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FACTORS INFLUENCING SEED GERMINATION OF WILD ONION AND CHICKPEA CULTIVARS

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Abstract

Wild onion (*Asphodelus tenuifolius* Cav.) is a major weed of chickpea in the sandy tract of Pakistan and elsewhere in the world, but its biology and physiology is less understood. Seeds of its four biotypes were collected from Bannu, Karak, Bhakkar and Mianwali districts of Pakistan for a series of laboratory experiments. Seeds were treated with a range of concentrations of gibberellic acid, potassium nitrate, thiourea or sodium azide and incubated at three temperatures 10, 20 and 30°C. The experiment was repeated once and was laid out in Completely Randomized design with factorial arrangement. Biotypes differed in dormancy status. Seeds of Mianwali biotype had the highest germination (74.5%) while Bhakkar (44.8%) and Karak (44.6%) biotypes produced lower germination at 10 and 20°C. Very little germination occurred at 30°C. In the second experiment the tolerance of five chickpea cultivars to Sodium azide was probed by treating their seeds with Sodium azide at 0, 0.76, 1.53, 2.30 and 3.07 mM for comparison. All chickpea cultivars showed 100% germination at all concentrations except 3.07 mM where the two cultivars KK-1 and KC-98 had 95% germination. While *A.tenuifolius* produced very little or no germination at all concentrations except 0 and 0.76 mM. Thus, to minimize the competition of wild onion in chickpea, sodium azide could be selectively applied to inhibit germination of wild onion in chickpea.

Introduction

Chickpea is the principal pulse and provides a major source of protein in the diet of the predominantly vegetarian population in Indo-Pakistan. While, wild onion (*A. tenuifolius* Cavi., Family *Asphodelaceae)* is a serious weed of 15 crops in 17 countries (Holm *et al.,* 1997). It is a notorious annual weed of sandy soils of Indo-Pak sub-continent (Mishra *et al.,* 2006). It has been observed as a serious weed of wheat (*Triticum aestivum* L.), mustard (*Brassica juncea* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medic.), and linseed (*Linum usitatissimum* L.) [Gupta *et al*., 1977; Poonia *et al.,* 2001; Tiwari *et al*., 2001]. Similarly it has been found to be the worst weed in chickpea (Hassan *et al.,* 2004; Hassan & Khan, 2005; Sultan & Nasir, 2003).

There are several features, which have rendered this weed species successful but the most important one is the seed dormancy, which enables the seeds to persist in the soil and survive under unfavourable conditions (Karssen, 1982; Harper, 1977; Holt, 1987; Taylorson, 1970). Benvenuti & Macchia (1995) showed that high CO_2 and low O_2 (hypoxia) induced dormancy in different weed species while Taylorson (1980/81) reported otherwise.

Tiwari *et al*., (2001) observed 80% yield reduction due to *A. tenuifolius* and Yaduraju *et al*., (2000) reported 56% losses in chickpea. Apart from competition this weed has the allelopathic potential to suppress the germination and growth of wheat, mustard, chickpea, and lentil (Mishra *et al.,* 2002). Sahi & Bhan (1991) reported that *A. tenuifolius* seeds can germinate in a range of temperatures from 10 to 35°C, with an optimum at 15°C. *A. tenuifolius* is a greater problem in chickpea as compared to wheat in the sandy districts of Pakistan. It is speculated that due to thermo-sensitive nature of the weed, it is more abundant in chickpea because the planting time of chickpea is during late September when the temperatures are higher enough for germination of wild onion while wheat planting is done in late October, the lower ambient temperatures at that time are conducive to the germination of *A. tenuifolius* which is uprooted during land preparation at the planting time. In order to understand the effect of temperature and germination promoters, seeds of *A. tenuifolius* and chickpea were exposed to various temperatures and germination promoters to investigate the most favourable temperature and effective chemical at different concentrations. Investigating the germination requirement of temperature and chemicals will enable us to formulate a package for its control. Chickpea cultivars were studied with various environmental concerns to know about the dormancy behaviour (Singh *et al*., 1987). There are various cultivars of chickpea cultivars which have different dormancy behaviour. . Dasht originated from a cross between C 44 and ICC 7770. C 44 is a local genotype well adapted to chickpea growing area of Punjab, whereas ICC 7770 is an *Ascochyta* blight resistant line obtained from ICRISAT, India (Ahmad *et al.,* 2005).

Material and Methods

Seed collection: In first experiment, the *A. tenuifolius* seeds were collected during chickpea harvesting in June 2005 from Bannu (32° N and 70° E), Karak (33° N and 71° E)] from Khyber Pakhtunkhwa and Bhakkar (31° N and 71° E) and Mianwali (32° N and 71° E) from Punjab. *A. tenuifolious* is a serious problem in these districts of the two provinces. The average annual rainfall in these regions is 250-300 mm. The seeds were cleaned and stored in paper bags at ambient temperature in the laboratory.

Chemicals treatments and temperature regimes: An experiment was conducted with the main plot being temperature regimes of 10°C, 20°C and 30°C. Subplots of Petri dishes containing 20 seeds on tissue paper in (CR) design with factorial arrangement with four biotypes and four chemicals (gibberellic acid (GA_3), potassium nitrate (KNO_3) thiourea (THU) and sodium azide (SA). Gibberellic acid was applied @ (0, 0.57, 1.15, 1.73 and 2.30 mM), potassium nitrate (0, 1.97, 3.95, 5.93 and 7.91 mM), sodium azide (0, 3.07, 6.15, 9.22 and 12.30 mM) and thiourea (0, 2.62, 5.25, 7.88 and 10.50 mM). Individual

Petri dishes were treated with 5 ml of solution on days 0, 3, 9 and then kept moist with distilled water throughout the experiment. Each treatment was replicated twice. Germination assays were performed over four weeks in a growth incubator (Model No.2020-2E, Shelab Manufacturing Inc., 300 N, and 26th Cornelius, OR 97113) with a 12 hr daily photoperiod.

In 2nd experiment, certified seeds of five chickpea cultivars viz., Sheenghar, Lawaghir, KK-1, KK-2 and KC-98 were collected from the Gram Research Station, Ahmad Wala, Karak Pakistan. These seeds were tested in comparison with the seed of *A. tenuifolius* biotypes of Karak and Bannu. The seed germination of both species was assayed by the procedure as outlined in the first experiment. Five seeds of each chickpea cultivar and *A. tenuifolus* biotypes were put for germination in Petri dishes. In comparison of seed germination of chickpea cultivars with *A. tenuifolius* the same protocol as highlighted in experiment 1 was followed except that only SA was applied at five concentration i.e. 0, 0.76, 1.53, 2.30 and 3.07 mM). In the previous experiments SA, was safe for chickpea and deleterious to *A. tenuifolious*, therefore this chemical was tested against chickpea. Both experiments were conducted under laboratory conditions at the Department of Weed Science, Khyber Pakhtunkhwa Agricultural University, Peshawar, Pakistan from July-October 2005 and were repeated under the same laboratory conditions during July-October 2006. Petri dishes were monitored twice a week for germinated seeds, which were then removed. Seeds were deemed to have germinated when two mm of radicle emerged.

Statistical model and data analysis: GENSTAT software was used for analysis of variance and the model used for analysis was completely randomized (CR) design with factorial arrangements and means were separated through Fisher's protected LSD test by using MSTATC. The mean data were then transferred to MS Excel for graphical presentations.

Results

In first experiment, germination of four biotypes of *A. tenuifolius* showed non significant differences during the course of experiment in both the runs i.e., July-Oct., 2005 and 2006 (Fig. 1). The data depicted that Mianwali biotype produced maximum (41.67 and 40.25%) germination in the either Run followed by Bannu biotypes and the remaining two biotypes produced minimum germination.

The germination of all *A. tenuifolius* biotypes (Bannu, Karak, Bhakkar and Mianwali) averaged across runs, chemicals and concentrations were differentially affected (Fig. 2) by temperature ($p<0.001$). The data indicated the nature of this interaction with significantly higher germination observed at 20°C than at 10°C for Mianwali and Bannu (88.4 and 66.0%), respectively. Mianwali biotype produced maximum germination among all the biotypes at all temperatures (51%). Minimum average germination was recorded for Karak (26%) while Bannu and Karak biotypes produced same germination. Higher temperature (30°C) produced minimum or almost no germination for all biotypes.

Analysis of variance of the data revealed that Gibberellic acid and its concentrations significantly $p<0.001$ affected the germination of the different biotypes (Fig. 3). The main effects of biotypes showed that maximum (52.0%) germination was recorded in Mianwali biotype followed by Bannu biotype, while minimum (25.25%) germination was produced by Karak biotype. However, it was statistically at par with Bhakkar biotype. Among the gibberellic acid concentration maximum (40.52%) germination was recorded at 0.57 mM. while minimum (34.16%) germination was recorded at 2.30 mM. However, it was statistically at par with 1.73 mM. In the interaction of biotypes and concentrations maximum (55.42%) germination was observed at 0.57 mM in Mianwali biotype. However, it was statistically at par with same biotype at 1.73 mM and 0 mM.

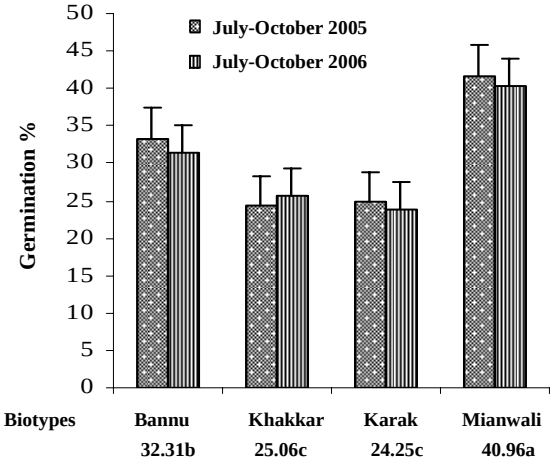

Fig. 1. Comparison of seed germination of *A. tenuifolius* biotypes.

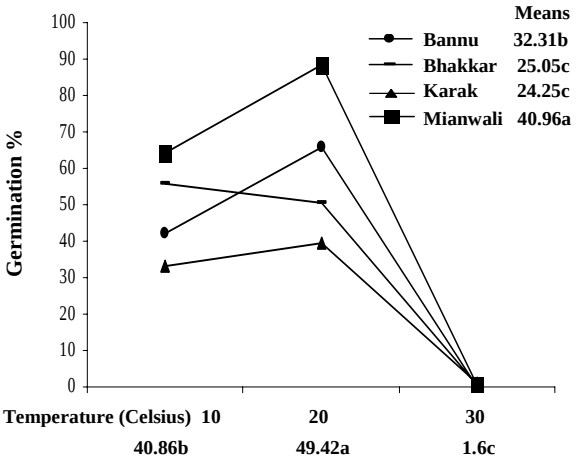

Fig. 2. Comparison of seed germination of biotypes at different temperature regimes.

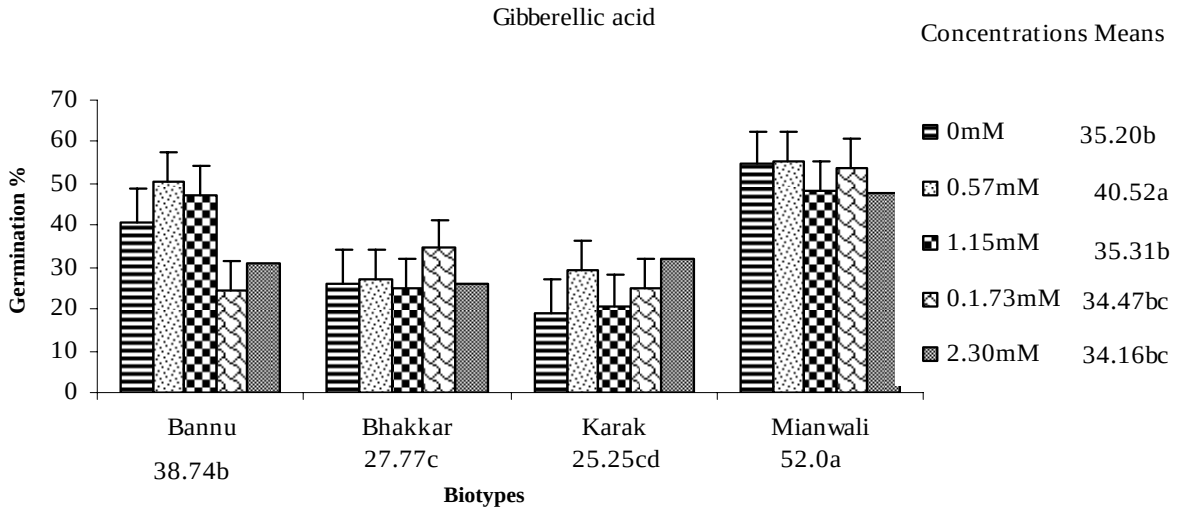

Fig. 3. Effect of different concentrations Gibberellic acid on the germination of biotypes.

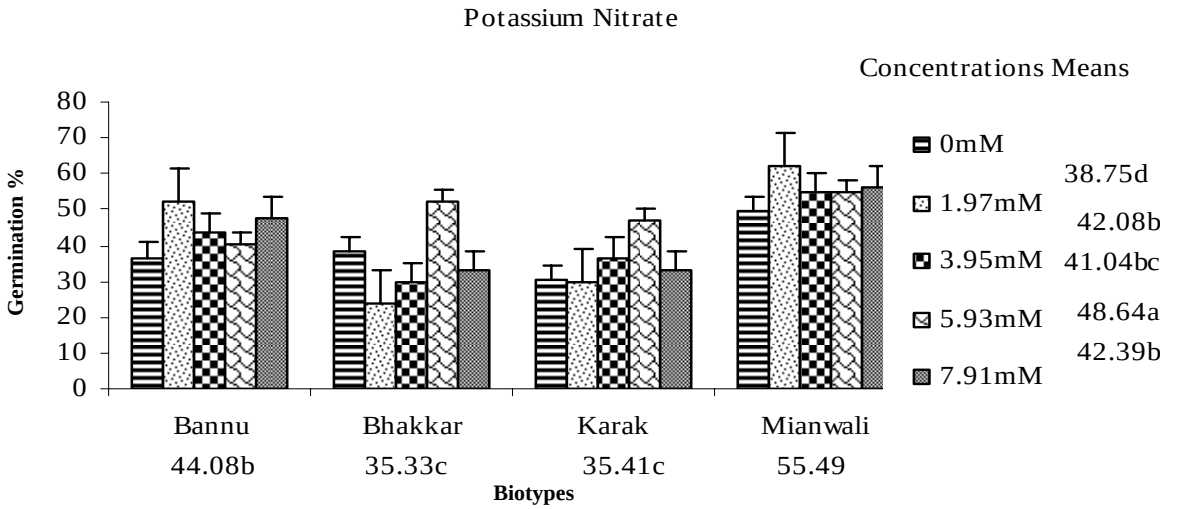

Fig. 4. Germination of biotypes as affected by Potassium nitrate acid and its concentrations.

Statistical analysis of the data revealed that Potassium nitrate and its concentrations significantly affected the germination of the different biotypes $p<0.001$ (Fig. 4). The main effects of biotypes showed that maximum (55.49%) germination was recorded in Mianwali biotype followed by Bannu biotype while minimum (35.41%) germination was produced by Karak biotype. However, it was statistically at par with Bhakkar biotype. Among the potassium nitrate concentration maximum (48.64%) germination was recorded at 5.93 mM while minimum (38.75%) germination was recorded at 0 mM. The remaining concentrations produced moderate germination. In the interaction of biotypes and concentrations maximum (62.08%) germination was observed at 1.97 mM in Mianwali biotype followed by the same biotype at 3.95 mM, 7.91 mM and 0 mM.

Biotypes germination was also significantly $p<0.001$ affected by Sodium azide and its concentration (Fig. 5). The main effects of biotypes showed that maximum (11.75%) germination was recorded in Mianwali biotype followed by Bhakkar biotype while minimum (5.58%) germination was produced by Karak biotype. Among the Sodium azide concentrations, maximum (2.29%) germination was recorded at 3.07 mM while the rest of the concentration produced very poor germination. In the interaction of biotypes and concentrations maximum (47.92%) germination was observed in Mianwali at 0 mM. All other biotypes produced significantly better germination at 0 mM, while very less or no germination at all concentrations of Sodium azide.

Thiourea and its concentrations also significantly $p<0.001$ affected the germination of different biotypes (Fig. 6). The main effects of biotypes showed that maximum (44.58%) germination was recorded in Mianwali biotype followed by Bannu biotype, while minimum (28.51%) germination was produced by Bhakkar biotype. However, it was statistically at par with Karak biotype. Among the Thiourea concentration, maximum (41.45%) germination was recorded at 10.50 mM while minimum (26.56%) germination was recorded at 2.62 mM. The remaining concentrations produced moderate germination. In the interaction of biotypes and concentrations maximum (49.58%) germination was observed at 10.50 mM in Mianwali biotype followed by the same biotype at all concentration except 2.62 mM.

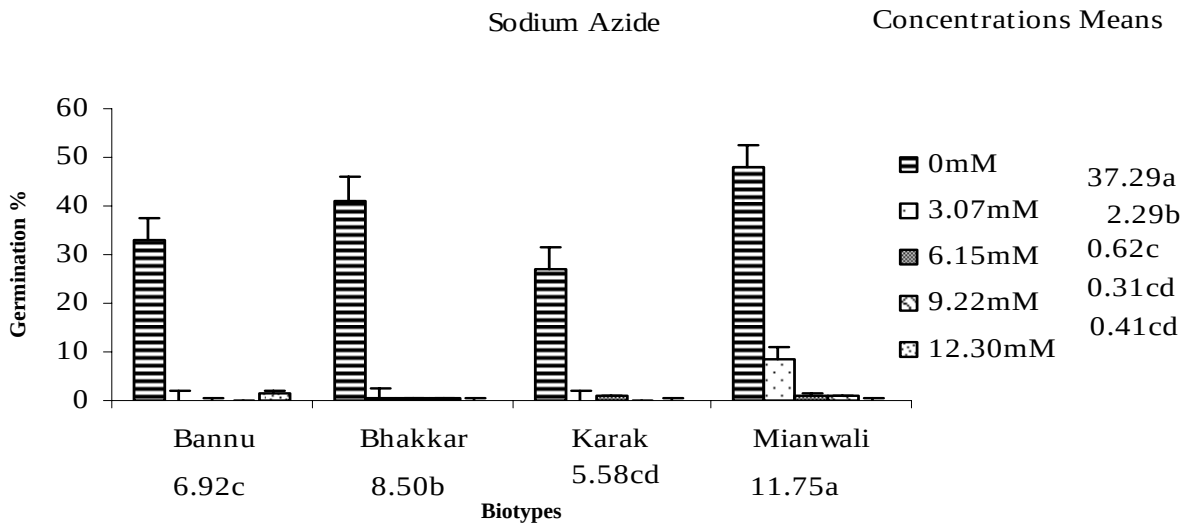

Fig. 5. Germination of biotypes as affected by Sodium azide acid and its concentrations.

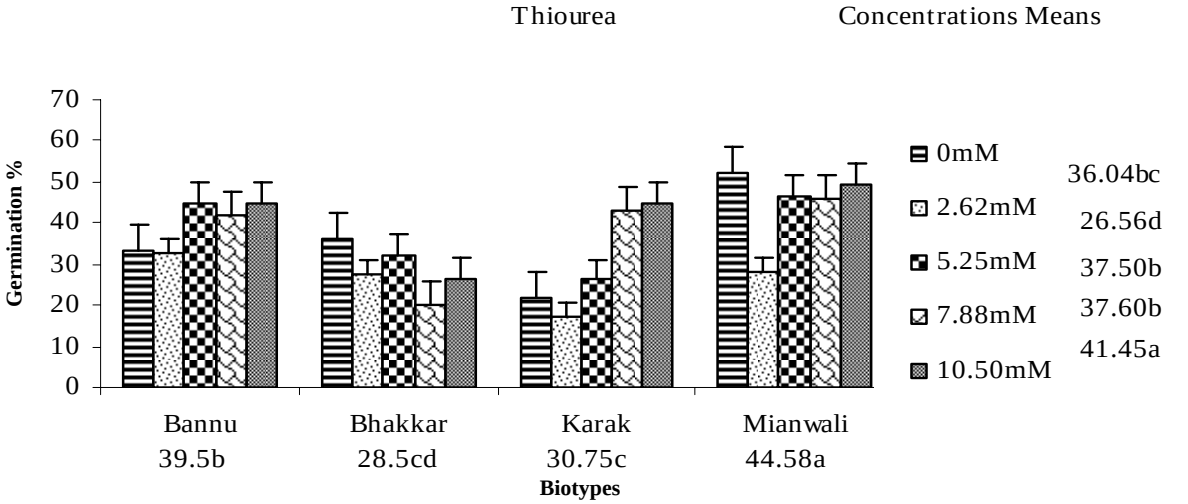

Fig. 6. Germination of biotypes as affected by thiourea acid and its concentrations.

In 2nd experiment Chickpea cultivars showed non significant differences to Sodium azide concentrations. The data (Fig. 7) indicated that 100% germination was recorded at all concentration except 3.07 mM when applied to Lawaghir and KK-1 (99% germination each) however these were statistically similar to other concentrations.

Sodium azide concentration affected the germination *A. tenuifolius* significantly ($p<0.001$) as shown in Fig. 8. The data indicated 3.07 mM of Sodium azide inhibited germination of both the biotypes tested by 100% followed by 150 1.53 mM at which Karak and Bannu biotypes germinated to the extent of 17.50 and 15% respectively. Control (0 mM) produced 100% germination followed by 65% in 50 mM and 60 of in 0.76 mM in both the biotypes.

Discussion

The study demonstrated that germination of *A. tenuifolius* biotypes was significantly affected by the temperature and dormancy breaking chemicals. Temperature being the most important factor in seed germination played a vital role in breaking seed dormancy. Intermediate temperature (20°C) was the optimum temperature for seed germination of two biotypes i.e., Mianwali and Karak; the same biotypes also produced comparatively higher germination at 10°C tested in this experiment. Little or no germination was recorded at 30°C, suggesting that the thermal optimum for *A. tenuifolius* had been exceeded at this temperature. Gorai *et al*., (2006) reported that exceeding the thermal optimum can either inhibit germination or may cause irreversible seed damage. These results provide the evidence for farmers to grow chickpea crop when the temperature falls below 20°C to minimize the competition of *A. tenuifolius.* However, it is cautioned that availability of moisture and lower chickpea yield due to delayed planting may be kept into focus. On the other hand, the growth regulators are also very important for breaking dormancy and we found that KNO_3 significantly promoted germination. Ecological difference among biotypes was also found. Mianwali and Bannu biotypes produced higher while Bhakkar and Karak biotypes produced lesser germination which is the additional management strategy for *A. tenuifolius* due to ecological differences in the germinability of the biotypes. Wild onion achieved 60 to 100% of its maximum vegetative growth at temperatures ranging from 18/11 to 30/23°C day/night (Patterson, 1996). The greatest biomass was produced at day temperatures of 18 or 24°C and night temperatures of 11 or 17°C.

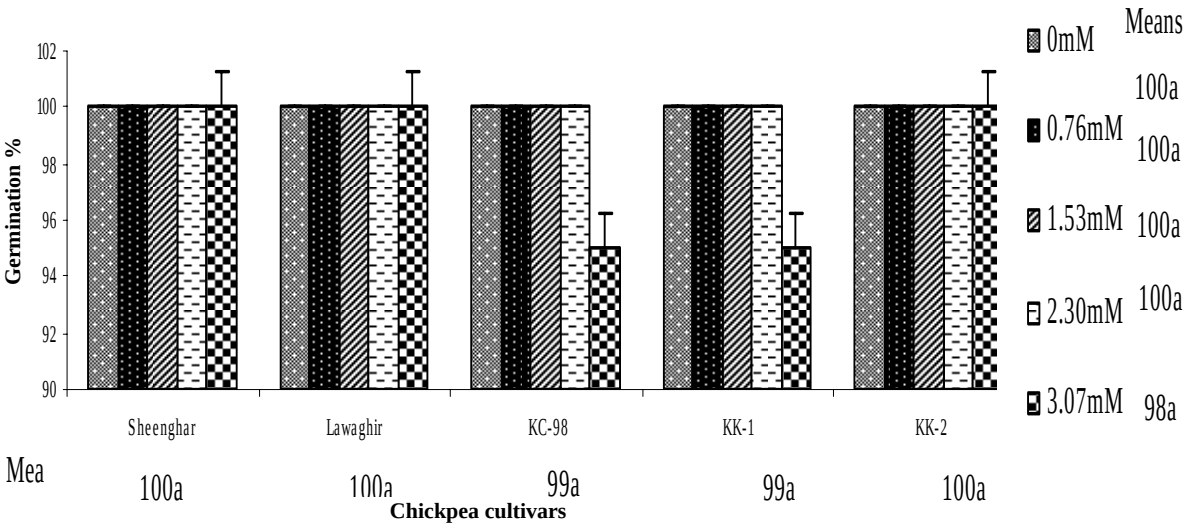

Fig. 7. Germination of chickpea cultivars treated with Sodium azide at different concentrations.

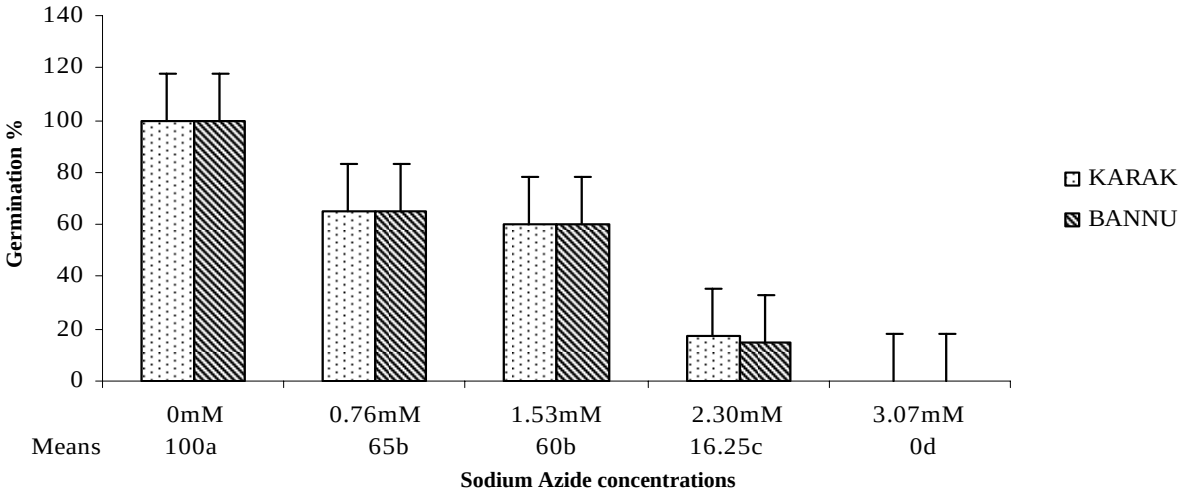

Fig. 8. *A. tenuifolius* biotypes seed germination as affected by Sodium azide concentrations.

Sahi & Bhan (1991) reported that temperatures ranging from 10-35°C favour germination of *A. tenuifolius* with maximum germination at 15°C (Hassan *et al;* 2004). Hassan & Khan (2005) investigated that GA_3 and KNO_3 break dormancy in many weed species. Mishra *et al.* (2002), Poonia *et al.* (2001), Yadav & Poonia (2005) and Yaduraju *et al.* (2000) also concluded that temperature is the most important parameter affecting germination. Bennvenuti & Macchia (1995), Karssen (1982), Harper (1977) and Holt (1987) claimed that temperature and growth regulators were important factors in seed germination. However, ecological differences among biotypes are also very important for the management of wild onion (*A. tenuifolius*). The instant results show that early sowing of chickpea or late sowing of wheat in rainfed areas in conjunction with other weed control methods could suppress this weed. In addition to these competitive cultivars, higher seed rates and rotation are also very important for the management of *A. tenuifolius* (Mishra *et al.*, 2002).

In experiment 2, all the chickpea cultivars germinated at all concentrations except 3.07 mM while, no germination was observed in *A. tenuifolius* biotypes at 3.07 mM of Sodium azide. The tested cultivars were found tolerant to all the concentrations of sodium azide except its maximum dose (3.07 mM). These studies provide the evidence that if Sodium azide applied to the field during seed bed preparation in will inhibit the germination of *A. tenuifolius* seeds. Further studies are suggested to investigate the feasibility of Sodium azide as a germination inhibitor of *A. tenuifolius* under the field conditions. Differential herbicides tolerance has been attributed to a differential uptake in wheat (DeFelipe *et al*., 1988), barely (Snipes *et al*., 1987) and other grasses (Derr *et al*., 1985). But such a tolerance even in other barley and wheat cultivars was assigned to rapid metabolism in tolerant cultivars (Fedtke & Schmidt 1988). Moreover, along with rapid metabolism, differential uptake and translocation was presented as the cause of tolerance in soybean (Connely *et al*., 1986). In many cases tolerance has been attributed to a varying target site (Stoltenberg *et al*., 1989).

Conclusions

The response of seed germination of *A. tenuifolius* biotypes to various temperatures and growth regulators

were differential. Mianwali biotype produced the best germination with Potassium nitrate at 20°C as compared to the rest of the biotypes. Overall 5.93 mM rate of Potassium nitrate was the most germinable concentration. Temperature (15-20°C) is the most favourable for the germination of *A. tenuifolius*. Among the chemicals, KNO_3 was the most effective in inducing germination while sodium azide emerged as inhibitory to *A. tenuifolius* germination.

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