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STUDIES ON GERMINATION ECOLOGY AND SEEDLING CHARACTERISTICS OF *CLEOME VISCOSA* AS AFFECTED BY VARIOUS ENVIRONMENTAL FACTORS

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Abstract

Knowledge about weed seed germination is essential for its control. By knowing the weed response to various environmental factors are helpful for weed management without the use of herbicides. The aimed of present study was to know the impact of several environmental related factors on germination and seedlings characteristics of *Cleome viscosa* under laboratory conditions. The temperature suitable for germination of *C. viscosa* was 30°C where 81.25% seed germination occurred. However, no or little germination was observed at 15 and 20°C, respectively. The germination of *C. viscosa* improved significantly from 5-7 pH, thereafter a decline was observed at pH 8 and 9 and completely inhibited at pH 10. Seed germination was somewhat salinity tolerant and germination observed at 250 mM NaCl (23.75%) which is much high but it was vulnerable to osmotic potential which completely prevented at -1.0 MPa. Seedling emergence responds substantially to burial depth. The seedling emergence was highest (87.5%) when seed retained at the surface of the soil and reduced to 30% at 4 cm depth. The germination and seedlings traits i.e. time to start germination, mean germination time (*MGT*) and time taken to 50% germination (T_{50}) germination index (*GI*) as well as root and shoot length, fresh and dry weight were affected significantly with all the studied environmental factors. The results obtained in this study will be useful to develop a comprehensive management program to control *C. viscosa*.

Key words: Burial depths, Osmotic stress, Salinity, Temperature and Weed seed

Introduction

Cleome viscosa Linn is an annual weed, can get the height of one meter and belongs to capparidaceae family (Frans, 1986). It grows mainly in tropical and sub-tropical regions as well as in coastal dunes and rangelands. The flowering starts in 3 to 4 weeks after the emergence of seedlings and life span is 3 months (Gilbey, 1975). It has sticky leaf and pungent smell that's why it is safe from insect pest attack and animals avoid for grazing.

Germination is the strategic feature for the formation of weeds in agro-ecosystem (Khan *et al.,* 2019). Seed germination is very important among the most basic stages in plant advancement at which the weed can seek a biological corner (Kegode *et al*., 2010) and is interfered by different ecological components, for example temperature, light, pH, osmotic and salt stress (Kegode *et al*., 2010). Temperature is considered the most imperative ecological signal controlling germination, species circulation and biological cooperation of weeds. It is variable for species inside genera and may be different for genotypes in a species (Debeaujon *et al*., 2000). Temperature range between 10-50°C was observed for weed seed production of similar or different species (Malik *et al*., 2000; Masin *et al*., 2010). Many weeds are photoblastic during germination, some are non-photoblastic while germination of some weed seed is reduced under light (Bewley & Black, 1994). Water stress is a major natural impulse which can inhibit seed germination and development of weeds (Norsworthy & Oliveira, 2006). Salinity is another reason for delay in germination of weed seeds or decline the plant growth and productivity (Chartzoulakis & Klapaki, 2000). Salt stress reduces the capacity of seed to germinate through osmotic impact and exceeds the salt fixation sufficiently higher to a harmful level (Nemati *et al*., 2011). There are some salt tolerant weed species; however for the greater part of weeds seed affected by high salt stress that may hinder the capacity of seed to assimilate water for germination and seed unable to grow (Koger *et al*., 2004). The growth of weed seeds and its development relies on the salt concentration and most weed species can tolerate to high salinity level (Ungar, 1991; Sattar *et al*., 2010). Some species of weed can develop on specific soil pH and concern in altering this condition resulted in in considerable effort in controlling weeds (Naylor & Council, 2002). For example, *Spergula arvensis* weed develop at low pH, so that liming is a good means of controlling these weed and stimulates the development of crop on acidic soil (Fried *et al*., 2008). Pattern of weed growth can be affected by a huge range of soil pH tolerant weeds (Nakamura & Hossain, 2009; Nandula *et al*., 2006). Weed can bear the pH range around 5 and 10 in their environment (Chejara *et al*., 2008; Chachalis *et al*., 2008). The reproductive capacity of weeds varies between species and depends mainly on weed size, light and water demand (Harrison *et al*., 2007). Burial depth of seed also influenced the emergence and germination of seedlings (Koger *et al*., 2004) by affecting light, humidity and temperature (Javaid *et al*., 2014).

Limited work has been available on germination ecology and seedlings characteristics of *C. viscosa*. The trial was planned to estimate the influence of environmental factors (osmotic stress, temperature, salinity pH and burial depth) on seed germination and seedling characteristics of *C. viscosa*.

Material and Methods

Site and seed collection: Experiments were performed in Agronomy labatory, College of Agriculture, University of Sargodha, Pakistan (32.0 ^{o}N and 72.6 ^{o}E) during 2014. Mature seeds were collected during March 2013 from several fallow farms near the experimental site, Punjab, Pakistan. At the time of collection, seeds were collected by cutting the stem of plant about 10 cm length with spike. After that paper bags were used to transport the seeds into the laboratory, seeds were detached from the spike of the plant, cleaned and air-dried at room temperature for 7 days. Seeds were stored in glass bottle unit used in the experiments.

General germination test protocol: *Cleome viscosa* seeds were sterilized in 10 mL sodium hypochlorite (NaHCl) and washed by distilled water for 5 times. *Cleome viscosa* seeds were sown in 9 cm diameter petri plates lined with Whatman filter paper No.10. A 3 mL distilled water or treatment solution was applied to each petri plate. Para-film was used to seal the petri plates to prevent the loss of water. All experimental trials were carried out at 25°C during 24 hours (day and night) except for temperature experiment. When radicle attained 2 mm length, seeds were considered germinated. Germination was recorded daily for 15 days. However, in the experiment of seed burial depths, seedlings were said to be emerged after the cotyledon comes out on soil surface.

Impact of temperature: To ascertain the impact of temperature on *C. viscosa* germination, 20 weed seeds were placed in a petri dish on a filter paper uniformly. For keeping the seed moist, distilled water of 3 mL was added in petri dish and then retained in incubator at temperatures of 20, 25, 30 and 35°C for the period of 15 days.

Impact of pH: The impact of pH on *C. viscosa* germination was determined by buffer solutions of 5, 6, 7, 8, 9 and 10 pH which was developed according to protocol defined by Chachalis & Reddy (2000).

Impact of salt stress: To ascertain the influence of salt stress on germination of *C. viscosa* seeds, 20 seeds were placed in the sealed petri dishes having NaCl solution of 0, 50, 100, 150, 200, 250, and 300 mM. However, distilled water was served as a control treatment.

Impact of osmotic stress: *Cleome viscosa* seeds were placed in petri plates having osmotic potential of 0, -0.2, -0.4, -0.6, -0.8 and -1.0 MPa. Osmotic potentials were developed by using polyethylene glycol (PEG 6000; Sigma-Aldrich Co., 3050, Spruce St., MO 63130) in distilled water. The equation described by Michel and Kaufmann (1973) was used for water potential calculation from known concentration of PEG 6000. Distilled water was used as the control treatment.

$$\text{Water potential} = -(1.18 \times 10^{-2})\, C - (1.18 \times 10^{-4})\, C^2 + (2.67 \times 10^{-4})\, 18\, CT + (8.39 \times 10^{-7})\, C^2 T.$$

where: T represents the temperature in centigrade while C is the PEG concentration.

Impact of seed burial depth: Impact of burial depth on seed emergence was determined in green house at College of Agriculture, University of Sargodha, Pakistan. A 20 seeds of *C. viscosa* were placed on surface of the soil and burried under soil (30% silt, 30% clay and 40% sand) at the depths of 0, 1, 2, 3, 4, 5, 6 and 7 in 15 cm diameter of plastic pots. In the entire experiment temperature of 25 ± 2°C was maintained the green house during the day and night. Water was applied to pots when required to maintained sufficient soil moisture. Seedlings were said to be emerged when cotyledon became visible at the soil surface.

Non-linear regression analysis was applied for data collected for germination or emergence percentage, osmotic stress, NaCl concentration or burial depths. A 3-parameter logistic model was applied to osmotic stress salt experiments. The fitted model was:

$$G(\%) = \frac{G_{\max}}{1+\left(\frac{x}{x_{50}}\right)^{g}} \qquad (1)$$

where G is total germination (%) at concentration x, x_{50} is NaCl concentration for 50% suppression of the highest germination and g denotes the slope and G_{max} is the maximum germination (%).

A 3-parameter logistic model:

$$E(\%) = \frac{E_{\max}}{1+\left(\frac{x}{x_{50}}\right)^{e}} \qquad (2)$$

was fixed to the seedling emergence (%) gained at various burial depths of 0-7 cm, where E is seedling emergence (%) at burial depth x, x_{50} is burial depth for 50% supression of the highest seedling emergence and e denotes the slope, E_{max} is the maximum seedling emergence (%).

Time taken to 50% germination or emergence was estimated by using a formula given by Coolbear *et al.* (1984):

$$T_{50}\, or\, E_{50} = t_i \frac{(\frac{N}{2} - n_i)(t_j - t_i)}{(n_j - n_i)} \qquad (3)$$

where N = final germinated or emerged seed. A n_j and n_i are the total germinated seed at times t_j (day) and t_i (day), respectively, when $n_i < N/2 < n_j$.

Mean germination or emergence time was estimated by equation of Ellis and Robert (1981):

$$MGTorMET = \frac{\sum Dn}{\sum n} \qquad (4)$$

where n = germinated seeds on day D

The germination or emergence index (*GI* or *EI*) was determined as described by the formula (Association of Official Seed Analysis, 1993):

$$GI \text{ or } EI = \frac{No\ of\ germinated \text{ or } emerged\ seedlings}{Days\ of\ first\ count} + \cdots + \frac{No\ of\ gerimnated \text{ or } emerged\ seedlings}{Days\ of\ final\ count} \quad (5)$$

Data regarding root and shoot length (cm), fresh and dry weight of root, shoot (g) of *C. viscosa* was collected by using standard procedures.

Statistical analysis

All experiments were carried out in CRD and there were four replications for each treatment. All the experiments were repeated twice. The collected data were subjected to one-way ANOVA. Treatment means were compared by LSD test at 5% probability level (Steel *et al.*, 1997). The graphical representation of the data was done by using Sigma Plot 2008 (11.0 Version).

Results

Impact of temperature: All tested temperatures significantly influenced the germination (%) of *C. viscosa* (Fig. 1). The germination of *C. viscosa* increased gradually when temperature rose from 20 to 30°C and highest germination (81.25%) of *C. viscosa* was observed at 30°C then declined to 46.25% at 35°C. However, no germination was occurred at 15°C and optimum temperature for germination was 30°C. The time to start germination of *C. viscosa* was significantly longer (4.5 days) at 20°C and least (3 days) at 30°C (Table 1). Shortest time (4.3 days) to achieve the 50% germination of the maximum (T_{50}) was taken at 30°C. However, mean germination time (*MGT*) and germination index (*GI*) were maximum at 30°C. The *MGT* increases and *GI* decreases at below or above 30°C (Table 1). The seedlings characteristics of *C. viscosa* were significantly influenced by all tested temperatures (Table 2). The maximum root and shoot length of *C. viscosa* were 2.57 and 2.12 cm, respectively at 30°C which was followed by 35°C (Table 2). At 20°C, the lowest shoot and root length was observed. In case of fresh and dry weight of *C. viscosa*, 30°C found to be the best to obtained higher values 1.19 and 0.24 g, respectively of these traits and lowest fresh and dry weight were produced at 20°C (Table 2).

Impact of pH: The germination of *C. viscosa* showed substantial response to various levels of pH buffer solution as shown in Fig. 2. At pH 7 the germination of *C. viscosa* was observed highest (78.75%) as compared to other applied levels of pH. The figure 2 also indicated that germination of *C. viscosa* improved significantly from 5-7 pH, thereafter a decline was observed at 8 and 9 and completely inhibited at pH 10. The shortest time to start germination and T_{50} were 2.5 and 4 days, respectively at pH 7 and statistically similar results were observed from pH 5-8 for both parameters (Table 1). However, the minimum (4.7 days) and maximum (8.4 days) *MGT* was recorded at pH 7 and 9, respectively and *GI* was observed highest at pH 7 over all other tested levels of pH buffer solution. In term of seedlings characteristics, the highest root (2.02 cm) and shoot (2.07 cm) length of *C. viscosa* was observed under the pH 7 as compared to all other pH levels (Table 2). However, fresh and dry weight of *C. viscosa* was found to be non-significant. Increase in pH from 5-9 did not affect the fresh and dry weight of *C. viscosa* (Table 2).

Impact of NaCl: A 3-parameter logistic model (G {%} = 79.8 [1 + $\{x/-158.5\}^{3.2}$], R^2 = 0.95) was fitted to ascertain the influence of NaCl concentration on germination data of *C. viscosa* (Fig. 3). The model revealed that germination reduced significantly when concentration of NaCl was increased from 0 to 250 mM. The highest (82.5%) *C. viscosa* seed germination was recorded where no salt stress was applied. The seed of *C. viscosa* was tolerated up to 100 mM NaCl and germination was 73.75 and 61.25% at 50 and 100 mM NaCl, respectively. Some germination (23.75%) was recorded even at 250 mM NaCl however, no germination was occured at 300 mM NaCl. The model indicated that 50% germination of the maximum was at 158.5 mM NaCl concentration. Compared to control, time to onset germination of *C. viscosa* was delayed approximately to 1 day at 50 mM NaCl and 100 and almost to 2 days at 150, 200, 250 mM NaCl (Table 1). The T_{50} and *MGT*of *C. viscosa* seed were decreased significantly under control (distilled water) treatment than each NaCl concentration that indicating fewer germination of *C. viscosa* under higher levels of NaCl. The rate of germination, *GI* was also maximum (4.0) under control treatment and reduced linearly by rise in NaCl concentration (Table 1). The seedlings characteristics of *C. viscosa* such as length of root, shoot fresh and dry weight were highest where no salt stress was applied. A significant reduction in these traits was also noted by enhancing the salinity level (Table 2).

Impact of osmotic potential: Influence of various osmotic potentials on germination (%) of *C. viscosa* was assessed by 3-parameter logistic model (G {%} = 85.6 [1 + $\{x/-0.53\}^{4.3}$], R^2 = 0.95) (Fig. 4). The model showed, when osmotic potential varied from 0 to -0.8 MPa the germination of *C. viscosa* reduced markedly from 87.5 to 27.5% and inhibited completely when osmotic potential of -1.0 MPa was applied. The model also estimated that 50% inhibition of highest germination was recorded at -0.53 MPa osmotic potential of this weed (Fig. 4). Osmotic potential of 0.6 to -0.8MPa markedly enhanced the onset of time for germination (4.5-5.7 days) as compared to 0 to -0.4 MPa (2.2-3.7 days). The T_{50} and *MGT* of *C. viscosa* were postponed with increasing the osmotic potential (Table 1). Compared to control, T_{50} and *MGT* were delayed nearly to 4-5 days when osmotic potential from -0.2 to -0.8 MPa was applied (Table 1). The *GI* of *C. viscosa* was decline when osmotic potential enhanced from 0 to -0.8 MPa, while, highest *GI* (4.2) was noted under control treatment. A linear decrease in root and shoot length of *C. viscosa* was observed when osmotic potential increased from 0 to -0.8 MPa (Table 2). The highest root (1.84 cm) and shoot (2.0 cm) length was observed where no stress was applied. The fresh and dry weight of *C. viscosa* was highest in control treatment and lowest values of these characteristics were observed at -0.8 MPa (Table 2).

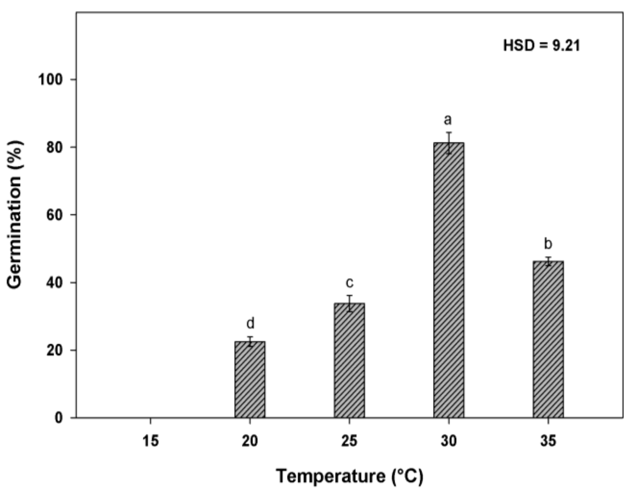

Fig. 1. Impact of temperature on germination of *Cleome viscosa*. Nail on the perpendicular bars symbolize the standard error of the means.

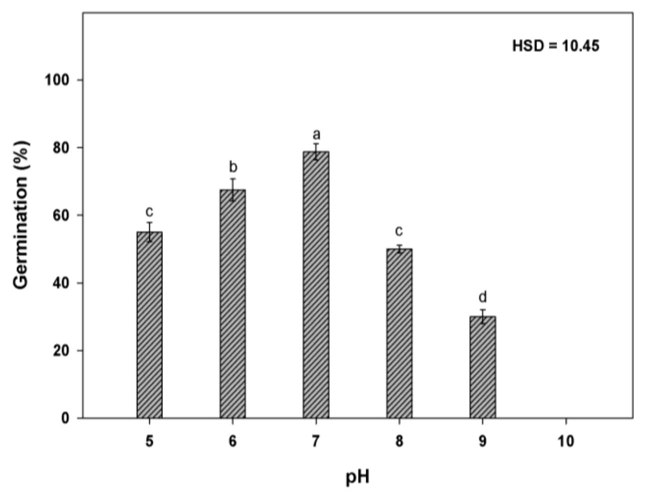

Fig. 2. Impact of pH on germination of *Cleome viscosa*. Nail on the perpendicular bars symbolize the standard error of the means.

Table 1. Impact of temperature, pH, NaCl concentration, osmotic potential and seed burial depths on germination parameters of *Cleome viscosa.*

Treatments		Time start to germination or emergence in days (SE)	T_{50} or E_{50} in days (SE)	MGTor MET in days (SE)	GI or EI (SE)
Temperature °C	15	NG	NG	NG	NG
	20	4.5^{a} (0.28)	5.7^{a} (0.14)	6.8^{a} (0.33)	0.7^{c}(0.04)
	25	4.2^{a} (0.25)	5.6^{a} (0.12)	6.5^{a} (0.16)	1.1^{c}(0.11)
	30	3^{b} (0.0)	4.3^{b} (0.12)	4.9^{b} (0.13)	3.5^{a}(0.18)
	35	3.7^{ab} (0.25)	5.6^{a} (0.29)	6.4^{a} (0.16)	1.5^{b} (0.03)
	HSD at 0.05	0.95	0.78	0.89	0.45
pH	5	3.5^{b} (0.28)	4.9^{b} (0.31)	5.6^{bc} (0.31)	2.1^{b}(0.24)
	6	2.7^{b}(0.25)	4.2^{b} (0.13)	5.0^{c} (0.11)	2.9^{a}(0.17)
	7	2.5^{b} (0.28)	4.0^{b} (0.39)	4.7^{c} (0.37)	3.7^{a}(0.28)
	8	3.5^{b} (0.28)	5.3^{b} (0.23)	6.4^{b} (0.21)	1.7^{b}(0.08)
	9	4.7^{a} (0.25)	8.2^{a} (0.82)	8.4^{a} (0.37)	0.8^{c} (0.08)
	10	NG	NG	NG	NG
	HSD at 0.05	1.19	1.96	1.29	0.82
NaCl concentration (mM)	Control (0)	2.5^{c} (0.28)	3.7^{d} (0.37)	4.5^{d} (0.29)	4.0^{a} (0.25)
	50	3.5^{bc} (0.28)	5.4^{cd} (0.28)	6.0^{c} (0.24)	2.6^{b} (0.12)
	100	3.7^{ab} (0.25)	6.9^{bc} (0.23)	7.3^{bc} (0.21)	1.8^{c} (0.11)
	150	4.5^{ab} (0.28)	7.5^{ab} (0.25)	8.1^{ab} (0.20)	1.3^{cd} (0.04)
	200	4.5^{ab} (0.28)	7.6^{ab} (0.23)	8.4^{ab} (0.19)	1.0^{de} (0.05)
	250	4.7^{a} (0.25)	9.3^{a} (0.79)	8.8^{a} (0.57)	0.6^{e} (0.07)
	300	NG	NG	NG	NG
	HSD at 0.05	1.24	1.86	1.41	0.57
Osmotic potential (MPa)	Control (0)	2.2^{d} (0.25)	3.9^{c} (0.12)	4.8^{d} (0.14)	4.2^{a} (0.27)
	-0.2	3.2^{cd} (0.25)	5.9^{b} (0.14)	6.4^{c} (0.10)	2.5^{b} (0.09)
	-0.4	3.7^{bc} (0.25)	6.0^{b} (0.14)	6.7^{c} (0.09)	2.0^{b} (0.07)
	-0.6	4.5^{b}(0.28)	7.0^{b} (0.22)	8.0^{b} (0.18)	1.3^{c} (0.11)
	-0.8	5.7^{a} (0.25)	8.8^{a} (0.55)	9.5^{a} (0.32)	0.6^{d} (0.05)
	-1.0	NG	NG	NG	NG
	HSD at 0.05	1.12	1.26	0.82	0.62
Seed burial depths (cm)	0	2.2^{c} (0.25)	3.5^{c} (0.18)	4.3^{d} (0.19)	4.6^{a} (0.30)
	1	2.7^{bc} (0.25)	4.4^{bc} (0.17)	5.4^{d} (0.19)	3.6^{b} (0.30)
	2	3.5^{b} (0.28)	5.5^{b}(0.18)	6.6^{c} (0.21)	2.2^{c} (0.12)
	3	4.7^{a} (0.25)	7.2^{a} (0.17)	7.8^{b} (0.28)	1.5^{cd} (0.04)
	4	5.5^{a} (0.28)	8.5^{a} (0.73)	9.1^{a} (0.39)	0.74^{d} (0.09)
	5	NE	NE	NE	NE
	6	NE	NE	NE	NE
	7	NE	NE	NE	NE
	HSD at 0.05	1.16	1.60	1.17	0.87

T_{50} or E_{50}, time to obtain 50% germination; MGT or MET, mean germination or emergence time; GI, germination index; EI, emergence index; NG or NE, no germination or emergence, NS, non-significant. The values within the column followed by different letters were significantly different at $p \leq 0.05$. LSD= Least significant difference. SE= Standard error of the means

Table 2. Impact of temperature, pH, NaCl concentration, osmotic potential and seed burial depths on seedling growth of *Cleome viscosa*.

Treatments		Root length (cm) (SE)	Shoot length (cm) (SE)	Fresh weight (g) (SE)	Dry weight (g) (SE)
Temperature °C	15	NG	NG	NG	NG
	20	1.37^{b} (0.03)	1.00^{b} (0.08)	0.99^{b}(0.04)	0.14^{b} (0.01)
	25	1.62^{b} (0.12)	1.19^{b} (0.06)	1.03^{b}(0.02)	0.17^{ab} (0.01)
	30	2.57^{a} (0.16)	2.12^{a} (0.25)	1.19^{a}(0.07)	0.24^{a} (0.02)
	35	1.82^{b} (0.17)	1.40^{b} (0.27)	1.07^{ab} (0.06)	0.19^{ab} (0.01)
	HSD at 0.05	0.60	0.58	0.12	0.06
pH	5	1.90^{ab} (0.10)	1.35^{b} (0.09)	0.82 (0.10)	0.21(0.05)
	6	1.37^{bc} (0.11)	1.75^{ab}(0.23)	0.77 (0.10)	0.20(0.06)
	7	2.02^{a} (0.10)	2.07^{a} (0.10)	1.00 (0.07)	0.28 (0.04)
	8	1.15^{c} (0.04)	0.87^{c} (0.02)	0.80 (0.04)	0.18 (0.00)
	9	0.95^{c} (0.09)	0.80^{c} (0.05)	0.62 (0.12)	0.12 (0.04)
	10	NG	NG	NG	NG
	HSD at 0.05	0.55	0.42	NS	NS
NaCl concentration (mM)	Control (0)	1.95^{a} (0.11)	1.75^{a} (0.22)	1.12^{a} (0.04)	0.28^{a} (0.02)
	50	1.60^{ab} (0.09)	1.52^{ab} (0.14)	1.07^{ab} (0.04)	0.25^{ab} (0.02)
	100	1.27^{bc} (0.10)	1.20^{bc} (0.04)	0.92^{bc} (0.04)	0.22^{abc} (0.01)
	150	1.07^{bc} (0.05)	0.95^{cd} (0.04)	0.84^{c} (0.02)	0.20^{abc} (0.00)
	200	0.95^{c} (0.04)	0.90^{cd} (0.02)	0.81^{c} (0.04)	0.19^{bc} (0.01)
	250	0.75^{c} (0.11)	0.77^{d} (0.12)	0.78^{c} (0.03)	0.14^{c} (0.00)
	300	NG	NG	NG	NG
	HSD at 0.05	0.56	0.41	0.19	0.08
Osmotic potential (MPa)	Control (0)	1.84^{a} (0.13)	2.00^{a} (0.02)	1.87^{a} (0.20)	0.79^{a} (0.04)
	-0.2	1.04^{b}(0.08)	1.38^{b} (0.03)	1.65^{ab} (0.11)	0.52^{b} (0.16)
	-0.4	1.00^{b}(0.04)	1.27^{b} (0.06)	1.37^{ab} (0.07)	0.44^{b} (0.05)
	-0.6	0.80^{c} (0.12)	1.02^{b} (0.20)	1.07^{b} (0.14)	0.31^{b} (0.23)
	-0.8	0.70^{c} (0.25)	0.97^{b} (0.24)	1.05^{b} (0.14)	0.17^{b} (0.19)
	-1.0	NG	NG	NG	NG
	HSD at 0.05	0.17	0.58	0.69	0.24
Seed burial depths (cm)	0	1.59^{a} (0.10)	1.70^{a}(0.16)	1.30 (0.07)	0.33^{a} (0.02)
	1	1.25^{ab} (0.09)	1.32^{ab} (0.04)	1.15 (0.02)	0.17^{ab} (0.00)
	2	1.05^{b} (0.15)	1.10^{b} (0.12)	1.00 (0.14)	0.15^{ab} (0.07)
	3	1.00^{b} (0.07)	0.87^{b} (0.00)	0.97 (0.07)	0.05^{b} (0.02)
	4	1.00^{b} (0.19)	0.85^{b} (0.03)	0.92 (0.09)	0.02^{b}(0.07)
	5	NE	NE	NE	NE
	6	NE	NE	NE	NE
	7	NE	NE	NE	NE
	HSD at 0.05	0. 42	0.57	NS	0. 22

T_{50} or E_{50}, time to obtain 50% germination; MGT or MET, mean germination or emergence time; GI, germination index; EI, emergence index; NG or NE, no germination or emergence, NS, non-significant. The values within the column followed by different letters were significantly different at $p \leq 0.05$. LSD= Least significant difference. SE= Standard error of the means.

Impact of seed burial depth: A 3-parameter logistic model ($E\{\%\} = 79.4\,[1 + \{x/-3.43\}^{5.5}]$, $R^2 = 0.95$) was used to know the effect of burial depth from 0 to 7 cm on emergence (%) of *C. viscosa* (Fig. 5). The model indicated that seeds of *C. viscosa* were sensitive to burial depth. The maximum seed emergence of *C. viscosa* was observed when seed placed at soil surface. The seed emergence of *C. viscosa* was decrease when burial depth extended from 0 to 4 cm and inhibited completely at 5, 6 and 7 cm. Emergence was highest (87.5%) when seed placed at soil surface and reduced progressively to 55 and 30% at 3 and 4 cm burial depths. The model also predicted that 50% emergence of the maximum emergence of *C. viscosa* was achieved at 3.43 cm seed burial depth (Fig. 5). Maximum time to start emergence (5.5 days) was recorded at 4 cm burial depth which was followed by 3 cm burial depth and this time was reduced by decreasing the seed burial depth up to 0 cm (Table 1). A similar trend was observed for 50% emergence time (E_{50}) and mean emergence time (*MET*). At 0 and 1 cm depth, E_{50} and *MET* were 3.5 and 4.3 days, respectively; whereas, by enhancing seed burial depth from 2 to 4 cm, the E_{50} and *MET* delayed approximately by 1 to 3 days. Emergence index was noted maximum (4.6) when the seed of *C. viscosa* was placed at soil surface (0 cm) and quick decline was observed at burial depth of 3 and 4 cm (Table 1). Seedling characteristics of *C. viscosa* illustrated in table 2 which indicated that root and shoot length of *C. viscosa* improved with decrease in the seed burial depth. A significant increase in the root (1.59 cm) and shoot (1.70 cm) length were observed when seed of *C. viscosa* grown on soil surface. *C. viscosa* fresh and dry weight was noted maximum at soil surface than all other burial depth (Table 2).

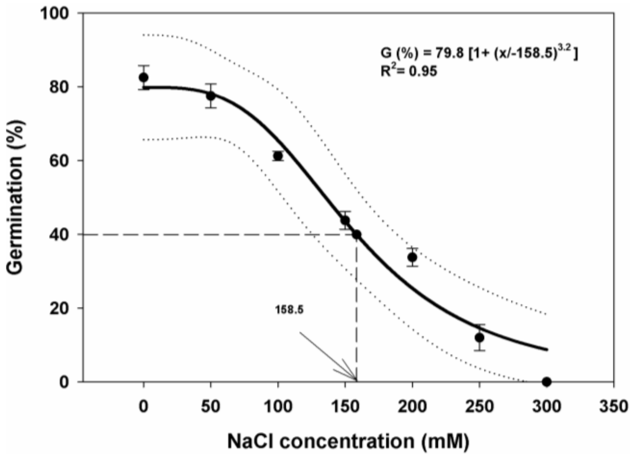

Fig. 3. Impact of NaCl concentration on seed germination of *Cleome viscosa*. Bold line represents a three-parameter logistic model {G (%) = G_{max} / [1 + (x/x^{50}) G_{rate}]} fitted to the data. Vertical dash line represents X-axis value at 50% of the maximum germination. Vertical bars represent ± standard error of the mean.

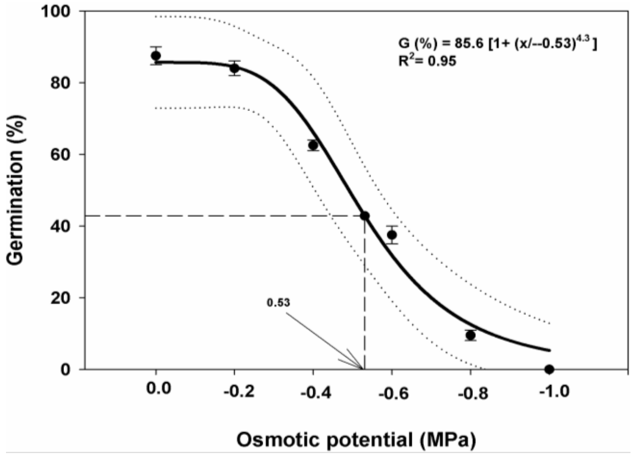

Fig. 4. Impact of osmotic potential on seed germination of *Cleome viscosa*. Bold line represents a three-parameter logistic model {G (%) = G_{max} / [1 + (x/x^{50}) G_{rate}]} fitted to the data. Vertical dash line represents X-axis value at 50% of the maximum germination. Vertical bars represent ± standard error of the mean.

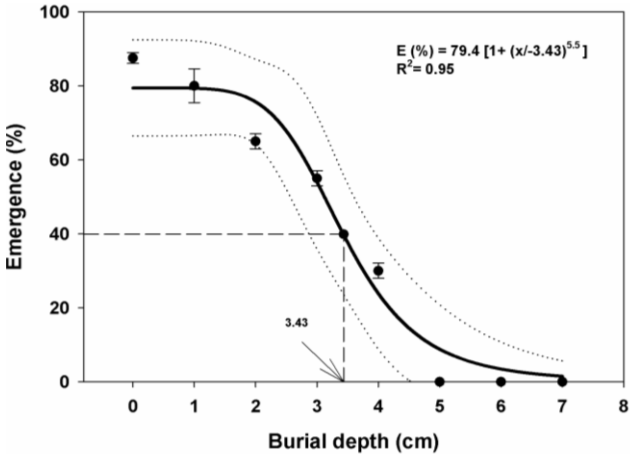

Fig. 5. Impact of seed burial depths on seed germination of *Cleome viscosa*. Bold line represents a three-parameter logistic model {E (%) = E_{max} / [1 + (x/x^{50}) E_{rate}]} fitted to the data. Vertical dash line represents X-axis value at 50% of the maximum germination. Vertical bars represent ± standard error of the mean.

Discussion

Cleome viscosa Linn. grows mainly in tropical, sub-tropical, coastal dunes and rangelands. It causes problem in many crops however, by knowing its germination ecology we can manage this weed in better way and reduce the crop yield losses. Our data indicated that weeds can germinate and emerge under different environments. The *C. viscosa* have highest germination at 30°C however, below or above this substantial germination was also observed along with germination traits (time to start germination/emergence, *T_{50} or E_{50}, MGT or MET and GI or EI*). These findings revealed that low temperature (< 20°C) affect the seed germination and seedlings traits of *C. viscosa* which may cause failure of *C. viscosa*. Our findings are supported by Benvenuti *et al.*, (2005) who revealed that seeds of *Cuscuta campastris* did not perform well at 10°C and maximum germination of seed was observed at 30°C. Kashmir *et al*., (2016) described that the maximum germination of *Avena fatua* and *Silybum marianum* Gaertn was recorded at a temperature of 25ºC. Benvenuti *et al*., (2004) also reported that 25°C was suitable temperature for maximum germination of *Leplochloa chinensis*. Red flower rag leaf weed is found in tropical and subtropical areas and can tolerate the temperature range of 10-30°C. Nakamura & Hossain (2009), Harrington (2009), Wei *et al*., (2009), Wing *et al*., (2009), Masin *et al*., (2010) also confirmed that up to 88% germination of different weeds under response of temperature. The seedlings root and shoot length, fresh and dry weight of *C. viscosa* was also recorded maximum at 30°C. The work of Abbas *et al.*, (2010) reported the increase in fresh weight of weed per unit area density of *Emex. australis*. Bebawi *et al*., (1984) described that weed dry weight differed significantly under different temperature ranges. Baskin and Baskin (1989) concluded that fresh and dry weight directly interlinked with biomass production at optimum ecology of specific weed. Maximum fresh and dry weight was recorded in henbit weed when all the requirements were ideal (Bewley *et al*., 1994). Chejara *et al*., (2008) found that optimum temperature resulted in increase in length of the shoot. Harrington (2009) studied that maximum shoot length of *Leptochloa chinensis* was accessed at 30ºC.

The *C. viscosa* can germinate under a range of soil pH (5-9), but pH 9 reduced its germination and seedlings characteristics significantly. The capability of this weed to tolerate a range of pH showed its adaptability under different soil conditions. Chachalis *et al*., (2008) and Wang *et al*., (2009) also have given the similar information about germination under different pH levels. Maximum germination of different weed species observed for pH 7, while minimum at pH 9. The work of the previous researcher indicated that pH range of 4-9 have no effect on weed species (Thomas *et al*., 2006; Chachalis *et al*., 2008; Wang *et al*., 2009) *Hibiscus trionum* (venice mallow) and coolatai grass (*Hyparrhenia hirta*) are also not effected under different pH levels (Chachalis *et al*., 2008; Chejara *et al*., 2008). Our findings are similar to Chejara *et al*., (2008) who reported that some species showed minute difference in length of root and shoot as well as fresh and dry weight over wide ranges of pH. If weed is not tolerant to pH, then its distribution is also affected which ultimately affects its root and shoot length (Nandula *et al.*, 2006).

Salinity imparts adverse effect on various physiological processes of plants. Our data indicated that *C. viscosa* showed tolerance to salt stress up to 250 mM NaCl concentration. However, the germination and seedlings traits of *C. viscosa* decrease with increase in salt concentration might be due to small quantity of water uptake and availability of appropriate environments for the entry of noxious ions in embryo. Weed germination and healthy seedlings establishment depends on concentration of salts (Ungar, 1991). The sodium ions replace the calcium and magnesium in anion conversation which change soil structure and fertility which cause water and nutrients stress (DiTommaso, 2004). Germination of *C. viscosa* under high salinity enables weed to grow in various types of soil. Rao *et al.*, (2008) also reported germination up to 80% under salt stress. So our results fall within the range found in literature. In accordance with our results, at higher NaCl concentration the seed of *Brassica tournefortii* Gouan and *Mimosa invisa* Mart. germinated (Chauhan *et al.*, 2006b; Chauhan & Johnson, 2008). Harrington (2009) described that increased dry weight of weed was observed by increasing NaCl concentration up to a specific level. Dry weight was statistically different in some weeds under different NaCl concentrations and more NaCl concentration resulted in lowest shoot length. Benvenuti *et al.*, (2003) reported that higher NaCl concentration resulted in lowest root length of *Hibiscus trionum*.

Stress of water is an imperative character that reduces the seed germination and seedlings characteristics of *C. viscosa*. These findings provide the information about relationship between rain and availability of water for germination and seedlings traits of this weed. Our results also revealed that both germination and seedlings parameters reduced by increasing the osmotic stress that may lead to weaker seedlings. According to Javaid & Tanveer (2014) *Emex australis* weed is very sensitive to osmotic stress and spread easily with the availability of water whereas, *E. spinosa* can tolerate the water stress to greater extent. Many weeds such as crafton weed (*Eupatorium adenophoum*); (Lu *et al.*, 2006), syndrella (*Syndrella nodiflora*); (Chauhan *et al.*, 2006b) and annual sowthistle (*Sonchus oleraceus*) and goat weed (*Scoparia dulcis* L.) (Jain & Singh, 1989) are very sensitive to osmotic stress. On the other hand, some species of weeds like venice mallow (*Hibiscus trionum*); (Chachalis *et al.*, 2008), trunnip weed (*Rapistrum rugosum*) (Chauhan *et al.*, 2006a) can tolerate well under low water potential. About 8 to 10% reduction in germination of *E. spinose* weed was observed when the osmotic stress was increased from 0 to -0.6 MPa (Javaid & Tanveer 2014). Chachalis and Reddy (2000) who described that dry weight (g m^{-2}) of weed significantly decreased by increasing osmotic stress at specific level and fresh and dry weight directly interlinked with biomass production at optimum ecology of specific weed. Maximum fresh and dry weight was recorded with distilled water when compared with osmotic stress treatments.

By increase in burial depth of seed beyond 0, seed germination as well as characteristics of *C. viscosa* was reduced. Seeding depth also suppresses the emergence of many weeds species (Koger *et al.*, 2004). Our data suggested that germination of *C. viscosa* was observed maximum at soil surface which showed that this weed can germinate easily under limited availability of water as documented above. Susko & Hussein (2008) and Rao *et al.*, (2008) also reported similar results under different seed depths. Our results also revealed that increase in the seed burial depth of *C. viscosa,* more time was required to start emergence, E_{50} and *MET* and reduce the *EI.* As deeper the seeds were sown of dove weed, E_{50} tended to decline and significantly reduced from 2 cm to 6 cm (Wilson *et al.*, 2006). Seed burial depth reduced the E_{50} and *MET* (Nakamura & Hossain, 2009). Maximum *MET* of *Fimbristylis miliacea* was investigated at 0.5 to 1 cm burial depth (Begum *et al.*, 2006). The *EI* and time to start emergence of weed seeds is affected by varying seeding depth (Boyd & Van Acker, 2003). The seedlings traits also deceased with increase in burial depth. These results are supported by the work of the previous researchers, who reported that increase in burial depth reduced emergence and seedling characteristics of many weeds (Benvenuti *et al.*, 2001; Benvenuti, 2003). Hoffman *et al.*, (1980) who described that weed dry weight significantly decreased as depth of seeding increased depending on size of seed. Hui *et al.*, (2009) concluded that fresh and dry weight depends on biological production of weed species and various burial depths.

Conclusion

Germination of *Cleome viscosa* is intensely affected by temperature and its optimum range was 30^{o}C. The measurements should be taken in summer when temperature is suitable for its germination. Seeds of *C. viscosa* germinate on the wide range of pH (5-9 pH, which represents most of the Pakistani soils) and indicated that pH have no significant effect on weed spread. It can tolerate to the wide range of salinity but it was profound to osmotic potential and totally prevented at -1.0 MPa. Seeds of *C. viscosa* produced highest germination when placed at soil surface instead of buried seeds. The germination and seedlings characteristics were reduced significantly with all the studied environmental factors. It becomes problematic and harmful weed under no-tillage conditions however, tilling the soil upto more depth could reduce the *C. viscosa* in the fields due to influence of burial depth on seed emergence.

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