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POTENTIAL ALLELOPATHIC IMPLICATIONS OFA RAIN-FED WEED *ASTRAGALUS* SPP. ON CHICKPEA CROP

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A three phase study was conducted for the determination of phytotoxic effect of *Astragalus* spp. (milkvetch or goat's-thorn) on the emergence and seedling growth of chickpea. In phase I, *Astragalus* whole plant, leaf, stem, fruit and root aqueous extracts were tested for their effect on chickpea seed germination. In phase II, and III different concentrations (1,2,3, 4 and 5%) of *Astragalus* leaf extract were tested for their phytotoxic influences on the emergence and early seedling growth of chickpea. Distilled water was applied as control treatment. Each experiment was conducted under completely randomized design (CRD). All extracts significantly inhibited the germination energy, germination index, caused delay in time to 50% emergence and mean emergence time of chickpea. Leaf aqueous extract application considerably reduced the root and shoot length and dry weight. P-coumeric, ferrulic, hydroxy, caffeic acid, meth oxybenzoic acid and syringic acid were the phytotoxins detected in leaf extract of *Astragalus* spp. Vanillic acid and syringic acid were determined in fruit extract. P-coumeric, M-coumeric acid and syringic acid were observed in root extract. Whereas, ferrulic, caffeic acid, M-coumeric acid and syringic acid were found in whole plant extract of *Astragalus* spp. Thus*Astragalus* species infestation in chickpeareleases allele chemicals in the rhizospherethat may cause poor germination and growth of chickpea.
Keywords: Ecology, phytotoxins, chromatography, *Astragalus*, chickpea.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is considered as a very nutritious source of food and feed for human and animals, respectively. Among pulses, it is placed at the third place all over the world and is mostly planted in dry and semi-dry areas in Pakistan, Iran, and India (Paolini *et al.,* 2006). In chickpea production, Pakistan (9.5%) ranks second position, after India (65%), which is followed by Turkey (6.7%) (Shah *et al.,* 2006). In Pakistan, chickpea is grown on an area of about 0.944 million hectares which produces 0.438 million tons of grains (Economic Survey of Pakistan, 2019).

About 90% of the total area grown in Pakistan lies in the Punjab province, out of which 92% of the area is rainfed named "Thal" which contributes about 77% in the total production of chickpea in Pakistan (Economic Survey of Pakistan, 2019). During the months of October and November, chickpea sowing is done in rain fed areas on the sandy soil with conserved moisture from the rains of summer season during the months of July to September, which also supports weed growth. The huge difference (2.63 tonnes per ha) is present as compared to the average achieved yield (0.67 tonnes per ha) at the farmer field and the potential yield (3.3 tonne sper ha), (Economic Survey of Pakistan, 2019) due to numerous biotic and a biotic factors including weeds.

The interaction of the biochemicals in agro-ecosystem which disturbs the germination and growth of the neighboring plants (inhibitory or stimulatory) by releasing secondary materials is named as allelopathy (Rice,1984). Weed allelopathy plays a significant role in agro-ecological systems, distress in germination and growth, quality and product of associated crop (Farooq *et al.,*2020). Some weeds release phytotoxic chemicals from waste decomposition, seeds, leachate, and instable excretions which affect the crops. Numerous undesirable plant species reduce the germination of crop plants as well as their growth and propagation (Dongre and Singh, 2007), while some weeds show promontory effects. It has been observed that the plant upper portion showed high effectiveness to inhibit the germination as compared to the ground portion and these influences are mostly reliant on the concentration(Hussain *et al.,* 2011).Inhibitory influences of phytotoxic water extracts of some broad leaved legume weeds including *Melilotusindica, Medicago denticulata, M. indica, Viciahirta, Lathyrus aphaca* and *M. polymorpha* at various concentrative levels on the emergence and seedling growth of non-legumes crops have been perceived (Khan *et al.,* 2012).

Reduction in the emergence, nodulation and seedling growth of leguminous crops like chickpea, garden pea, soybean, green gram, horse bean and cow pea with aqueous extracts of *Ageratum conyzoides* (Batish *et al.,* 2006), *E. odoratum*, *Asphodelus tenuifolius* (Babar *et al.,* 2009), *Chenopodium murale* (Batish *et al.,* 2007), *Solanum nigrum* (Kadioglu *et al.,* 2005), *Tectona grandis*, *Tridaxpro cumbens*, *Trianthema portulacastrum* and *Euphorbia helioscopia* (Tanveer *et al.,* 2010)in pot and field bioassay has been reported in literature.

Astragalus spp. is a xerophytic bushy plant belonging to *Fabaceae* family which has normal height of about 40 cm. This genus is mostly distributed all over the world especially in Asia, Australia, Europe, South and North America, and Northern Africa (Zarre and Azani., 2013). This weed was particularly chosen because it is permeating in typical single cropping system (chickpea-chickpea) of Pakistan. It was assumed that poor germination, stunted emergence and less production of rainfed chickpea in the presence of *Astragalus* weed is due to its allelopathic expression in addition to competition for light, space, water and mineral nutrition. Hence, the objective of this experimental study was to evaluate the phytotoxic influences of *Astragalus* spp. on germination, emergence and seedling growth of chickpea.

MATERIALS AND METHODS

The lab study was conducted at Department of Agronomy, University of Agriculture, Faisalabad (UAF), Pakistan, in three phases with the following procedure. At maturity, the field grown *Astragalus* spp. plants were uprooted from chickpea field by using random sampling technique and separated into different parts. Different plant parts (fruits, leaves, stem, and root) and whole plants were shade dried for one week, chopped down into small pieces and then oven dried at 70°C for 2 days. At room temperature, all the plant parts and whole plants were soaked for one day by combining of 1 g dried plant material: 20 ml of distilled water ratio (Hussain and Gadoon, 1981). *Astragalus* whole plant, leaf, stem, fruit and root aqueous extracts were acquired by passing the mixture through the mesh sieves. Five different concentrative levels (1, 2, 3, 4 and 5%) were prepared from the stock solution of aqueous extract of various plant parts.

In phase-I, 25 chickpea seeds were sown in petri dishes at room temperature having diameter of 9 cm evenly lined with Whatman No. 10 filter paper. Equal amount of distilled water and aqueousextracts of different plant parts were added to respective petri dish throughout the experiment as per treatment plan to keep them moist.

In phase-II of the experiment, 25 chickpea seeds were sown in petri dishes having diameter of 9 cm evenly lined with Whatman No. 10 filter paper. Ten ml of each concentration (1, 2, 3, 4 and 5%) of *Astragalus* leaf extract and distilled water as a control treatment was applied to particular petri dish accordingly. Phase III, was carried out in the sand filled pots having10 cm diameter and 10 cm depth, and ten chickpea seeds were evenly sown in individual pot. During crop growth durations, different leaf extract concentrations (1, 2, 3, 4 and 5 %) of *Astragalus* leaf and distilled water were applied to respective pot according to required treatments. In all phases of the experiment, completely randomized design (CRD) with factorial arrangement was followed and there were four replicates for each experiment. There were two repeats in each phase. Throughout the experiment period, the moisture conditions were monitored carefully and plant extracts or distilled water was supplied for the avoidance of the drying out of germinating seeds and growing chickpea seedlings.

When the radicle tip (2mm) had grown from the seed coat, the seed was considered as a germinated seed. The counts of the germination were taken on daily basis throughout three weeks. Seedling emergence was measured when the cotyledon has appeared on the soil surface. Seedling growth data was recorded for consecutive fifteen days. The calculation of germination/emergence percentage was carried out after counting all the germinated/emerged seeds, by using the formula developed by Association of Official Seed Analysis (AOSA, 1990).

$$\text{Germination or emergence \%age} = \frac{\text{Germinated or emerged seeds}}{\text{Total seeds}} \times 100 \quad [1]$$

The calculation of the germination/emergence index (GI/EI) was calculated as defined by AOSA (1990).

$$\text{GI} = \frac{\text{No of germinated seeds}}{\text{Days of first count}} + \cdots + \frac{\text{No of germinated seeds}}{\text{Days of final count}} \quad [2]$$

The time taken to 50% germination/emergence of seedlings (T_{50}) was calculated by the formula of (Coolbear *et al.,* 1984).

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - t_i\right)(t_j - t_i)}{n_j - n_i} \quad [3]$$

Where all the germinated/emerged seeds count is represented by N, while the cumulative germinated/emerged seeds counts (n_i) and adjacent seeds counts (n_j) at times $t_i$andt_j, when $n_j > N/2 > n_i$.

The mean germination and mean emergence times (MGT and MET) was calculated by using the Ellis and Roberts's equation (1981).

$$\frac{\text{MGT}}{\text{MET}} = \sum \frac{D_n}{\sum n} \quad [4]$$

Where "n" stands for the number of germinated/emerged seeds or seedlings counts and the total days counts/numbers noted from the germination/emergence starts to the completion of the experiment is denoted by D (days).

On 4th day of seed sowing, the calculation of the germination energy (GE) was calculated as reported by Farooq *et al.,* 2006. GE was calculated in percentage for all the

emerged/germinated seeds recorded after four days of sowing divided by all the seeds sown (Ruan *et al.*, 2002).

$$\text{Germination/emeregence Energy} = \frac{\text{Total germinated/emerged seeds at } 4^{\text{th}}\text{day}}{\text{Total seeds}} \times 100 \quad [5]$$

After fifteen days of sowing, root and shoot length was measured by measuring tape from the connective joint of root and stem. Roots and shoots of the seedlings were separated by scissors and weighed after drying at 65ºC in oven for 24 hours.

In High Tec Lab of UAF, all the soluble phenolics were identified through the expressed procedure (Randhir and Shetty, 2005) and were defined equal to the gallic acid. Micro syringe filters were used for the filtration of plant and soil extracts. *Astragalus*s pp. leaf water extract was analyzed chemically due to high inhibition potential land the determination and quantity of their suspected phenolics by Shimadzu HPLC system (Model SCL-10A, Tokyo, Japan). Peaks were detected by using UV detector. The running of suspected phytotoxin standards (Aldrich, St Louis, USA) was done similarly for the determination and quality (Table 1).

Table 1. Phytotoxinsidentified in *Astragalus* spp. extracts.

Phenolics compounds	*Astragalus* spp. part				
	Leaf	stem	Fruit	Root	Whole plant
Chromatotropic					
Chlorogenic					
P-coumeric	√			√	
Ferrulic	√				√
Galic acid					
Caffeic acid	√				√
Hydroxy	√				
Methoxybnzoic acid	√				
M-Coumeric acid				√	√
Syringic acid	√	√	√	√	√
Vanillic acid			√		

***Statistical analysis*:** The analysis of the data was done by using the Fisher's analysis of variance function of MSTAT and the treatment means were compared by using LSD ($p \leq 0.05$)(Steel *et al.*, 1997).Minitab 16 software (Minitab, State College, PA) was used for the completion of figures as well as the regression analyses, where the components such as cubic, linear and quadratic were efficiently examined for significance level of data and involved if the sum of residual squares were decreased significantly ($p < 0.05$). The best suitable model of regression between leaf extract concentrations (%) of *Astragalus* spp., mean germination/emergence time, length and dry weight of seedling of chickpea was first order linear (Eq.7), However the relationship between leaf extract concentration (%) of *Astragalus* spp. and root length was adequately described by the linear third-order polynomial (Eq. 8). For the explanation of relationship between the water extract concentration (%) of *Astragalus* leaves and chickpea's germination, the very fitting regression model was the nonlinear Asymptotic Regression (Concave) (Eq. 9). The NLIN Procedure of SAS (2008) was used for the estimation of the nonlinear model's parameters.

$$y = \beta_0 + \beta x + \varepsilon \quad [7]$$

$$y = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 + \varepsilon \quad [8]$$

$$y = \theta_1 - \theta_2 e^{\theta_3 x} \quad [9]$$

Where, the dependent (response) and independent variable are denoted by y and x, respectively. While, ε is the error term which is expected to have normal distribution with constant variance. By the residuals' examination for each response, the assumptions of model's validity were proved as defined by Montgomery (2009).

RESULTS AND DISCUSSION

The assayed extracts of different parts of *Astragalus* species plants significantly inhibited germination of chickpea seed as compared to distilled water control and inhibitory effects varied from 13 to 53% (Fig. 1) in petri dish bioassay experiments.

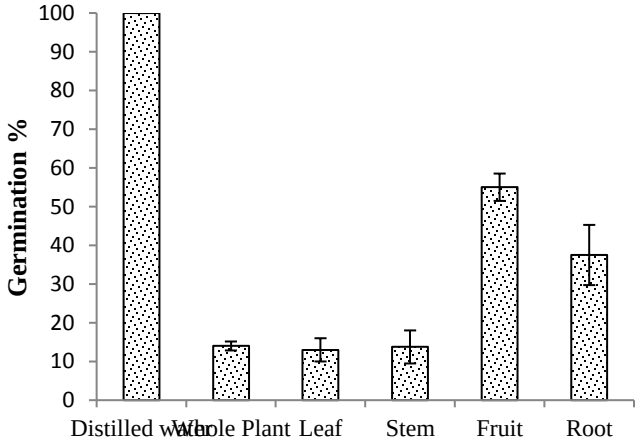

Figure 1. Allelopathic effect of extracts of different plant parts of *Astragalus* spp. on germination percentage of chickpea seed in petri dish bioassay.

Astragalus species leaf extract was the most suppressive but was statistically equal to stem and whole plant aqueous extracts. Leaf extracts also reduced GE and increased time taken to 50% emergence/germination and MGT with minimum GI (Table 2). The germination of the seeds was very high in the control treatment (distilled water) in comparison with the other treatments. Due to presenceof allelochemicals (P-coumeric, ferrulic, syringic acid, caffeic acid, hydroxy, methoxybenzoic acid, vanillic acid and M-coumeric acid), the availability of water in extracts might be

very low which controlled the imbibition of water and stuck the emergence (Table 1). Singh and Sangeeta (1991) exposed thatthe germination of black gram and chickpea was affected by the phytotoxic influences of water extract of *Parthenium hysterophorus*. The suppression of the seed germination might be attributed to the interference of respiration by mitochondria, metabolic enzyme's activities involved in the pathway of oxidative pentose phosphate and glycolysis (Abrahim *et al.,* 2003). *Chenopodium album, Solanum nigrum* and *Matricaria chamomilla* caused 10 to 90% inhibition in chickpea seed germination (Kadioglu *et al.,* 2005). Similarly, the germination of chickpea was inhibited by the phytotoxic effects of *Eupatorium odoratum* and *Ageratum conyzoides* (Batish *et al.,* 2006). Germination percentage as well as germination index of chickpea was highly reduced by the leaf leachate of *Asphodelus tenuifolius* (Babar *et al.,* 2009; Tanveer *et al.,* 2010). Different concentrations of *Astragalus* leaves extracts inhibited the germination of chickpea seed (Fig. 2) in petri dish bioassay experiment.

Table 2. Allelopathic effect of extracts of different plant parts of *Astragalus* spp. on germination traits of chickpea seed.

Treatments	GE	GI	MGT(d)	T_{50} (d)
Distilled water	100.00a	11.31a	2.37d	1.54d
Whole Plant extract	5.00d	0.74d	4.79c	4.06bc
Leaf extract	0.00d	0.34d	9.82a	8.83a
Stem extract	2.50d	0.45d	6.41b	5.68b
Fruit extract	51.2b	3.89b	3.04d	2.50cd
Root extract	32.50c	2.30c	3.46d	2.66cd
LSD	9.135	0.807	1.201	1.854

Means not sharing a letter in common in a column differ significantly at 5% level of probability. GE: Germination energy; GI: germination index; MGT: Mean germination time; T_{50}: Time taken to 50 % germination; LSD: Least significant difference.

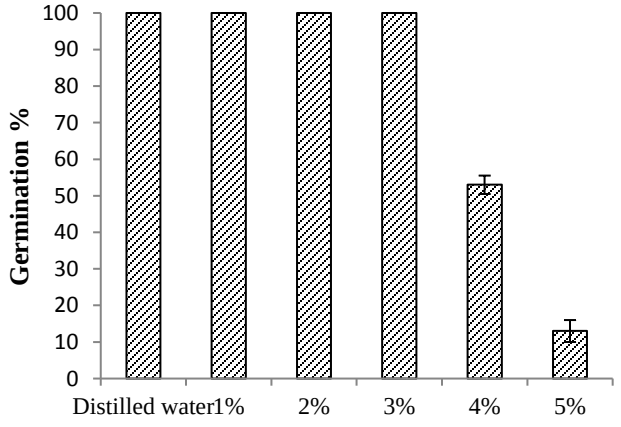

Figure 2. Allelopathic effect of *Astragalus* spp. leaf extract concentrations on germination percentage of chickpea seed in petri dish bioassay.

The inhibitory effect was found very dependent on the different levels of concentration. By the dilution, the inhibitory effect of extracts was lessened on the seed germination. Chickpea seed germination was not affected by 1, 2 and 3% concentrations of *Astragalus* leaf extract matched with control treatment (distilled water). Germination of chickpea seed decreased significantly at 4% extract concentration and maximum decrease was recorded at 5% concentration of *Astragalus* species leaf extract.

Relationship between *Astragalus* leaf extract concentration and seed germination (%) of chickpea was adequately described by the Asymptotic regression model (concave) (Fig. 3F). It indicates that there is strong relationship between *Astragalus* leaf extract concentration and chickpea seed germination; signifying that fitted model revealed in Fig. 3F can be applied to expect germination at 0-5%*Astragalus* leaf extract concentrations. The plots fitted with first order linear, third order polynomial and nonlinear regression models. Within each plot, the fitted models equations, P and R^2 values are shown which depict a progressive rise in T_{50} and MGT and linear decline in GI and GE with increasing concentrations of *Astragalus* spp. leaf extract (Table 3).

Table 3. Allelopathic effect of *Astragalus* spp. leaf extract on germination traits of chickpea seed.

Treatments	GE	GI	MGT (d)	T_{50} (d)
Distilled water	100.00a	11.31a	2.37e	1.54c
1% leaf extract	88.00b	7.75b	3.49d	2.62bc
2% leaf extract	83.00b	6.90c	3.90cd	3.31b
3% leaf extract	52.00c	5.74d	4.59c	3.94b
4% leaf extract	6.00d	1.89e	8.57b	8.60a
5% leaf extract	0.00e	0.34f	9.82a	8.83a
LSD	5.193	0.560	1.072	1.715

Means not sharing a letter in common in a column differ significantly at 5% level of probability. GE: Germination energy; GI: germination index; MGT: Mean germination time; T_{50}: Time taken to 50 % germination; LSD: Least significant difference

Chickpea seeds MGT prolonged by increasing leaf extract concentrations of *Astragalus* spp. (Figure 3A) exhibiting a strong linear relationship. The linear relationship between *Astragalus* leaf extract concentration and MGT was R^2 = 0.89 (Fig.3A).In a study conducted by Hoque *et al.* (2003), it was reported that among *Acaciaauriculi formis*aqueous leaf extract concentrations of 0, 10, 25, 50, 75 and 100%, the highest suppression (90.39%) on the germination of chickpea seeds was recorded with leaf water extract at 100% concentration. Among all the plant parts extracts, leaf aqueous extract showed maximum suppressive effect on the seed emergence of *C. lacryma-jobi* and its seedling growth (Li and Jin, 2010).

The result indicated that leaf aqueous extract at different concentrations did not bring any significant change on the emergence of chickpea seeds in soil bio assay (data not

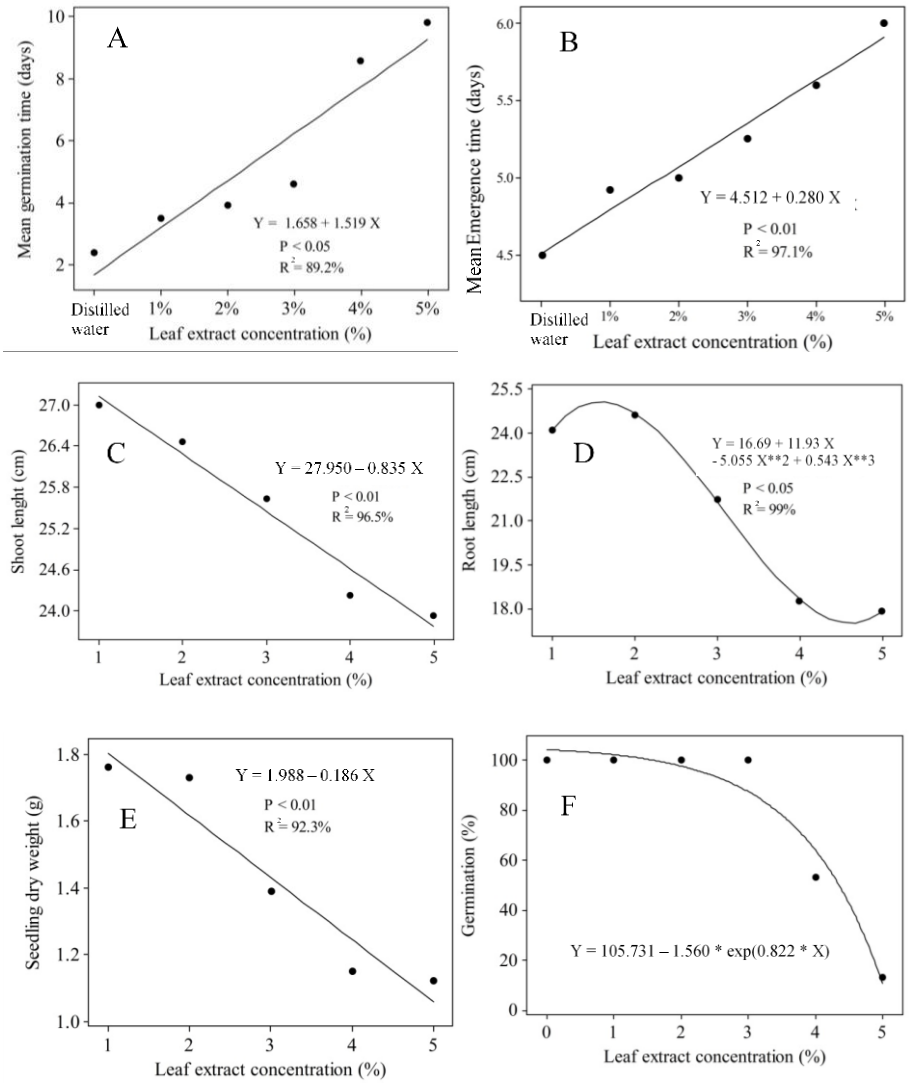

Figure 3. Effect of *Astragalus* spp. leaf extract concentration on mean germination time, mean emergence time, shoot/root length, seedling dry weight and germination of chickpea.

shown) but the remarkable decrease in GE and GI was observed when the extract concentration was increased. *Astragalus* 5% concentrated leaf extract resulted in significantly prolonged MGT and T_{50} germination of chickpea seeds (Table 4). By increasing the concentration of *Astragalus* spp. leaf extract, the MGT of chickpea seed was increased (Fig. 3B) exhibiting a strong linear relationship. The linear relationship between *Astragalus* leaf extract concentration and MET was $R^2 = 0.97$ (Fig.3B).

Table 4. Allelopathic effect of *Astragalus* spp. Leaf extract concentrations on emergence traits of chickpea seed in soil.

Treatments	EE	EI	MET (d)	T_{50} (d)
Distilled water	55.00a	2.26a	4.50e	3.91d
1% Leaf extract	17.50b	2.05b	4.92d	4.45c
2% Leaf extract	12.50bc	2.02bc	5.00d	4.51c
3% Leaf extract	10.00bc	1.93c	5.25c	4.66bc
4% Leaf extract	5.00cd	1.82d	5.60b	4.97b
5% Leaf extract	0.00d	1.68e	6.00a	5.48a
LSD	8.211	0.104	0.304	0.336

Means not sharing a letter in common differ significantly at 5 % level of probability. EE: Emergence energy; EI: Emergence index; MET: Mean emergence time; T_{50}: Time taken to 50 % emergence; LSD: Least significant difference

Aqueous leaf extract significantly inhibited shoot length of chickpea seedlings at 4 and 5% over the 1, 2 and 3% concentration (Table 5). Whereas, leaf extract started its suppressive effect from 3% concentration on shoot length of chickpea seedling. Leaf extract inhibited root length more than shoot length. Root and shoot lengths were always

Table 5. Allelopathic effect of *Astragalus* spp. leaf extract concentrations on shoot length, root length, fresh and dry weight of chickpea seedling in soil.

Treatments	Shoot length (cm)	Root length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)
Distilled water	29.54 a	32.10 a	11.43 a	2.30 a
1% Leaf extract	26.99 a	24.12 ab	8.93 ab	1.76 a
2% Leaf extract	26.46 ab	24.63 a	9.03 a	1.73 a
3% Leaf extract	25.63 abc	21.73 b	7.71 bc	1.39 ab
4% Leaf extract	24.23 bc	18.26 c	6.65 cd	1.15 b
5 % Leaf extract	23.93 c	17.90 c	6.29 d	1.12 b
LSD	2.281	2.789	1.239	0.465

Means not sharing same letter in a column differ significantly at 5% level of probability.

smaller than application of distilled water control. Significant effect of different concentrations of *Astragalus* leaf extract was noted on the seedling of the chickpea and their dry and fresh weights. *Astragalus* leaf extract of 3% caused highest decrease in fresh and dry weight of chickpea seedlings compared with 1 and 2% extracts. However, maximum reduction in seedling weight was observed at 5% leaf extract.

A strong linear relationship was also observed between shoot length (decreased), seedling dry weight (decreased) of chickpea and *Astragalus* leaf extract concentration when analyzed through linear regression (Fig.3 C and E). The relationship for the *Astragalus* leaf extract concentration and chickpea root length was very strong (R^2 = 0.99). The 3rd order polynomial can be used for effective description (Fig. 3D). The fitted linear models can be used to predict MGT, seedling lengths and seedling dry weights of chickpea. The suppression in the growth of root and shoot of chickpea seedling by leaf extracts might suggest interaction between the endogenous chemicals of chickpea seeds and exogenous compounds in leaf extract of *Astragalus*. Chickpea seedling growth and dry weight reduction in this study is in agreement with Singh *et al.* (2004) who observed similar allelopathic effect of *Ageratum conyzoides* extracts on chickpea. In another research work, *Pisum sativum* and *C. arietinum* emergence and seedling growth were gradually reduced by increasing the *Chenopodium murale* weed residues in the soil (Batish *et al.*, 2007).

***Conclusion*:** Aqueous extract of *Astragalus* species contain allelochemicals that inhibit the germination and growth of chickpea. Aqueous extract of leaf have more inhibitory effect as compared to other plant parts: inhibitory effect increased with increasing concentrations.

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