

A STUDY OF DIFFERENT PARAMETERS OF OSMOTIC POTENTIAL COMPARED WITH WEED (*CHENOPODIUM ALBUM*) ON WHEAT AND CHICKPEA CROP

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Abstract

The study was conducted to evaluate the effect of osmotic pressure on germination and radicle growth of two test species wheat and chickpea along with the aqueous extract of different concentration of weed (*Chenopodium album* L.). In the first experiment two test crops were treated with the weed extract in the concentration of 1%, 3% and 5% while in the second experiment four levels of osmotic pressure (22 kpa, 33 kpa, 44 kpa, 66 kpa) were created artificially using mannitol and treated the seeds of two test crops. This study focus that the responses of seeds or seedling to plant extract is not only or entirely by allelopathy the possibilities also exist that the extract may also exert negative osmotic effect. Results showed that germination percentage was not affected by osmotic pressure as compared to weed extract. However, the radicle elongation of both species was significantly reduced in similar concentration of weed extract. Generally the aqueous extracts were more effective in inhibition of seed germination, radicle growth and percent inhibition in radicle growth as compared to the mannitol solution. Results suggested that aqueous extract of *C. album* had an allelopathic effect on wheat and chick pea and osmotic pressure play little effect as *C. album* is concerned.

Key words: Osmotic pressure, Mannitol, *Chenopodium album*, Allelopathys.

Introduction

Plants have been an indispensable part of human life for ages. Ever since ancient times, their fruits, seeds, roots and branches have been used to meet personal and social needs such as food, curing diseases and beautifying the planet (Ercisli, 2009; Erturk *et al.*, 2010; Canan *et al.*, 2016; Hricova *et al.*, 2016; Yazici & Sahin, 2016).

The term allelopathy refers to the detrimental effects of one species (the donor) on the germination, growth or development of another species (the recipient). Many researchers reported the direct role of allelopathy in agriculture and considered the effect of harvest residue decomposition on weeds and crops yield. Many allelopathic bioassay results are difficult to apply in field condition because methods used do not always reflect actual ecological situations (Stowe, 1979; Putnam, 1985; Weston, 1996; Wardle *et al.*, 1998; Inderjit, 2002; Kruidhof *et al.*, 2009).

Chenopodium album L. is a widespread weed with wheat (Le-Tourneau *et al.*, 1956; Saeed *et al.*, 1987) and is responsible for reduction in both shoot and root growth of wheat, when it grown in 2% (W/V) extract of common lambsquarters. It also caused inhibitory effect on the radicle growth of corn, bean and wheat. Bukolova (1971) found that wheat, rye and garden cress show reduction in mitotic activity in their roots if associated with this weed. Alam (1996) reported that weed affected the dry matter yield of wheat and rice. It is the most problematic weed of wheat, indicated by many workers of agricultural fields in Pakistan. Weed infestation is the main cause of low wheat yield in Pakistan and is reported to cause yield reduction by 25-30% (Anjum & Bajwa, 2010). Various investigators such as Bell (1974) and Wardle *et al.*, (1992) reported that osmotic pressure of cell sap might also inhibit germination and radicle growth therefore it was hard to explain that inhibitory effect was due to allelopathy. The highest mannitol ($12.2 \pm 2.1\%$ dw) content was found in

Sargassum mangarevense during the austral winter (Zubia *et al.*, 2008). Mannitol is a polyol. It is metabolically inert in human and occurs naturally as a sugar or sugar alcohol and it is also osmotic diuretic (www.dmglib.com). The species that metabolize mannitol have several advantages over that have exclusively translocate sugars. One advantage is the increase in tolerance to salt and osmotic stress as a result of mannitol functions as "compatible solute". Another advantage is in pathogen attack (Stoop *et al.*, 1996). Mannitol is also reported to promote seedling growth of orchid (Ernst, 1967). In Pakistan, no one included osmotic pressure of weed during allelopathic bioassays investigations except Salam (2015).

The objective of present study is to study the role of osmotic pressure along with different concentrations of *C. album* on two test species i.e. wheat and gram.

Materials and Methods:

To check any inhibitory effect due to osmotic pressure along with different concentrations of weeds extract on test crops (wheat and chickpea) experiments were conducted in the green house Department of Botany, Federal Urdu University of Art, Science and Technology Karachi-Pakistan. The germination experiments were carried out in laboratory (Petri dishes) by the following methods.

Collection of material

Plant of *Chenopodium album* were collected from sites of University of Karachi, Federal Urdu University, different waste places, road sides of Karachi and Hyderabad. Sufficient amount of plants of *C. album* were collected to obtain extract for different concentrations. This trial was conducted in Department of Botany, FUUAST, Karachi. Test crops seeds were obtained from Atomic Energy Commission and Agricultural Research

Station, Tandojam. The seeds of test crop were cleaned and stored in paper bags and kept in dry place at room temperature in laboratory. The data of the germination and radicle growth elongation of test crops were obtained from petri dishes experiments. Calculation of percent germination, speed of germination 'S' by Khandakar and Bradbeer (Khandakar & Bradbeer, 1983), percentage of inhibition on radicle growth by Surendra & Pota (1978) by the following formula:

$$\text{Germinability (G\%): } G\% = \frac{\text{Total No. of seed germinated}}{\text{Total No. of seeds}} \times 100$$

$$\text{Speed of germination index (S): } S = \left[\frac{N_1}{1} + \frac{N_2}{2} + \frac{N_3}{3} + \dots + \frac{N_n}{n} \right] \times 100$$

where $N_1/1, N_2/2, N_3/3, \dots, N_n/n =$ Proportion of seeds with germinated on day 1,2,3,.....n.

$$\text{Inhibition \%: } I = 100 - \frac{E_2 \times 100}{E_1}$$

where: I= % inhibition, $E_1 =$ Response of control plant, $E_2 =$ Response of treated plant

The test species were tested by using Mannitol solution of varied concentrations to test the effects of possible osmotic potential.

Preparation of mannitol solution: Aqueous solution of mannitol were prepared in four different concentrations i.e. 16, 24, 32 and 50 g.L⁻¹ which corresponding 22.0, 33, 44 and 66 kPa (osmotic potential) and deionized water was used as a control, to assess their relative effects on seed germination and radicle elongation (Wardle *et al.*, 1992). Milk cryoscope was used to determine osmotic pressure of mannitol solution. Each concentration of mannitol solution prepared in 400 mL distilled water. These test crop seed germination bioassays were conducted in laboratory. All glassware was sterilized with 0.1% HgCl₂ for 1 minute and 3 to 4 time washed with distill water. Five replicates per treatment were employed with 10 sterilized seeds were kept in 9 cm diameter Petri dish and Whatman No. 1 filter paper. Seeds of test crop arranged at uniform distance in Petri dishes. The Petri dishes were covered with glass covering and sealed by

insulating tape. Normally the temperature of the trial was 20-30°C. These trials were carried out side by side as (1) control (2) with mannitol solution (3) with weed extracts. Three mL mannitol solution and deionized water were used on alternate days in each control and treatment. The trial was terminated after ten days. Germination of seeds in each Petri dish of test crop was daily recorded and radical length measured on alternate day. Cumulative percent germination, speed of germination (S) and radical length inhibition were calculated according to the formula described above.

Preparation of weed extract: Sufficient amount of weed plants were collected, washed and dried at room temperature for 7-10 days and ground by Willey Mills to 1 mm (40 mesh) size. Ten g of whole plant material mixed with 100 mL distill water and left for 24 hours in dark in laboratory at room temperature (Average 25-30°C during day) for extraction. A filtrate obtained from mixture and final volume up to 100 ml, 10% aqueous extract considered as a stock solution adjusted to pH 6.8 with 1N HCL (Rice, 1972). A series of dilution (1%, 3% and 5%) prepared from the stock solution were used for bioassay trials. Petri dishes were regularly checked for moisture, germination of seeds and radicle length till the trial terminated.

Data Analysis: Data of each experiment were analyzed and subjected to analysis of variance (ANOVA) one way (Gomez & Gomez, 1984). The means were compared by Duncan's multiple range test (DMRT) at $p < 0.05$.

Results and Discussion

In Table 1 showed the effect of different osmotic solution of mannitol on germination, radicle elongation and % inhibition on wheat. It is shown that no significant effect and associated from zero (control) to 22 osmotic potential, however from 33 kPa to 66 kPa considerable reductions is recorded in seed germination and % inhibition (Fig. 1). Radicle elongation is significantly reduced in 33 to 66 kPa mannitol. It is also evident from the Table 1.

Table 1. Effect of osmotic stress and weed extract on seed germination and seedling growth of wheat.

Treatments	Final germination	Speed of germination	Radicle elongation (cm)	% Inhibition radicle elongation
Mannitol				
Control	100 ± 0	86.3	11.65 ± 0.51a	0
22 kPa	94 ± 4	74.5	11.65 ± 0.51a	0
33 kPa	86 ± 6	73.3	10.52 ± 0.27b	9.69
44 kPa	88 ± 5.83	77.6	8.68 ± 0.27b	25.4
66 kPa	94 ± 4	70	8.21 ± 0.55b	29.52
Chenopodium album extract				
Control	94 ± 2.44	86.6	12.02 ± 0.71a	0
21 kPa (1%)	48 ± 15.93	49	1.90 ± 0.54b	84.2
32 kPa (3%)	56 ± 10.77	36	1.16 ± 0.13b	90.35
64 kPa (5%)	44 ± 15.03	33	0.79 ± 0.23b	93.43

Table 2. Effect of osmotic stress and weed extract on seed germination and seedling growth of gram.

Treatments	Final germination	Speed of germination	Radicle elongation (cm)	% Inhibition radicle elongation
Mannitol				
Control	100 ± 0	70.6	10.48 ± 0.33a	0
22 kPa	96 ± 2.4	60	9.3 ± 0.32b	11.25
33 kPa	90 ± 6.3	60	9.24 ± 0.38b	12.21
44 kPa	92 ± 3.7	58	8.68 ± 0.31bc	17.17
66 kPa	88 ± 5.8	50	7.84 ± 0.53c	27.67
<i>Chenopodium album</i> extract				
Control	84 ± 6.78	58	9.06 ± 0.72a	0
21 kPa (1%)	64 ± 5.09	37	3.06 ± 0.35b	66.3
32 kPa (3%)	56 ± 8.71	31	1.91 ± 0.21bc	79.1
64 kPa (5%)	50 ± 3.16	8.5	1.57 ± 0.19c	87.7

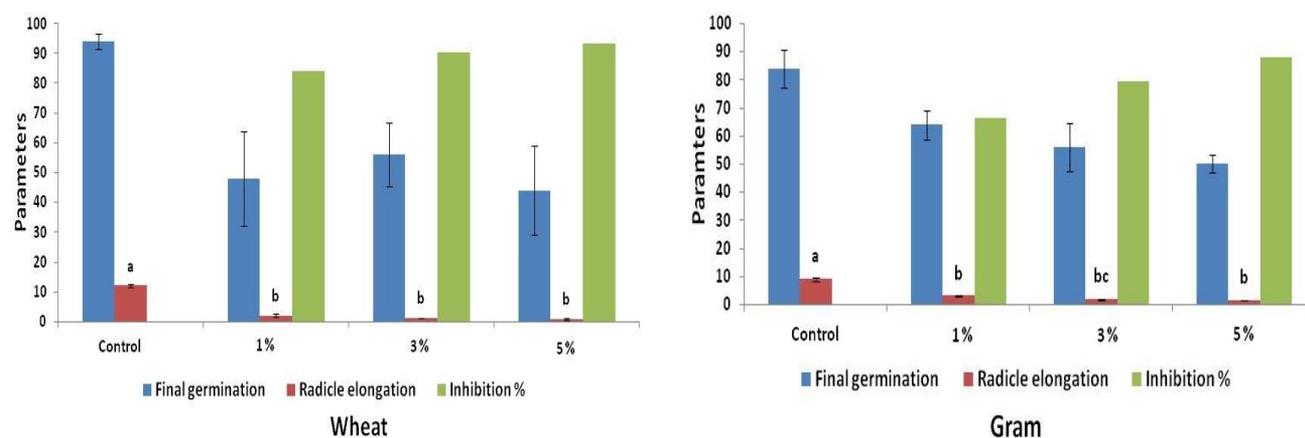
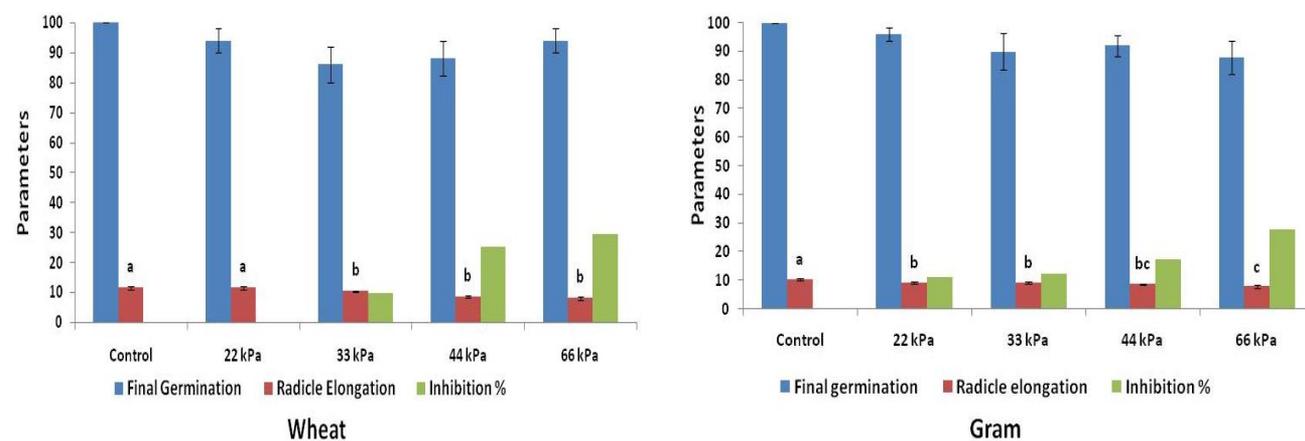


Fig. 1. Effect of osmotic potential on germination, radicle elongation and inhibition % in radicle elongation of wheat and gram

Fig. 2. Effect of different concentration of aqueous extract of *Chenopodium album* whole plant of wheat and gram on germination, root length and % inhibition in root length.

That effect of osmotic potential from 33 kPa to 66 kPa is same and not significantly different in radicle elongation. In wheat, the effect of different concentrations of *Chenopodium album* plant extract on germination, radicle elongation and % inhibition of test species was observed. It is evident that higher reduction in germination, speed of germination, radicle elongation and % inhibition were recorded even in to 1% weed extract which is equivalent to only 22kPa. In 5% solutions (66kPa) of mannitol and plant extract, pronounced reduction in germination (94 to 44), speed of germination (70 to 33), radicle elongation (8.21 to

0.79) and % inhibition (30 to 93) were recorded in plant extract. It is apparent that “Wheat” is highly sensitive to *Chenopodium album*.

In Table 2, gram did not show sensitivity to germination in all ranges of osmotic potential. This showed higher the osmotic potential, higher the reduction in germination. Effect on germination, speed of germination, radicle growth and % inhibition of test species using control (0.0 kPa) and different concentrations of mannitol solutions. However gram also showed the similar trend like wheat. These results are highly significant if compared with control (Fig. 2).

Both (Table 2) showed significant effect of osmotic potential and plant extract. However plant extract effects (allelopathic) are stronger than osmotic effect. If 5% concentration solution of mannitol (66Kpa) and plant extract is considered 8 to 2cm in radicle growth and 28 to 88% inhibition were recorded in plant extract.

Germination and early seedling growth is the most critical phase in the establishment of plant species which is influenced by a number of factors under temperature, water stress, light, etc (Hampson and Simpson. 1990). Different osmotic pressures due to mannitol solution were used in comparison with weed (*Chenopodium album*) extract for different growth parameters of two test species (wheat and gram). Osmotic pressure solutions due to mannitol only slightly effected the percent germination in two test species. These results were consistent with Gharoobi *et al.*, (2012), who showed that germination of corn, barely and canola was not affected by osmotic potential. Smith *et al.*, (1989) also reported that germination in wheat and other crop decreased with increasing moisture tension.

In our study radicle lengths were significantly reduced throughout in different levels of mannitol solution. Similar results were reported by Khan (1992) that osmotic pressure should be so adjusted to allow all the biochemical and physiological process of germination to take place but to inhibit the cellular development and consequently root emergence even after weeks of contact between the seed and the osmotic solution. Similar results were found by Serraj and Sinclair (2002). Osmotic adjustment in all plant tissues result in water uptake and it maintain the cell turgor pressure and hence influence their physiological processes such as stomatal regulation, photosynthesis and cell expansion etc. In whole studies highest levels of osmotic potential have negatively affected the radicle length of test species. These results are more or less similar with Chaves *et al.*, (2003) who found the reaction of the plant to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development. Bell (1974) reported that the response of seeds or seedling to plant extracts is due entirely to allelopathy however the possibly exists that the extracts may also exert negative osmotic effects on the test species. In present studies wheat results at higher osmotic concentration (66Kpa) are in line with Akber *et al.*, (2009) who reported that the plumule and radicle growth reduced with highest level (16gL⁻¹) of PEG osmoticum. Similar results were reported by Redhouane (2007) who stated that pearl millet seed exposure to osmotic stress affect germination as well as root and shoot length. The result of this study is similar to Neto *et al.*, (2004) reported interaction between osmotic treatment (Mannitol concentrations) and Soyabean. Germination was not reported to decrease but hypocotyl and root length gradually reduced in other treatments. Osmotic stress on the gram radicle growth in higher concentration of mannitol. Similar results of Pratap & Sharma (2010) who reported inhibition of gram seedling in PEG. The inhibition of germination and seedling growth in weed extracts may presumably due to the

presence of diverse allelochemicals in the extract. Many scientists have suggested phenolics as the main cause of inhibition of metabolic process during germination (Williams & Hoagland, 1982; Kuiters, 1989).

Conclusion

Present studies show that osmotic pressure inhibit germination and radicle growth in higher concentration but *C. album* inhibit both in low concentration, therefore, it may concluded that inhibition by this weed is due to the allelopathy. It is also recommended that during allelopathic investigations osmotic pressure should be included for better conclusion.

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