Plant allelopathic effects are different due to environmental conditions in which they grow and also their genetic characteristics[49].

Conclusion

Our result demonstrated that the ethanolic extracts of *M. azedarach* more phytotoxic activity to *C. arietinum* plants compared with petroleum ether extracts. The shoots were found more sensitive to the organic extracts of *M. azedarach* than the roots. The organic extracts of *M. azedarach* on *C. arietinum* showed a stimulatory response in all aspects in our study in 5% concentration compare to all other concentration including control. Data also, indicate the important role of organic extracts of *M. azedarach* concentration on parameters growth of *C. arietinum* plants. Moreover, the higher the concentration was the higher the reduction in parameters growth and vice versa.

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