

Comparative Allelopathic Effects of Different Parts of *Parthenium hysterophorus* L. on Seed Germination and Biomasses of *Cicer arietinum* L.

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Parthenium (*Parthenium hysterophorus* L.) has become an invasive woody plant in recent times in many parts of the world. It is rapidly spreading in Pakistan, causing severe damage to the agriculture productivity of the country. A laboratory scale study was conducted in order to evaluate the allelopathic effect of exotic herb *Parthenium* on chickpea (*Cicer arietinum*) by morphological approaches. Experiments were carried out in petri plates supplemented with aqueous extracts of root, stem, leaves and flowers of *Parthenium*. Pronounced variations were noticed for allelopathic activity of different plant parts at different concentrations. *Parthenium* extracts either inhibited or promoted the germination of seeds and seedling growth of chickpea. Results showed that aqueous extracts of root, leaves and flowers significantly reduced the germination and seedling growth. The maximum inhibition was observed with the extract of leaves and flowers as well as the highest inhibition was observed at higher concentrations i.e. 7.5 and 10%. It is concluded that aqueous root, stem, leaf and flower extracts of *Parthenium* contain allelopathic compounds inhibits seed germination and seedling growth of chickpea at higher concentrations.

Key words: allelopathic, weed, Parthenium, chickpea, biomasses

With the burgeoning population of the world, achieving the food security has become a challenge to mankind; as a result, only yield maximization is becoming the last word of modern agriculture. This system is mostly based on the use of heavy doses of chemicals like fertilizers or pesticides to satisfy nutrient deficiency and to control pest attack, respectively. Successful breeding for disease and insect resistant cultivars in combination with development of integrated pest management systems have led to a reduction in the demand for fungicides and insecticides, while herbicide use is still increasing worldwide. Continuous use of heavy doses of chemicals is encouraging resistance development in different pests and endangering the ecosystem. Most agricultural systems collectively use three million tons of herbicides per year. The use of herbicides causes another problem that of the selective growth of weeds (Caamal *et al.*, 1996).

Allelopathy refers to the beneficial or harmful effects of one plant on another plant through the release of biochemical, known as allelochemicals by leaching, root exudation, volatilization, residue decomposition, and other processes (Fraenkel, 1959). In other word, any direct or indirect effect of plants on other plants through the release of chemicals and plays an important role in many agro-ecosystems (Kohli and Rani, 1994). The potential use of allelochemicals as herbicides, such as alkaloids, benzoxazinones, cinnamic acid derivatives, cyanogenic compounds, ethylene and other seed germination stimulants, and flavonoids which had been isolated from over 30 families of terrestrial and aquatic plants. All these chemicals possess actual or potential phytotoxicity (Putnam, 1988). Selection of allelopathic plants is a good and commonly used approach for identification of plants with biologically active natural products (Duke *et al.*, 2000). The Allelopathic nature of any plant is due to the presence of different types of Allelochemicals in it (Rizvi and Rizvi, 1992).

Parthenium is considered to be one of the ten worst weeds of the world (Oudhia, 2002). In Western Australia, it has been declared a 'noxious' weed of category A – Eradication (Navie *et al.*, 1996). The Allelopathic potential of *Parthenium* weed results from the release of phytotoxic substances such as, ferulic,

caffeic, vanillic, chlorogenic, *p*-coumaric and *p*-hydroxybenzoic acids, parthenin, ambrosin and coronopilin, which inhibits germination and growth of several crop plants and multi-purpose trees (Srivastava *et al.*, 1985; Swaminathan *et al.*, 1990) and also cause allergic eczematous contact dermatitis and respiratory problems in humans and livestock (Auld and Medd, 1987).

The inhibitory effects of *Parthenium* weed on germination and seedling growth can probably be attributed to the allelopathic nature of the extracts. Different kinds of Allelopathic chemicals were reported to be released from *Parthenium* roots (Guzman, 1988) and leaves (Swaminathan *et al.*, 1990; Stephen and Sowerby, 1996), Sesquiterpene, lactones and phenolics are from stems (Mersie and Singh, 1988). Ought to be the water-soluble compounds involved in allelopathic activities (Picman and Picman, 1984; Swaminathan *et al.*, 1990). These chemicals were reported to have allelopathic potential on various agronomic crops and weeds (Sarma *et al.*, 1975; Mersie and Singh, 1987; Stephen and Sowerby, 1996), vegetable crops (Mersie and Singh, 1988) and multipurpose trees (Swaminathan *et al.* 1990). According to Tomaszewski and Thimann (1966), cell division is affected as allelopathic chemicals are found to inhibit gibberellin and indoleacetic acid function.

Chickpea (*Cicer arietinum* L.) is an important pulse crop, which belongs to leguminous family, ranking third after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.) (Dhar and Gurha, 1998). Even most of the people of the region are not aware about *Parthenium* problem although it is found to be grown gregariously along their fields of chickpea in the area. Therefore, the present study was conducted with the aim to explore the allelopathic potential of *Parthenium* on chickpea that will aid in the development of control and management strategies.

MATERIALS AND METHODS

Seed collection and sterilization

Healthy uniform grains of chickpea were obtained from Barani Agriculture Research Centre Karak, Khyber Pakhtunkhwa. Before germination test the seeds of

chickpea were surface sterilized with 5% alcohol for 20 min, then rinsed with the distilled water for several times to remove excess of chemical.

Plant extract

The *Parthenium* plants were dried with blotting paper and immediately separated into root, leaf, stem and flowers. Each part of the fresh plant was cut into 2–3 cm and/ or smaller pieces and were dried at room temperature for seven days. The dried plant materials were then grounded using mortar and pestle. Then a powder of each plant part of the sample were weighed as 2.5, 5, 7.5 and 10 g and each amount of powder and added water to prepare 100 ml of solutions i.e. 2.5, 5, 7.5 and 10%. The mixture was stirred for 10 minutes every 2 h interval and kept at room temperature in the laboratory overnight. After 24 h of soaking, the extracts were filtered through Whatman No. 1 filter paper. The solution was then kept in bottles and stored for further use.

Seed germination

In the Petri dish the filter papers were kept and 5ml of the various concentrations of the *Parthenium* plant extract were poured on top of dishes. Then ten seeds of plant were kept in each Petri dish. A separate series of control was set up using distilled water. The germination of the seed was noticed after every 24 h up for one week.

Biomasses

The root and shoot length of the seedlings was analyzed after two weeks with the help of scale. To measure the fresh weight, the root and shoot portions of seedlings were weighed then dried in a hot air oven at 70°C for 24 h and the dry weight was taken by using a digital balance.

Statistical analysis

The results were calculated for three independent determination with their means and standard deviations. The data was analyzed with one way ANOVA. The difference was considered to be significant if p value was less or same to 0.05 ($p \leq 0.05$).

RESULTS

Effects of root extract on seed germination and seedling growth

Germination data of chickpea, treated with aqueous root extracts of *Parthenium* is summarized in Fig. 1.

After 24 h no germination was recorded while after 48 h, only 7.5% treatment showed significant inhibition of 36.66% as compared to control having 56.66% germination. Similarly, germination after 72 h showed significant inhibition i.e. 50, 46.6 and 40% in the treatments of 5%, 7.5% and 10%, respectively as compared to 83.3% in the control. Germination recorded after 96 h and 120 h showed inhibition in 5% and 10% treatments while in 148 h, only 5% treatment posed significant inhibition. The results showed a slightly inhibitory or no effect of *Parthenium* in case of 2.5% as compared to control.

The root length (cm) in control was 8.2 cm while it was recorded 7.5, 6.2, 4.3 and 5.45 cm for the concentration of 2.5, 5, 7.5 and 10% extract, respectively (Fig. 1A). All the tested concentrations inhibited root length significantly. The shoot length of gram seedling in control was 6.3 cm while it was recorded 9.5, 7.4, 4.6 and 5.5 cm for the concentrations of 2.5, 5, 7.5 and 10%, respectively. Interestingly, lower concentrations i.e. 2.5 and 5% promoted the shoot development while exposed to 7.5 and 10% resulted in stunted shoot growth (Fig. 2). The shoot fresh weight in control was 0.9 g, while it was recorded 1.8, 1.7, 1.2 and 1.3 g in 2.5, 5, 7.5 and 10% treatments, respectively. Increase in shoot fresh weight was observed in all the treatment especially in 2.5% as compared to control (Fig. 2). The shoot dry weight estimated for different concentration of plant extract showed increase among all the treatments of extracts except 10% where a significant decrease was observed. The root fresh weight in control was 0.23g while it was recorded 0.16, 0.16, 0.13 and 0.16g in 2.5, 5, 7.5 and 10% extract, respectively. Decrease ($p \leq 0.05$) in root dry weight was found in all the treatments as compared to control (Fig. 2).

Effects of stem extract on seed germination and seedling growth

Results indicated that stem extracts significantly ($p \leq 0.05$) reduced the germination of chickpea when exposed to 7.5% and 10% concentrations at 48, 72 and 96 h interval (Fig. 3). At 48 h interval the inhibited germination of 36.6% was observed both in 7.5 and 10% concentrations with compared to the untreated control where 70% germination was recorded. After 72 h the

inhibited germination of 37.6 and 43.3% was recorded in seeds exposed to 7.5 and 10% treatments, respectively while in untreated control it was 73.3 %. Similar trend was observed in 96 h interval in the seed germination exposed to 7.5 and 10% treatments. Germination data after 120 and 148 h, the difference between treated and untreated seeds was reduced and no significant inhibition was observed with compared to control.

The root length in control was 10.6 cm while it was recorded 12.4, 10.3, 9.4 and 8.2 cm in 2.5, 5, 7.5 and 10%, respectively (Fig. 4). The lower treatment i.e. 2.5% increased ($p \geq 0.05$) the root length of chickpea seedlings while higher concentrations (7.5 and 10%) caused decline in the root length. The shoot length of 4.6 cm was recorded in control while it was recorded 3.5, 4.2, 3.5 and 3.2 cm in 2.5, 5, 7.5 and 10% extracts, respectively (Fig 4). In case of root fresh and dry weight, except 2.5% treatment, all the higher concentrations significant ($p \leq 0.05$) reduced the biomasses. Similarly, in case of shoot fresh weight, 2.5% treatment improved fresh weight while rest of all treatment decline the fresh weight. Similar trend was observed in dry weight where all the treatments caused reduced dry weight except 7.5% (Fig. 4).

Effect of aqueous leaf extract on seed germination and seedling growth

Seeds that were exposed to the leaf extract of *Parthenium* showed more inhibition as compared to root

and stem extracts. Data collected at different time intervals i.e. 48, 72, 96, 120 and 144h showed inhibitory effect with all the tested treatments with exception of 2.5% as compared to the untreated control. Maximum inhibition ($p \leq 0.05$) was observed in 7.5 and 10% concentrations. The only exception was the treatment with 2.5% extract where increase in seed germination occurred (Fig. 5). The root length in control was 11.5 cm while it was recorded 20.22, 0.43, 0.15 and 0.17cm in 2.5, 5, 7.5 and 10% extract, respectively. The highest value was found in 2.5% concentration which showed promotory effect (Fig. 6). A highly significant decrease ($p \leq 0.05$) was observed at higher concentrations of leaf extract. Similar to that of root length, shoot length showed same trend i.e. an increase in shoot length was observed in seedlings treated with 2.5% extract, while the higher concentrations (5, 7.5 and 10%) significantly inhibited shoot length in chickpea seedlings (Fig. 6).

The root fresh weight showed maximum value with untreated control, but a gradual decline in the fresh weight was observed with increase in extracts concentrations. The weight of root was also affected with increasing extract concentrations except 2.5% treatment. (Fig. 6 C and D). Fresh weight of shoot was slightly effected with higher concentrations while lower concentrations improved the fresh weight. Similarly, dry weight of shoot also affected by exposure to concentrated extracts (Fig. 6).

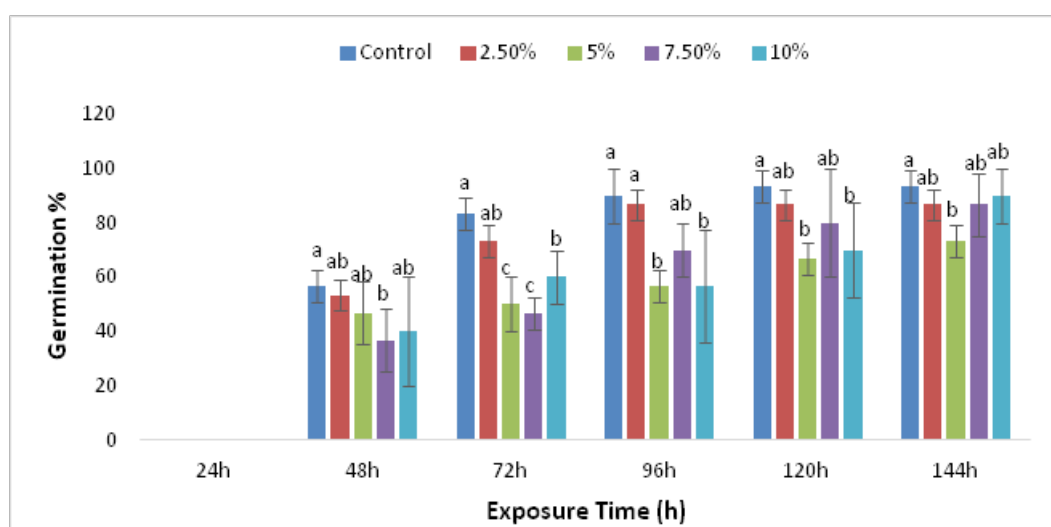


Figure 1. Effect of *Parthenium* aqueous root extract on seed germination of *Cicer arietinum*. Each data point shows mean of three replicates with 10 seeds in each replica. Vertical bar indicates SD at ($p \leq 0.05$) and alphabets shows significant difference.

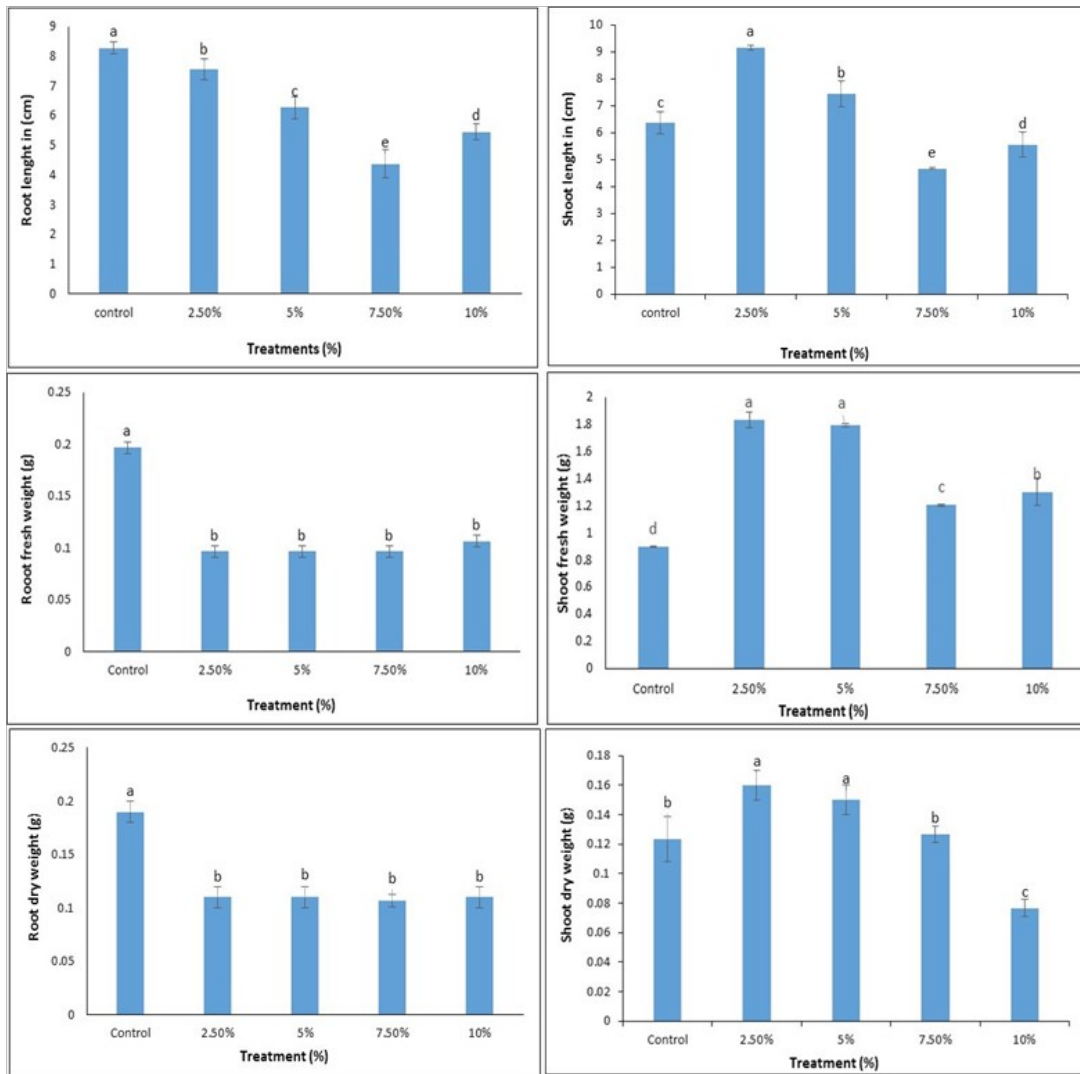


Figure 2. Effect of *Parthenium* aqueous root extract on root length (A), shoot length (B), root fresh weight (C), shoot fresh weight (D), root dry weight (E) and shoot dry weight (F) of *Cicer arietinum*. Each data point shows mean of three replicates with 10 seeds in each replica. Vertical bar indicates SD at ($p \leq 0.05$) and alphabets shows significant difference..

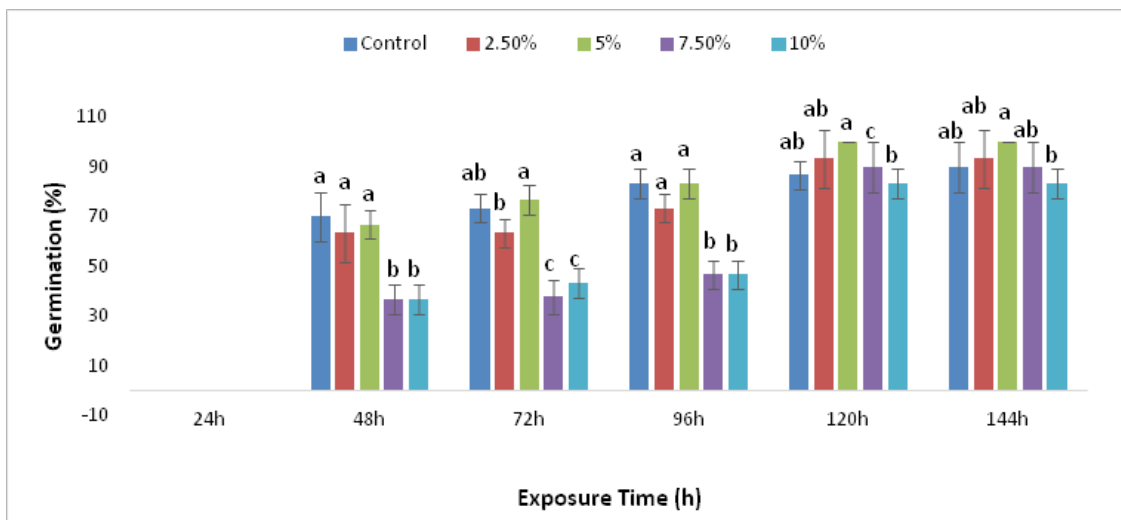


Figure 3. Effect of aqueous stem extract of *Parthenium* (2.5, 5, 7.5 and 10%) on percentage of seed germination of chickpea and alphabets shows significant difference.

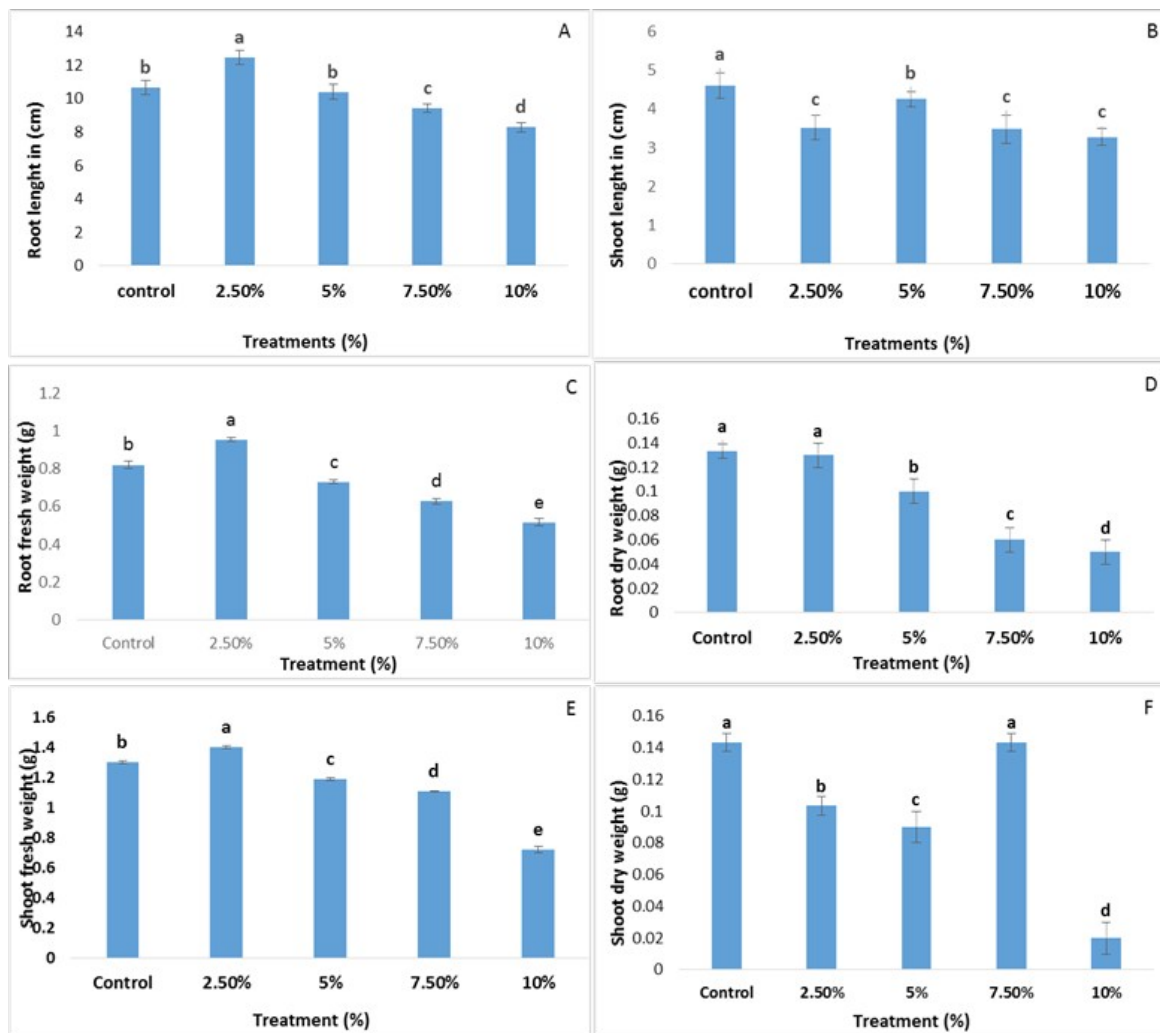


Figure 4. Effect of aqueous stem extract of *Parthenium* on root length (A), shoot length (B), Root fresh weight (C), Root dry weight (D), Shoot fresh weight (E) and Shoot dry weight (F) of *Cicer arietinum*. Each data point shows mean of three replicates with 10 seeds in each replica. Vertical bar indicates SD at ($p \leq 0.05$) and alphabets shows significant difference.

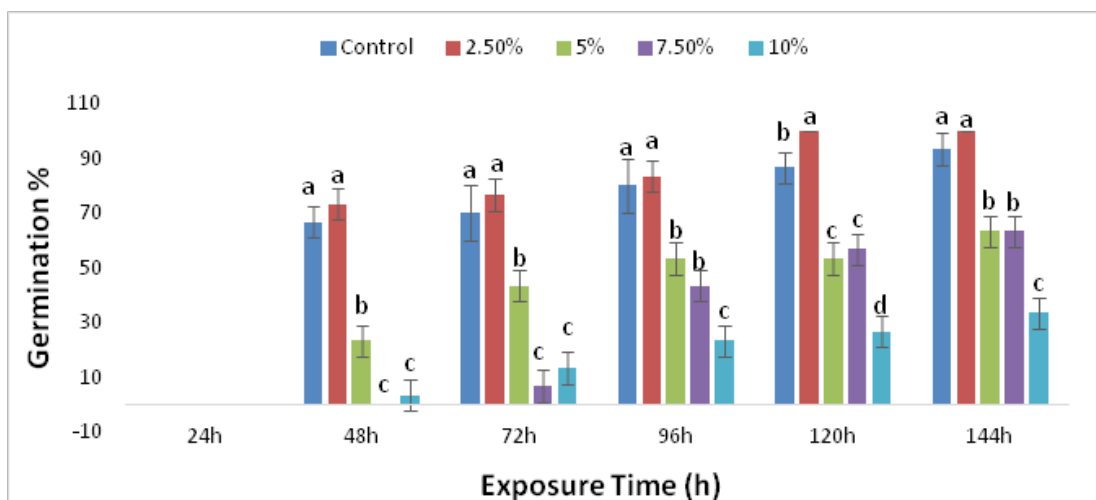


Figure 5. Effect of aqueous leaf extract of *Parthenium* (2.5, 5, 7.5 and 10%) on percentage seed germination of *Cicer arietinum* and alphabets shows significant difference.

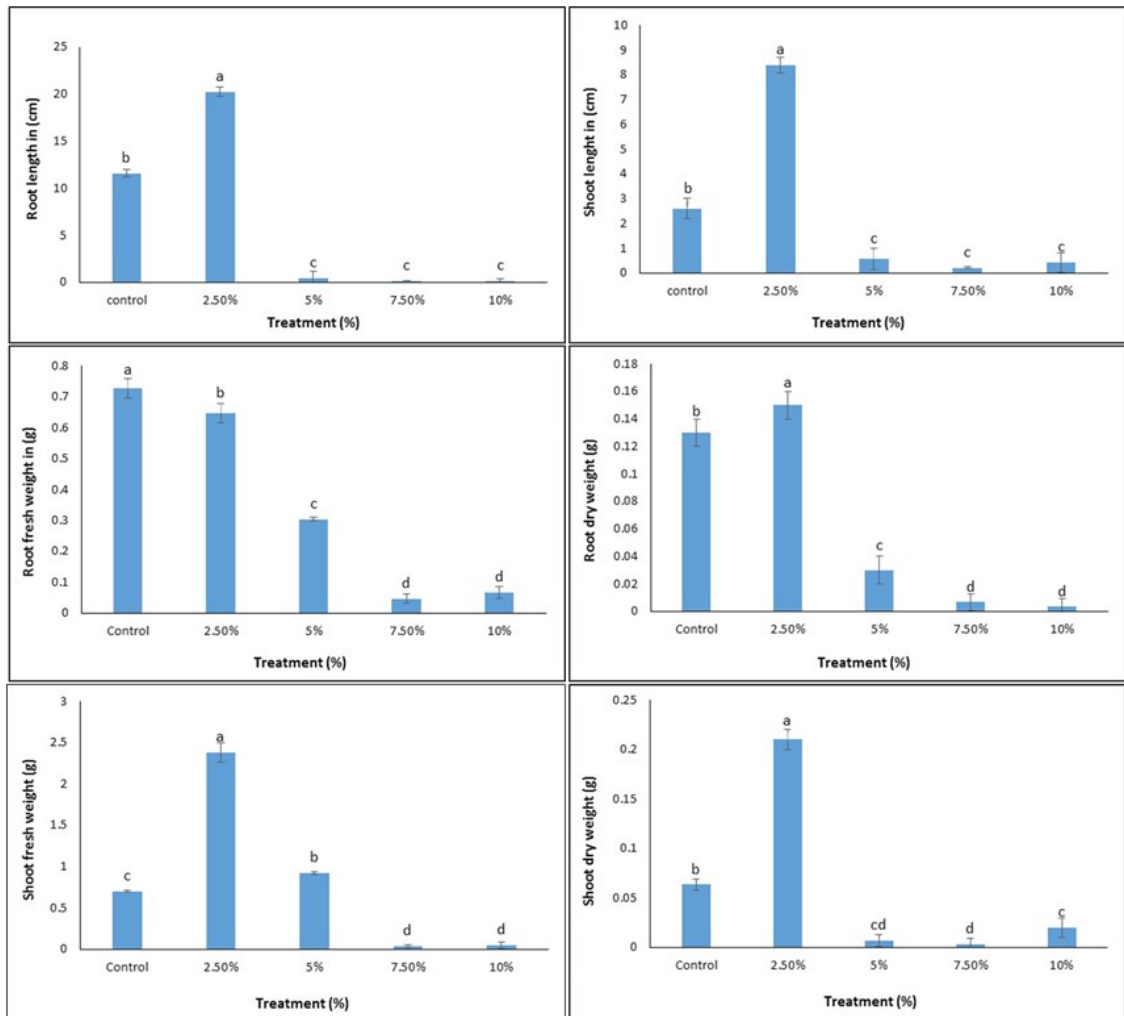


Figure 6. Effect of aqueous leaves extract of *Parthenium* on root length (A), shoot length (B), Root fresh weight (C), Root dry weight (D), Shoot fresh weight (E) and Shoot dry weight (F) of *Cicer arietinum*. Each data point shows mean of three replicates with 10 seeds in each replica. Vertical bar indicates SD at ($p \leq 0.05$) and alphabets shows significant difference.

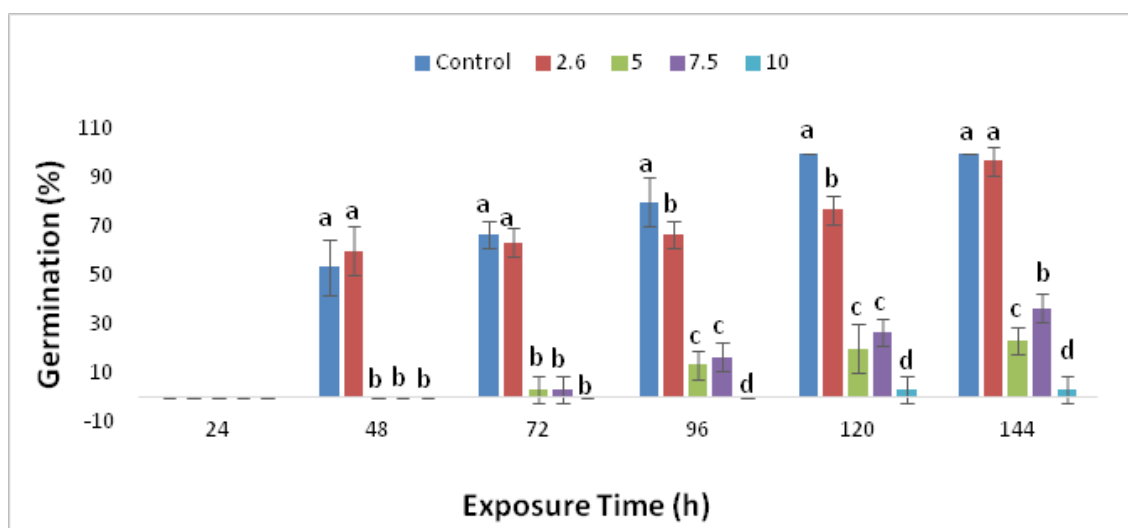


Figure 7. Effect of aqueous flower extract of *Parthenium* (2.5, 5, 7.5 and 10%) on percentage seed germination of *Cicer arietinum* and alphabets shows significant difference..

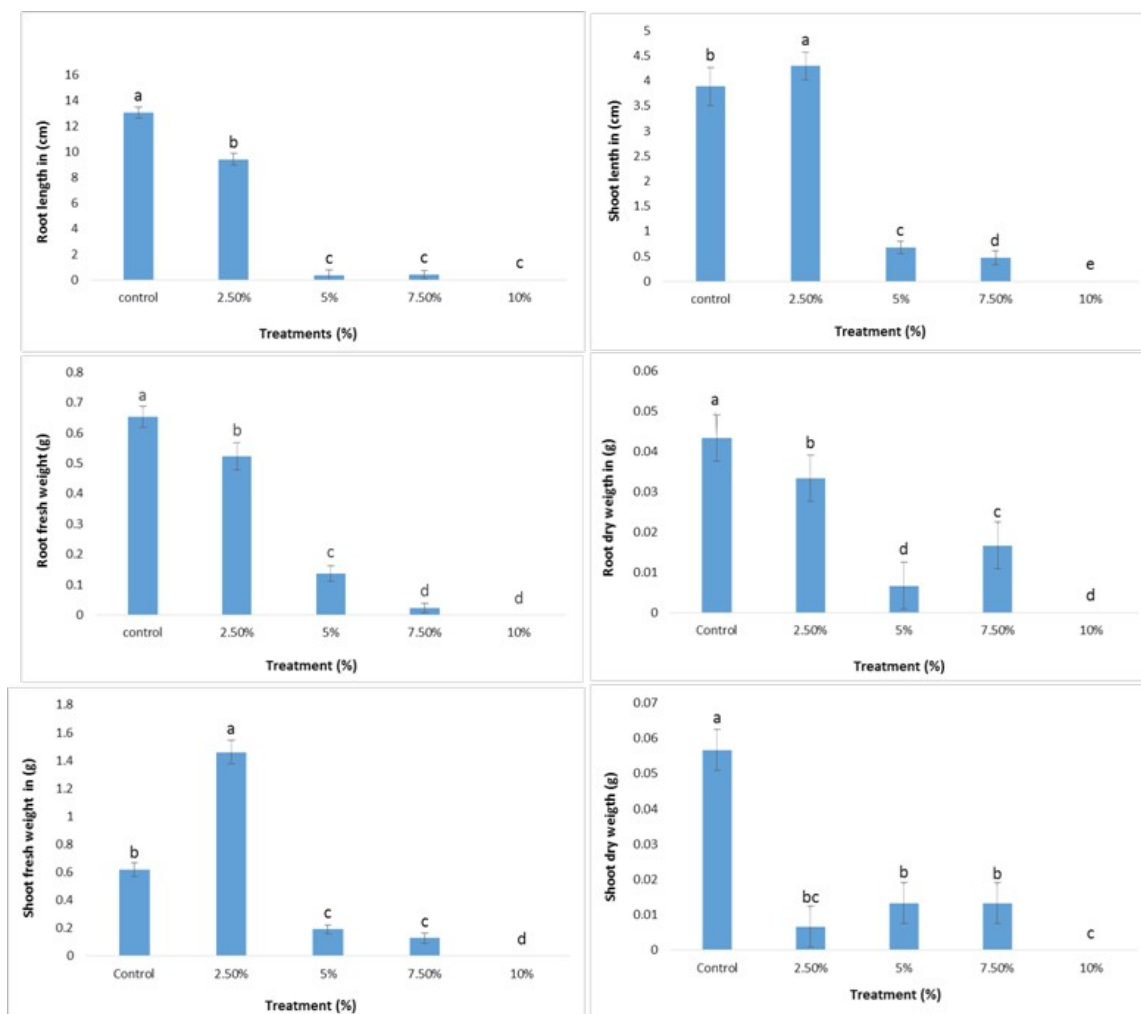


Figure 8. Effect of aqueous flower extract of *Parthenium* on root length (A), shoot length (B), Root fresh weight (C), Root dry weight (D), Shoot fresh weight (E) and Shoot dry weight (F) of *Cicer arietinum*. Each data point shows mean of three replicates with 10 seeds in each replica. Vertical bar indicates SD at ($p \leq 0.05$) and alphabets shows significant difference..

Effect of flower extract on seed germination and seedling growth

Similar to leaf extract, results of flower extract treated seeds revealed that various concentrations (5, 7.5 and 10%) significantly suppressed ($p \leq 0.05$) the germination of seeds in chickpea. The effect was severe in higher concentrations (Fig. 7). Present results also indicated that leaf extract posed more severe inhibition in seed germination with compared to the other extracts. The root length was also decreased significantly as compared to the control where it was 13.08 cm while in 2.5, 5, 7.5 and 10% extracts the length were 9.04, 0.38, 0.41, 0 cm, respectively (Fig. 8). Similar trend was observed in the shoot length where except 2.5%, all the treatments declined the length of shoot. The results of root fresh and dry weight were indicated in Fig. 8. A

decrease in the root fresh and dry weight was observed when subjected all tested concentrations of flower extracts. A decrease was observed in shoot fresh weight in plants subjected to 5, 7.5 and 10% while, 2.5% increased the biomasses in term of shoot fresh and dry weight (Fig. 8).

DISCUSSION

The present study was conducted to investigate the effect of aqueous extract of different parts of *Parthenium* on seed germination and other morphological characters of *Cicer arietinum*.

Obtained results revealed that high concentrations of aqueous root extract of *Parthenium* inhibited seed germination of chickpea (Fig. 1, 3, 5 and 7). *Parthenium* extracts of root, stem, leaves and flowers showed inhibitory effect especially at high concentrations at 48,

72 and 96h intervals but an increase in germination was observed at lower concentrations. The study also demonstrated that extractions obtained from leaves and flowers posed more significant inhibition on seed germination as compared to extractions obtained from root and stem. Significant inhibition ($p < 0.05$) was observed in 7.5 and 10% concentrations. The only exception was the treatment with 2.5% extract where increase in seed germination occurred. Similarly, the various concentrations (5, 7.5 and 10%) of flower significantly suppressed ($p < 0.05$) the germination of seeds of chickpea. This indicates the availability of high level of the inhibitory chemicals in higher concentration in leaves and flowers as compared to roots and stems.

This study revealed that allelopathic effect varied among the concentration levels of the extracts and the parts of the weed from which they were extracted. Its effect on germination and growth suggests that different parts, especially leaves and flowers of *Parthenium* may act as a source of allelochemicals after being released into soil or after decomposition ultimately, control the growth neighboring of successional plants.

Similar results were also obtained by (Maharjan et al., 2007): who observed that the increasing concentration of *Parthenium* water extract exhibited inhibitory impacts on seed germination of cereal crops. Earlier works reported that *Parthenium* has deadly allelopathic effects on rice, wheat, chickpea, soybean and mustard (Karim and Forzwa, 2010; Biswas, 2010). There was a gradual decrease in seed germination with the increase in concentration of extracts obtained from different parts of *Parthenium*. These results showed that leaf aqueous extract of *Parthenium* exhibited significant inhibitory effect on seed germination had adverse effect than the stem as reported by Tefera (2002). It is also reported that aqueous extracts of leaf and flower of *Parthenium* caused complete failure of seed germination of chickpea. Naeem et al., (2012) demonstrated similar finding on wheat. However, lower concentrations of the tested extracts gave significant increase on the germination of seeds. This might be the protective mechanism that lower allelochemicals concentration could stimulate the germination and growth of other plants as supported by several studies (Reigosa et al., 2000; Hong et al., 2004; Nektarios et al., 2005).

Furthermore, few studies have found that some allelochemicals could perform stimulatory effects on other plants (Chen, 1999). A small number of nutrients brought by root extracts also might partly contribute to the stimulative effect (Khan et al., 1999).

Studies showed that higher concentration of *Parthenium* retard the germination and growth of plant which might be due to inhibition of cell division as allelopathic chemicals have been found to inhibit gibberellin and indole-acetic acid function (Tomaszewski and Thiemann, 1966). The inhibition of shoot elongation caused by allelochemical lead to reduced plumule length. This positive relation between extract concentration of *Parthenium* and reduction in seedling length followed the finding in lettuce (Wakjira, 2009). Root exudates are an important source of allelopathic chemicals (Lee and Monsi, 1963; May and Ash, 1990). Which could have significant allelopathic effects on other plants in the plantation. The results that seed germination and early seedling growth were inhibited by *Parthenium* root extracts. In addition, relatively higher seed germination rates and early growth of target species were observed in the treatment with lower root extract concentration compared to distilled water treatment.

The present study also showed that aqueous extract of (root, leaf and flower) of *Parthenium* inhibited radical and plumule length (Fig. 2, 4, 6 and 8) except for lower concentration of the extract where root and shoot length was promoted. The inhibitory effect was found more serious in treatments with leaf and flower extract especially at higher concentrations (7.5 and 10%). The effect of different dilutions of different parts of *Parthenium* was found more prominent on root elongation as compared to shoot elongation. This was probably due to direct contact of root with the extract and subsequently with inhibitory chemicals (Bhowmik and Doll, 1984; Quasem, 1995). Some earlier workers have also reported that *Parthenium* extract reduces root and shoot length of chickpea and *Zea mays* (Bhatt et al., 1994). Studies have also shown that the phenolic compounds found in leaf of *Parthenium* have inhibitory effect on nitrogen fixing and nitrifying bacteria which in turn reduces the growth plants (Kanchan, 1975; Kanchan and Jayachandra. 1979).

In chickpea, the dry matter accumulation was also decreased with increasing concentration of *Parthenium*. The shoot and root fresh and dry biomass of chickpea also showed an increase when subjected to the lower concentration of *Parthenium* stem extract as compared to control. At higher concentration, especially 7.5 and 10%, significant inhibition was recorded. This could occur only when some allelochemicals present in high concentrations in leaf and flower extract prevented the growth of embryo or caused death of embryo. The extract of *Parthenium* induced a variety of chromosomal aberration in dividing cells, which increased significantly with increasing concentration and duration of exposure (Rajendiran, 2005).

Similar to our findings, Maharjan, *et al.* (2007) found that the biomasses of the tested species was significantly reduced with the increase in the concentration of leaf and flower aqueous extract of *Parthenium*. Few other studies also presented the same trend (Auld and Medd, 1987; Sarma *et al.*, 1975; Srivastava *et al.*, 1985; Evans, 1997). Tamado *et al.* (2002), observed similar effect on maize and sorghum, multi-purpose trees, pumpkin, and tomato.

Present results attributed the release of different kinds of phytotoxic compounds, viz. phenolic, sesquiterpenes and lactones from root and vegetative part of plants as well as from the achen by exudation and leaching, respectively (Auld and Medd, 1987; Sarma *et al.*, 1975; Guzman, 1988; Evans, 1997; Belz *et al.*, 2007). The present study also showed that, aqueous extracts of flower at all concentration levels and leaf at intermediate and higher concentration levels induced the highest allelopathic effects as indicated by complete failure of germination and seedling growth. This could be due to the presence of high level inhibitory compounds in these plant parts as compared to shoot and roots (Kanchan and Jayachandra, 1980). Similarly, Belz *et al.*, (2007), reported that *Parthenium* releases compounds to the soil during decomposition of leaves might be leading toxic compounds caused phytotoxicity.

The findings of this study demonstrated that *Parthenium* weed has allelopathic effect on chickpea as indicated by its inhibitory effect on germination, germination rate, growth and dry matter production of

the seedlings.

CONCLUSION

The present investigation revealed that aqueous extract of *Parthenium* at different concentration level possess strong inhibitory effect on the seed germination percentage, root and shoot length and fresh and dry biomass of chickpea. The allelopathic effect of *Parthenium* leaf and flower extracts was observed to have significant inhibitory effect on seed germination, root and shoot length, fresh and dry weight of chickpea as compared to root and stem extract of *Parthenium* which showed inhibition only at higher concentrations while comparatively caused less inhibition at lower concentrations. Complete failure in seed germination of chickpea in treatments with leaf and flower extracts were recorded at 7.5% and 10% extract of *Parthenium* while 2.5% treatment showed promotory effect but non-significant. Since in Pakistan, *Parthenium* is very extensively spreading in all open localities, wastelands and roadsides and is emerging as a real threat. For this purpose early and appropriate measure to stop its invasion are not taken in time then it may become a major weed problem in our cultivated lands.

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