Exercise 1

Artificial saline water and saline soil preparation (Soil salinity and plant tolerance)

Soils containing an excess concentration of soluble salts or exchangeable sodium in the root zone, it is called as salt-affected soils (Conway 2001; Denise 2003; Jim 2002). Salt-affected soils (Usara/ Kalar) can be broadly categorised into three types based on their salinity and sodicity (Gonzalez et al., 2004) Table-5.1. When soils contain excessive concentration of water-soluble salts containing positive charge cations such as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) along with negative charge anions chloride (Cl^-), sulphate (SO_4^{2-}), nitrate (NO_3^-), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), these are called saline (Rhoades and Miyamoto, 1990). These dissolved salts cause the harmful effect on seed germination, plant growth and yield when the concentration in the root zone exceeds critical level (Conway 2001; Denise 2003).The more soluble salts such as sodium chloride (NaCl), sodium sulfate ($NaSO_4$), sodium bicarbonate ($NaHCO_3$), and magnesium chloride ($MgCl_2$) cause more plant stress than less soluble salts such as calcium sulfate ($CaSO_4$), magnesium sulfate ($MgSO_4$), and calcium carbonate ($CaCO_3$). Irrigation water and saline soils were classified into four and five major groups respectively, depending on salinity levels (Table-5.2). The electrical conductivity (EC) or EC of the saturated soil paste (ECe) is an important parameter because this value is used to characterise crop salt tolerance. Salt susceptible (glycophytes /sweet plants) and tolerant plants (halophytes/ salt tolerant plants) are classified into four groups viz, sensitive, moderately sensitive, moderately tolerant and tolerant (Fig-5.1 and Table-5.3).

Table-5.1 Classification of salt-affected soils

Class	pH	ECe (dS/m)	SAR	ESP
Normal	6.5 -7.5	<4	<13	<15
Symptom	No visible symptom and normal growth of the plant			
Saline	<8.5	>4	<13	<15
Symptom	White crust on the soil surface. Water-stressed plants. Leaf tip burn/ non-sodic soil with sufficient soluble salts to interfere with the growth of most crops			
Sodic	>8.5	<4	>13	>15
Symptom	Poor drainage. Black powdery residue on soil surface. Soils with sufficient exchangeable sodium to interfere with the growth of most plants, but without appreciable quantities of soluble salts			
Saline-Sodic	<8.5	>4	>13	>15
Symptom	Grey-colored soil. Plants showing water stress. Soils with sufficient exchangeable sodium to interfere with the growth of most plants and containing appreciable quantities of soluble salt			

(Source: Horneck *et al.* 2007)

Table-5.2. Crop response to salinity, measured as the electrical conductivity of the soil saturation extract (ECe)

Soil depth	Saline Soil Classes/ Interpretation (Classification of irrigation water salinity)				
	Non-Saline/ salt-free	Weakly Saline/ Slightly saline(Low salinity water)	Moderately Saline(Medium salinity water)	Strongly Saline (High salinity water)	Very Strongly Saline (Very high salinity water)
	ECe (dS/m) at 25 ^{o}C [(ECw (dS/m)]				
0-60 cm (0-2 ft)	0-2 (up to 0.7)	2-4 (0.7- 2.5)	4-8 (2.5-7.5)	8-16 (7.5-22.5)	>16 (> 22.5)
60-120 cm (2-4 ft)	<4	4-8	8-16	8-16 (7.5-22.5)	>16 (> 22.5)
Crop response	Salinity effects mostly negligible, except in very sensitive plants	Yield of very sensitive crop restricted	Yield of most crop restricted	Only tolerant crop yield satisfactorily	Only a few tolerant crops yield satisfactorily

(In parenthesis indicate irrigation water salinity: ECw)
USDA classification of irrigation water salinity (adapted from Richards, 1969)

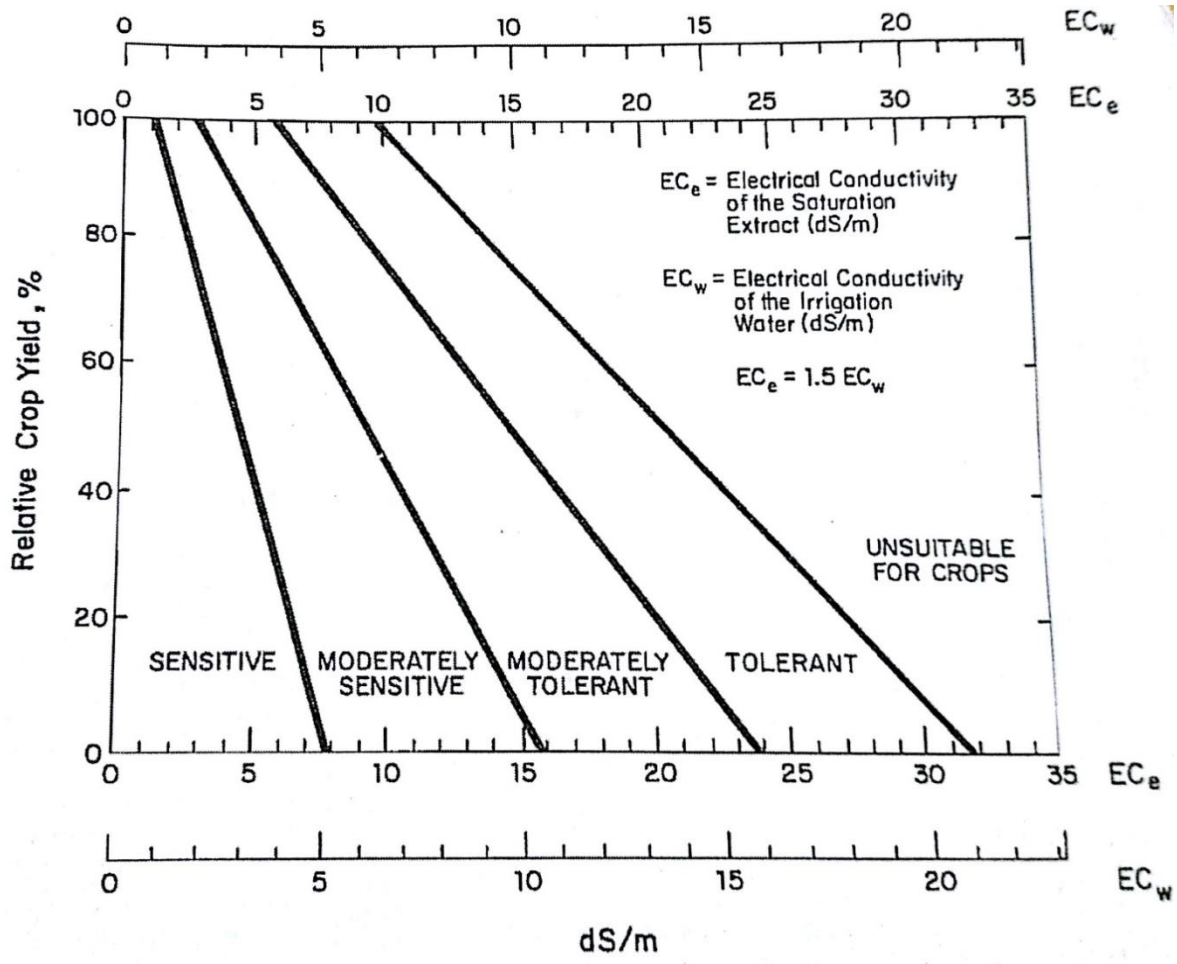

Fig: 5. 1 Relative crop yield (or yield potential) as a function of average root zone salinity (dS/m) grouped according to relative tolerance or sensitive to salinity. Source: Adapted from Maas and Grattan 1999; Grieve et al .2012)

Table- 5.3 Salt tolerance ratings of various crops

Sensitive	Moderately sensitive	Moderately tolerant	Tolerant
Rice	Chickpea	Sorghum	Barley
Sesame	Corn and Corn (forage)	Soybean	Canola
Gram, Black or urd bean	Peanut	Sunflower	Cotton
Pigeonpea	Sugarcane	Wheat	Guar
Walnut	Alfalfa	Barely (forage)	Oats and forage Oats
Mango	Berseem	Guinea	Rye and forage Rye

		grass	
Banana	Cowpea (forage)	Dhaincha	Triticale
Apple	Buffel grass		Wheat (semidwarf)
			Wheat (durum)
			Kallar grass
			Date palm

Source: Adapted from Maas and Grattan 1999; Grieve et al. (2012)

A) Preparation of saline water (Source: USDA Hand book No-60)

Known standard mixtures of salt ratios are used for conducting the experiment under (specify your actual experiment-test tube, hydroponics, pot, and field) for screening the salt tolerant/transgenic cultivars based on Table 5.4, Fig.5.2 (A and B) and Table 5.5 Fig-5.3 using the following formula:

Desired EC = mEq or ME x MW

Where,

mEq or ME = milli equivalent for desired EC

MW = molecular weight of the salt

Desired mixture of salts and its ratios: NaCl, Na_2SO_4, $MgCl_2$, and $CaSO_4$, 13:7:1:4 respectively

Level of desired saline EC (dS/m): 4, 8, 12, 16

Ex: NaCl at 4 EC at 4 EC = 45meq L^{-1} (Fig.5.2 (A and B)

= Concentrations of salt (me L^{-1}) × salt ratio

Total salt ratio

ME $= \frac{45}{25} \times 13 = 23.43$

Test the EC of the water before using it to saturate the soil, germination paper (Test the EC of the water before using it to saturate the soil, germination paper (salinity levels raised on germination paper)

Table: 5.4.Computed salt requirements for desired saline water levels given for various types of experiment (Test tube, hydroponics, pot, and field soils)

EC (dS/m)	ME for all 4 salts	ME for individual salt				MW				Salt required (g) /liter)= ME x MW			
		NaCl	Na_2SO_4	$MgCl_2$	$CaSO_4$	NaCl	Na_2SO_4	$MgCl_2$	$CaSO_4$	NaCl	Na_2SO_4	$MgCl_2$	$CaSO_4$
4	45	23.4	12.6	1.8	7.2	58	142	203	172	1.4	1.8	0.4	1.2
8	95	49.4	26.6	3.8	15.2	58	142	203	172	2.9	3.8	0.8	2.6
12	150	78.0	42.0	6.0	24.0	58	142	203	172	4.6	6.0	1.2	4.1
16	200	104.0	56.0	8.0	32.0	58	142	203	172	6.1	8.0	1.6	5.5

Note: This prepared saline solution/or saline water directly used for germination study in petri dish/germination paper study/ in vitro test tube method or hydroponic study (Hoagland solution) or saline irrigation method- mostly useful/preferable to laboratory conditions, but not good for pot/field conditions. This is why because soil ECe generally comes down into lower than desired or targeted saline soil ECe

Table: 5.5. Electrical conductivity (EC) of pure solutions at 20°C (dS/m) equivalent with mM solution

Solution	EC (dS/m)
10 mM NaCl	1.0
100 mM NaCl	9.8
500 mM NaCl	42.2
10 mM KCl	1.2
10 mM $CaCl_2$	1.8
10 mM $MgCl_2$	1.6
50 mM $MgCl_2$	8.1

The solutions represent those of salts found in soils or in seawater. Data from the Handbook of Physics and Chemistry (CRC Press, 55th edition, 1975).

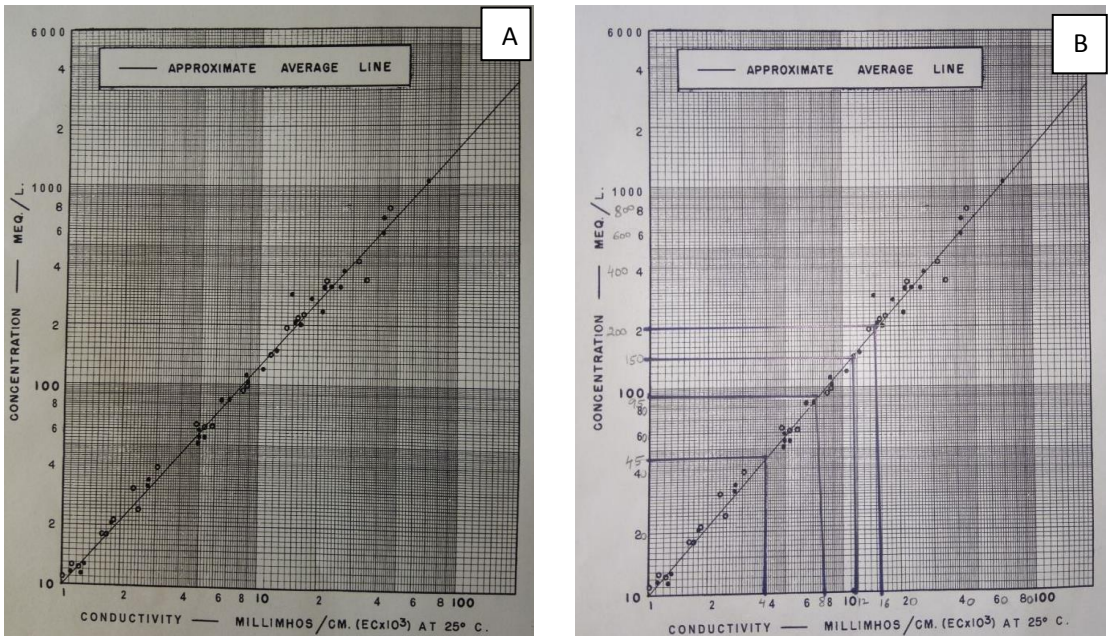

Fig.5.2 (A and B) Concentration of saturation extraction of soil in milliequivalents per liter as related to Electrical conductivity (Conductivity v/s. concentration Source USDA Hand book No-60)

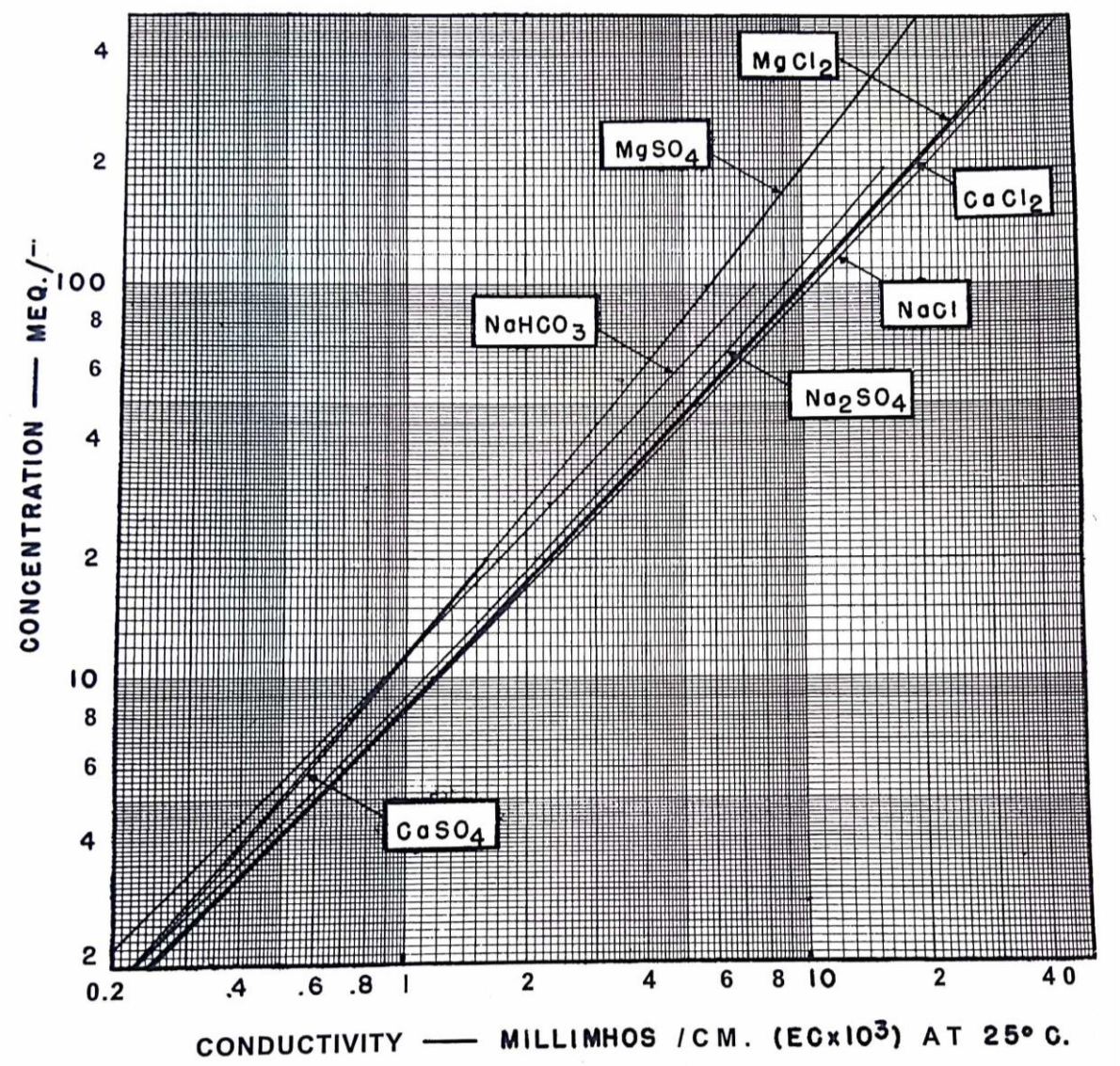

Fig-5.3 Concentration of single-salt solutions in mill equivalents per liter as related to electrical conductivity

B. Preparation of artificial saline soil (I.C. Gupta *et al* 2012)

Artificial saline soils are usually used in pots and micro plot experiments. To develop a given salinity level, application of salts like NaCl, $CaCl_2$ and Na_2SO_4 dissolved in the ratio of 7:2:1, gives good results as it is the ratio in which these salts are found in semi-arid areas. Other composition of salts could be used depending upon the kind of [(Ex. NaCl, Na_2SO_4, $MgCl_2$, and $CaSO_4$, (13:7:1:4 ratio) for petri dish, test tube, hydroponic, pot/pit experiments] experiments. In this case, take dry, grounded and sieved (2mm) known weight of soil in the pots.

Desired level of EC (dS/m): 4, 8, 12, 16

To calculate the salts required to prepare a soil with ECe of 4, 8, 12, 16 dSm^{-1}

Calculate the salt required for1 litre of 4, 8, 12, 16 (each) EC water (Table-5.6) that would be able to saturate about 2.5 kg of the soil if the porosity of the soil is taken as 0.4 by weight (for semi-arid soils). The calculation of salinity depends on the percentage saturation of the soil which needs to be estimated individually for the type of soil used for the experiment.

Table: 5.6.Computed salt requirements for desired salinity levels given various types of soils

	A mixture of salt ratios			Equivalent weight			ME=EC X Salt ratio			Salt required (g) /liter) = ME x MW		
EC	NaCl	$CaCl_2$	Na_2SO_4	NaCl	$CaCl_2$	Na_2SO_4	NaCl	$CaCl_2$	Na_2SO_4	NaCl	$CaCl_2$	Na_2SO_4
4	7	2	1	59	56	71	28	8	4	1.6	0.4	0.3
8	7	2	1	59	56	71	56	16	8	3.3	0.9	0.6
12	7	2	1	59	56	71	84	24	12	4.9	1.3	0.9
16	7	2	1	59	56	71	112	32	16	6.6	1.8	1.1

Dissolved NaCl and $CaCl_2$ in approximately half of the total water and Na_2SO_4 in the remaining half of the water.

Test the EC of the water before using it to saturate the soil.

[Note- 1: Equivalent weight of salt = $\frac{Moleculat\ weight}{Net\ charge\ on\ Ion}$,

Note-2: $Na_2SO_4 = \frac{142}{2} = 71$]

Note: Initial checking of ECe is required to know the salt concentration already present

Note: This prepared saline soil, directly used for sowing/transplanting in pot conditions. The soil containing salts should is irrigated with ordinary water. The drain holes in the pot should be plugged or seald with M-seal. An equal volume of water should be added to the pots having different ECe (dS/m) soils. Before planting seedlings /root slips, the pot should be watered for two weeks, and salts should be allowed to distribute within the pot uniformly. Check the EC of irrigation water. If the water is saline, then the salts will get added to the soil salinity. So before planting/sowing, measuring the EC of watered soil is warranted.

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Exercise 2

Measurement of water content in soil and plant tissue

A) Measurement of water content in soil

Quantification of available water in the soil is mandatory in the studies related to water management, irrigation scheduling, development of drought-tolerant varieties and studies concerned with stress physiology. Usually, the moisture content at field capacity and the wilting point is -0.3 bar and -15.0 bar respectively. The soil moisture held between field capacity and the permanent wilting point is called available water; called available water should not be less than 50% for healthy plant growth. There are several methods of determining the soil moisture content. Field capacity plant available water and the permanent wilting point (Fig-9.1). These levels of soil water content can be expressed in inches of water per foot of soil (Table-9.1) as well as in bars.

Following methods are commonly employed ones:

1. Gravimetric method
2. Time domain reflectometry
3. By Neutron probe

The energy regarding either soil matric potential or soil moisture potential can be measured by the following method also

1. Resistance block
2. Tensiometer
3. Psychrometer

Field capacity (FC): the field capacity of the soil is described as the water content of the downward flow of gravitational water has become very slow, and water content has become relatively stable. This situation exists several days (1-3) after the soil has been wetted by rain or irrigation.

Permanent wilting point (PWP): this is the soil water content at which plants remain wilted unless water is added to the soil. Richards and Wadleigh (1952) found that the soil water potential at wilting ranged from -10 to -20 bars, with the average at about -15 bars which are used as an approximation of soil water.

Plant-Available Water (PAW): The amount of water held in the soil that is available to plants; the difference between field capacity and permanent wilting point. Since field

capacity and PWP represent the upper and lower limit of soil water availability, this range has considerable significance in determining the agricultural values of soils. The following methods can measure the quantity or content of water in the soil. As a general rule, plant available water is considered to be 50 percent of the water holding capacity.

A). Estimation of soil moisture by gravimetric method

Aim: to determine the moisture content of the soil by gravimetric method

Materials: Screw augar, aluminium tins (moisture tins), oven, balance

Procedure:

1. Take Soil samples with the help of a screw type auger at 0-15, 15, 30 and 50 and 75cm depths from the control and stress plot

2. After determining the wet soil weight, the soil samples were dried in a hot air oven at 80 ^{o}C for 72hours, and the dry weight recorded. The soil moisture content expressed in percent soil moisture availability.

Percept moisture content=$\frac{Weight\ of\ moist\ soil\ (g) - weight\ of\ dry\ soil\ (g) \times 100}{Weight\ of\ moist\ soil\ (g)}$

Advantages:

1. Cheap method
2. Accurate method than other methods
3. Used for calibration of other instruments

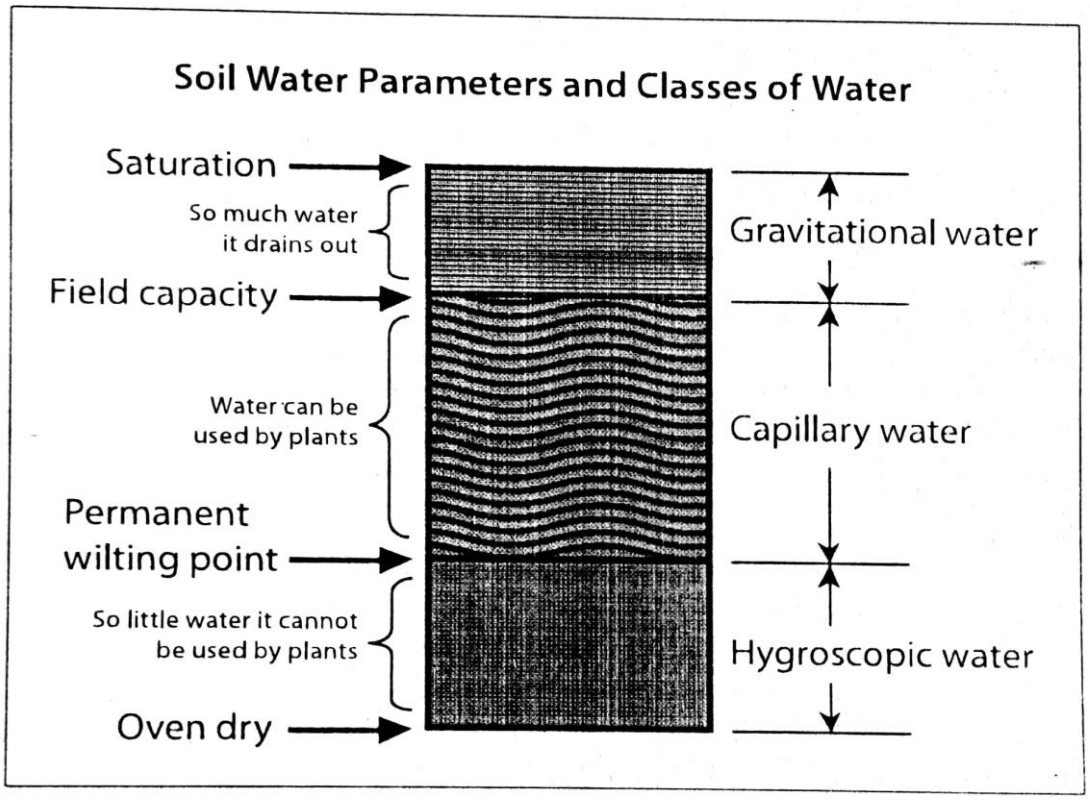
Disadvantages:

1. Destructive sampling
2. Labour requirement at each sampling
3. Not applicable to field conditions
4. More time is require

Table- 9. 1. Soil water content parameters for different soil textures

Soil texture	Field capacity (in./ft)	Plant available water (in./ft)	Permanent wilting point (in./ft)
Sand	1.2 (0.10)*	0.7 (0.06)	0.5 (0.04)
Loamy sand	1.9 (0.16)	1.1 (0.09)	0.8 (0.07)
Sandy loam	2.5 (0.21)	1.4 (0.12)	1.1 (0.09)
Loam	3.2 (0.27)	1.8 (0.15)	1.4 (0.12)
Silt loam	3.6 (0.30)	1.8 (0.15)	1.8 (0.15)
Sandy clay loam	4.3 (0.36)	1.9 (0.16)	2.4 (0.20)
Sandy clay	3.8 (0.32)	1.7 (0.14)	2.2 (0.18)
Clay loam	3.5 (0.29)	1.3 (0.11)	2.2 (0.18)
Silty clay loam	3.4 (0.28)	1.6 (0.13)	1.8 (0.15)
Silty clay	4.8 (0.40)	2.4 (0.20)	2.4 (0.20)
clay	4.8 (0.40)	2.2 (0.18)	2.6 (0.22)

Numbers in parenthesis are volumetric water content expressed as foot of water per foot of soil. (Source: Hanson 2000)



1. **Fig: 9.1 Soil water parameters and classes of water (Source Juan *et al*** E-618 08/12)

Determination of Relative Water Content (RWC) in leaf tissue

The relative water content (RWC) is one of the reliable parameters to know the water status in plants and it decreases gradually with increases in severity of drought stress. Decline of RWC as response of stress were reported by several investigators under different stress conditions (Barr and Weatherley, 1962). Further it has been suggested that the plants to retain a high RWC during stress period are conspired as tolerant once (Barr and Weatherley, 1962). The relative water content (RWC; or 'relative turgidity) of a leaf is a measurement of its hydration status (actual water content) relative to its maximal water holding capacity at full turgidity. RWC provides a measurement of the 'water deficit' of the leaf and may indicate a degree of stress expressed under drought and heat stress. A genotype with the ability to minimise stress by maintaining turgid leaves in stressed environments will have physiological advantages (e.g., this allows turgor dependent processes such as growth and stomatal activity, and to protect and maintain the photosystem complex). The term was introduced by Weatherly in 1962, is a modification of an older term, water saturation deficit (WSD). This term expresses the leaf water content as a percentage of turgid water content and is calculated by the following equation.

$$\text{RWC (\%)} = \frac{Fresh\ weight - Oven\ dry\ weight}{Turgid\ weight - Oven\ dry\ weight} \times 100$$

WSD and RWC are related; RWC = 100-WSD or RWC+WSD=100%. Barrs and Weatherly (1962) have found 4 hours to be the optimum time for floating leaf discs or whole leaves in water to determine turgid weight. Hewlett and Kramer (1963) found entire leaves are more satisfactory than discs for some species.

Procedure:

1. Collect the leaf sample; usually fully expanded topmost leaf is preferable. Time of sampling 11-12noon is desirable.
2. Immediately after sampling place the sample in a polythene bag and seal properly to minimizing water loss from the leaf.
3. Samples should reach the lab as soon as possible and place these sample in picnic cooler (temperature around10-15 °C)
4. Cute 5-10 cm length mid-leaf sections or 5-10 cm leaf discs of around 1.5cm in diameter or take the several leaf lets depending upon the plant species (in smaller composite leaves). Avoid the midribs and veins
5. Weight the samples and quickly to record the fresh weight.

6. Hydrate the samples to full turgidity by floating on DDH_2O or de-ionized water or normal tap water in a closed petri-dish for 4hrs at normal room temperature and light
7. Add 0.01% Tween 20 in case the leaf sample surface is waxy and not getting wet by water.
8. After 4hrs take out the samples; remove the surface moisture quickly and lightly with filter paper or blotting paper and immediately weigh to obtain fully turgid weight
9. Keep the sample in an hot air oven for 48 hours at 75-80 ^oC and record the oven drying weight of the sample

Advantages:

1. Simple and needs no sophisticated equipment

Disadvantages:

1. Unfortunately, a given water deficit or RWC does not represent the same level of water potential in leaves of different species or ages or from different environments. Leaf and cell characteristics (thickness, elasticity) can cause changes in RWC although water potential may be unaltered, particularly as the leaf matures
2. Time consuming

Note:

1. With good and careful work the method should normally result in about 2% to 3% of RWC being a statistically significant difference between treatments.
2. Estimation of relative water content (RWC) in large size of population/genotypes is not possible, so first short out the germplam by Plant Water Content [(PWC) whole plant] or Leaf Water Content (only leaf):

Formula

PWC (g/g) = (FW-DW)/DW

Whereas FW-Fresh weight, DW-Dry weight

Observation sheet

S,No	Sample ID	Fresh weight (g) (A)	Turgid weight (g) (B)	Dry weight (g)	RWC %= [(A-C/B-C)]x 100
1	Control	0.95	1	55	89
2	Stress	0.90	1	45	82

References:

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Exercise 3

Imposition of moisture stress by gravimetric approach

Objective: To generate drought/moisture stress induced plant tissues for assessing various physiological and molecular assays.

Materials: Pots or battery containers, garden soil, sand and manure, mobile weighing devices, seed/plant material, rain-out-shelter (ROS) or polythene sheet covered on net house

Procedure:

1. Weigh the empty pots and record the accurate weights for each pot **(A)**

2. Fill the pots with soil: sand: farmyard manure mixture in the ratio of 2:1:1. or 2:1 ratio of soil: farmyard manure mixture. While filling the pots, makes sure that the soil mixture is not compacted

3. Weigh the pot along with soil **(B)** and deduct the empty pot weight to obtain the dry soil weight **(C)**.

C= B-A

4. Carefully flood the pot with water (not splashing the soil from the pot). Allow it for overnight to drain excess water and attain field capacity (FC).

5. Take the pot weight after saturation **(D)** and deduct empty pot weight **(A)** to get full soil weight (**E)** at field capacity.

E=D-A

6. Subtract the dry soil weight from the full soil weight to get the amount of water required to attain 100% FC **(E-C)**.

7. Sow seeds of the crop under investigation in the pots. Maintain two to four seedlings in each pot and water regularly to maintain moisture level at desired level of FC viz 100% FC, 75% FC, 60% FC etc., Ensure to protect the pots from rains or any other source of water by keeping them under rain out shelter (ROS)

8. At four or six-leaf stage or at good foliage, impose drought stress by withholding irrigation (please refer the diagrammatic representation given below). Weigh the pots at regular intervals to monitor water status at different FCs, Replenish the water every

time by adding the required amount of water depending on the loss of water occurred previously and also based on the set FC value. The amount of water to be replenished to maintain the required FC in the containers can be arrived at based on the formula given below.

To maintain 100% FC, X ml of water is required. Therefore, to maintain Y% FC, it is

$$Y\%\ FC = \frac{Y\% \times X \text{ ml of water}}{100\%}$$

For example, the amount of water required to maintain 100% FC = 200ml

Therefore, the amount of water required to maintain 80% $FC = \frac{80 \times 200ml}{100} = 160ml$

The plants under different treatments are to be grown for a week or longer depending on the crops. During this period, soil water potential (Mpa) and osmotic potential (Mpa) are measured with Dew Point Potentiometer and Osmometer respectively. Similarly, Relative water content (RWC %) is quantified according to Barrs and Weatherly (1962) to assess the tissue water status and Electrical conductivity (EC %) is quantified to assess the stress-induced cell damage.

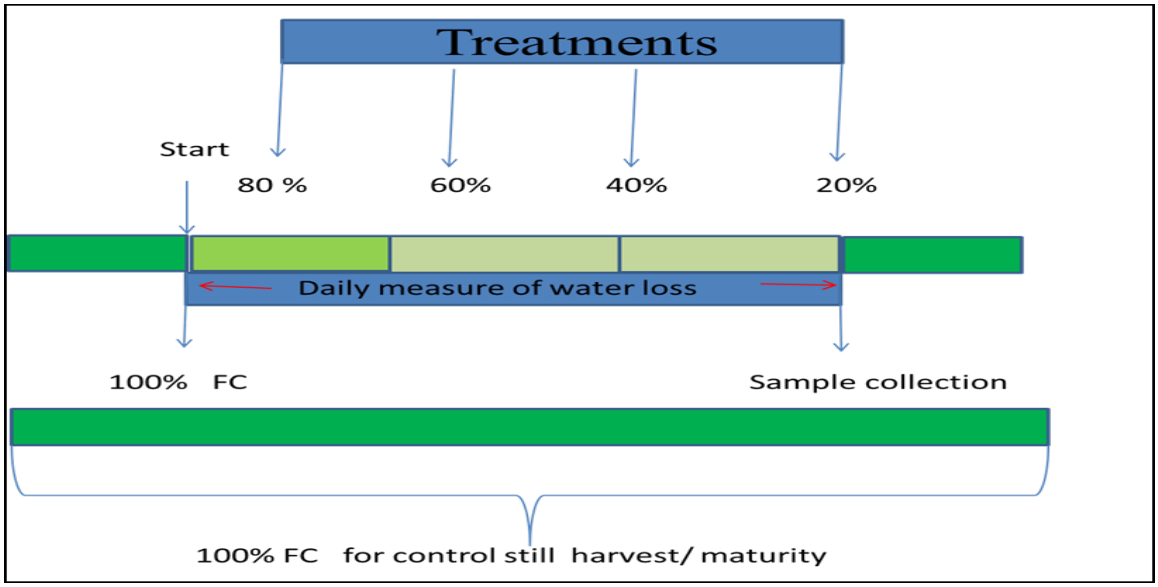

Figure 10.1: Diagrammatic representation of gravimetric approach followed for imposing precise levels of moisture stress/ drought.

Note: Better terms are Available soil moisture (ASM) or Soil Moisture depletion (SMD), instead of Field Capacity (FC)

Ex: In the literature, Available Soil Moisture (ASM) between 40 -50% or Soil Moisture depletion (SMD) between 50-60%, 40-50% has been used as Field Capacity (FC) whereas this should be treated as ASM or SMD instead of Field Capacity.

T2 (20% ASM) T1 (40% ASM) Control (80% ASM)

Figure.10.2. Diagrammatic representation of gravimetric approach followed for imposing precise levels of drought (Berseem crop). ASM- Available Soil Moisture

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Germination ecology of *Emex spinosa* and *Emex australis*, invasive weeds of winter crops

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Summary

Emex spinosa and *Emex australis* are invasive dicotyledonous weeds. The effects of various environmental factors on the germination of these weeds were investigated under laboratory and glasshouse conditions. Germination response of both species was lower at warmer temperature, and maximum germination was recorded at 20/12°C (day/night). Light stimulated germination in both species, but considerable germination also occurred under darkness. More than 80% of *E. spinosa* seeds germinated at pH between 6 and 9, whereas *E. australis* seeds germination was considerably decreased at pH 9. *Emex spinosa* was fairly tolerant to salinity as compared with *E. australis* and germination (21%) of *E. spinosa* occurred even at 200 mM NaCl. Both species were sensitive to osmotic stress, but *E. spinosa* tolerated more osmotic stress than *E. australis*. Temperature above 20/12°C (day/night) and low osmotic potential increased time to start germination and mean germination time (MGT), as well as decreased germination index (GI) of both species. Darkness resulted in increased MGT and decreased GI in both species when compared with 10 h photoperiod. Salt stress strongly increased time to obtain 50% germination and reduced GI of both species. In both species, an increasing burial depth decreased emergence percentage and emergence index and increased time to start emergence, although some seed emerged even at 10 cm burial depth. It was concluded that both species can germinate over a wide range of environmental conditions. However, *E. australis* was more sensitive under adverse environmental conditions compared with *E. spinosa*. This information on germination ecology may aid in developing tools and strategies for management.

Keywords: spiny emex, devil's thorn, doublegee, three-cornered jack, germination, light, osmotic stress, pH, salt stress, temperature, weed seed.

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Introduction

Emex spinosa (L.) Campd. (spiny emex, devil's thorn) and *Emex australis* Steinh. (doublegee, three-cornered jack) are closely related invasive winter weed species in Pakistan that belong to the Polygonaceae. *Emex spinosa* is native to the Mediterranean region and Asia Minor, whereas *E. australis* is native to southern Africa (Steinheil, 1838). Both weeds may co-exist in a field and are difficult to distinguish at early growth stages. *Emex australis* has fruit double in size with spines double as long as those of *E. spinosa*. Both species are serious weeds of crops and pastures in major areas of the world (Gilbey & Weiss, 1980; Abbas *et al.*, 2010) and their spiny fruit contaminate grain and cause injury to livestock.

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The ability of the weeds to grow, invade and persist in winter cereals can be attributed to high seed production and establishment of persistent seedbank (Cheam, 1987). The weeds only propagate by seed and their spiny fruits cling to grazing animals and automobile tires and are thereby carried to new localities (Hagon & Simmons, 1978). They are difficult to control because of a fleshy taproot that absorbs and stores moisture for their development. In addition to competitive effects of the weeds (Abbas *et al*., 2010), they are problematic in winter crops because their spiny seeds and abundant biomass production adversely affect harvest efficiency and crop quality (Weiss & Julien, 1975).

Seedling emergence is one of the most critical phases in plant development at which the weed can compete for an ecological niche and is mediated by various environmental factors such as temperature, light (Andersson *et al*., 2002), pH, osmotic and salt stress (Bewley & Black, 1994). Temperature and light are considered the most important environmental signals regulating germination, species distribution and ecological interaction (Ebrahimi & Eslami, 2012). Temperature is a major determinant of germination percentage and rate when other factors (water, salinity and acidity) are not limiting (Martinkova *et al*., 2006). Temperature effects are variable for species within genera (Van Assche *et al*., 2003) and may also differ between genotype within species (Debeaujon *et al*., 2000). Many plants require light for germination, some are insensitive to light and others are inhibited by light (Bewley & Black, 1994).

Moisture stress may delay, reduce or prevent germination and growth of plant (Norsworthy & Oliveira, 2006). Ability to germinate under conditions of moisture stress may enable a weed to take advantage of the condition that limit the growth of other species. The impact of soil moisture on germination varies among weed species. In addition, field condition may differ spatially and temporally, depending on rainfall, temperature and soil type. Salt stress is a major constraint in cereal production worldwide. Germination is delayed and reduced when salt stress exceeds a critical level by decreasing the ease with which seeds imbibe water or facilitating the entry of ions to toxic levels (Romo & Eddleman, 1985). The level of salinity at which germination is reduced varies with species, genotype, environmental conditions, osmotic potential and specific ions (Ungar, 1991).

Plants can tolerate a pH range in their environment between 5 and 10 (Chachalis *et al*., 2008); beyond this range, high concentration of ions can be directly toxic to plants (Chejara *et al*., 2008). Some weeds germinated in a wide range of pH (Ebrahimi & Eslami, 2012). Weed seed location in the soil seedbank influences germination and emergence (Oliveira & Norsworthy, 2006).

Biological and ecological information, specifically germination ecology of a specific weed, is necessary to optimise weed control and maximise the efficiency of management tactics (Ebrahimi & Eslami, 2012). In southern Punjab, most soils are sandy loam and saline with limited water availability and average temperatures range from 15 to 20°C at the time of wheat sowing. Crop rotation in this area is wheat-cotton-wheat or wheat-millet-wheat. Seedbeds for wheat are typically prepared with conventional cultivators working to a depth of 10 cm. To understand the expansion of the geographic range of *E. spinosa* and *E. australis* in Pakistan, we need to know how their seeds respond to varied climatic factors. To date, little research especially on the germination ecology of these weeds has been conducted in Pakistan. The objectives of this study were to determine the effects of temperature, light, salt and osmotic stress, pH and burial depth on germination and seedling emergence of *E. spinosa* and *E. australis*.

Materials and methods

Seed description and germination tests

Experiments were conducted at the Department of Agronomy, University of Agriculture Faisalabad, Pakistan under laboratory and glasshouse conditions during 2009. In April 2009, seeds of *E. spinosa* and *E. australis* (10 collections were made from an area of 100 km diameter) were collected at maturity, at onset of senescence, from several distantly located farmer fields of wheat in the District Layyah, Punjab, Pakistan and a bulked sample was prepared. Working samples were drawn from this composite sample. The mature seed colour was brownish. The seeds were cleaned and dried for 7 days at room temperature (25°C) and then stored in paper bags at room temperature for 20 days and used in all experiments.

Germination was determined by placing 25 seed evenly in a 9 cm diameter Petri plate containing filter paper Whatman No. 10, moistened with 5 mL distilled water or a treatment solution. Seeds of *E. spinosa* and *E. australis* were surface sterilised by soaking in 10% sodium hypochlorite (NaOCl) for 5 min, followed by five rinses with distilled water before the start of each germination trial. Petri plates were sealed with Parafilm to reduce water loss. All the experiments (except temperature experiment) were conducted at 20/12°C day/night temperature with 10 h photoperiod (except light experiment). Cool white fluorescent bulbs (FL40SBR; National, Tokyo, Japan) were used to

produce a photosynthetic photon flux density of 200 μmol m^{-2} s^{-1} for all the experiments, except dark treatment in the light experiment. Germinated seeds with a radicle at least 2 mm long were counted and removed daily for a period of 3 weeks. Petri plates assigned to darkness treatment were opened in a dark room equipped with a green safe light.

Effect of temperature

Seed germination was determined in germination cabinets under fluctuating day/night temperature (15/10, 20/12, 25/15, 30/20, 35/25°C) with 10 h photoperiod for 3 week. period. These temperature regimes were selected to reflect temperature variation during winter to autumn. The Petri plates were kept in a germination cabinet (Seedburo Equipment Company, Chicago, IL, USA).

Effect of light

To evaluate the effect of light and dark on germination, seeds were exposed to 10/14, 24/0 and 0/24 h light/dark regimes per 24 h cycle at 20/12°C day/night temperature. Treatments with 24 h light regime were uncovered to allow continuous light exposure. Petri plates assigned to the dark treatment (24 h dark regime) were covered with a double layer of aluminium foil. Treatments with 10/14 h light/dark were left uncovered for 10 h to allow light exposure, and light was provided by white fluorescent bulbs with a photosynthetic photon flux density of 200 μm m^{-2} s^{-1}. For the dark treatment, water addition to the Petri plate and daily germination counts were conducted in a dark room with a green safe light. Exposure to dim green light was for <30 s.

Effect of pH

To examine the effect of pH on germination, buffer solutions of pH 6–9 were prepared according to the method described by Chachalis and Reddy (2000). A 2 mM solution of MES [2-(*N*-morpholino) ethanesulfonic acid] was adjusted to pH 6 with 1N sodium hydroxide (NaOH). A 2 mM solution of HEPES [*N*-(2-hydroxy-methyl) piperazine-*N*-(2-ethanesulfonic acid)] was adjusted to pH 7 or 8 with 1N NaOH. A pH 9 buffer was prepared with 2-mM TRICINE [N Tris (hydroxymethyl) methylglycine] and adjusted with 1 N NaOH. Unbuffered deionised water (pH 6.2) was used as a control.

Effect of salt stress

Seeds of *E. spinosa* and *E. australis* were incubated in sodium chloride (NaCl) solutions of 0, 50, 100, 150, 200 and 250 mM to examine the effect of salt stress on germination. To distinguish the salinity and osmotic effect of NaCl, the non-germinated seeds from both species at higher NaCl concentration were transferred to Petri dishes containing 5 mL of distilled water and placed in the germination cabinet. The seeds were rinsed before being transferred.

Effect of osmotic stress

Emex spinosa and *E. australis* seed were tested for germination in aqueous solution with osmotic potential of 0, −0.2, −0.4, −0.4, −0.6, −0.8 and −1.0 MPa. Osmotic potentials were prepared using Polyethylene glycol (PEG 6000) in distilled water. The following equation (Michel & Kaufmann, 1973) was used for calculation of water potential from known concentration of PEG 6000:

$$\text{Water potential} = -(1.18 \times 10^{-2})\,C - (1.18 \times 10^{-4})\,C^2 + (2.67 \times 10^{-4})\,18CT + (8.39 \times 10^{-7})C^2T \quad (1)$$

where C is the concentration of PEG (g kg^{-1} distilled water) and T is temperature (°C).

Effect of burial depth on seedling emergence

The effect of seed burial depth on seedling emergence was studied in the glasshouse. For soil media, clay (30%), silt (30%) and sand (40%) were thoroughly mixed. Twenty-five seeds of each species were placed on the soil surface or covered with soil at depths of 2, 4, 6, 8 and 10 cm in 15 cm diameter plastic pots. Glasshouse temperatures were 23 ± 2°C during the day and 15 ± 2°C at night with a 10 h photoperiod (200–500 μmol photons m^{-2} s^{-1}). Pots were left opened and watered as needed to maintained adequate soil moisture. Each pot had a hole at the bottom for drainage. Seedlings were considered emerged when a cotyledon was visible at the soil surface. Seedling emergence was recorded daily for 21 days. The time to obtain 50% germination or emergence (T_{50} or E_{50}) was calculated according to the following formula of Coolbear *et al.* (1984):

$$T_{50} \text{ or } E_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{(n_j - n_i)} \quad (2)$$

where N is the final number of germinated or emerged seed, and n_j and n_i are the cumulative number of seed germinated by adjacent counts at times t_j (day) and t_i, (day), respectively, when $n_i < N/2 < n_j$.

Mean germination or emergence time (MGT or MET) was calculated according to the equation of Ellis and Roberts (1981):

$$\text{MGT or MET} = \frac{\sum Dn}{\sum n} \quad (3)$$

where n is the number of seed that had germinated on day D, and D is the number of days counted from the beginning of germination experiment. The germination or emergence index (GI or EI) was calculated as

described by the Association of Official Seed Analysis (1990) using the following formula:

$$\text{GI or EI} = \frac{\text{No of germinated or emerged seedlings}}{\text{Days of first count}} + \ldots + \frac{\text{No of germinated or emerged seedlings}}{\text{Days of final count}} \quad (4)$$

Analysis

A completely randomised design with four replications was used in all experiments. Data were subjected to analysis of variance (ANOVA). The model structure of ANOVA was

$$Y_{ij} = \mu + T_i + \in_{ij} \quad (5)$$

where Y_{ij} is the observed response variable, μ is an overall mean, T_i is the explanatory variable and $\in_{ij}$ is the error. The significant differences among treatment means were identified using Fisher's LSD at $P < 0.05$ (Steel *et al.*, 1997). A square root arcsine transformation was used to stabilise the variances for percentage data before analysis. Nonlinear regression analysis was used to determine how NaCl, osmotic stress or burial depth affected percentage germination or emergence. Germination (%) values at different concentrations of NaCl and osmotic potential were fitted to a functional three-parameter logistic model using SIGMA PLOT 2008 (version 11.0, SyStat Software GmbH, Schimmelbuschstrasse 25 D-40699 Erkrath Germany). The model fitted was

$$G(\%) = G_{\max}/[1 + (x/x_{50})^g], \quad (6)$$

where G is the total germination (%) at concentration x, $G_{\max}$ is the maximum germination (%), x_{50} is the NaCl concentration or osmotic potential for 50% inhibition of the maximum germination and g indicates the slope. A three-parameter logistic model:

$$\{E(\%) = E_{\max}/[1 + (x/x_{50})^e]\} \quad (7)$$

was fitted to the *E. spinosa* seedling emergence (%) obtained at different burial depths of 2–10 cm, where E is the total seedling emergence (%) at burial depth x, $E_{\max}$ is the maximum seedling emergence (%), x_{50} is the burial depth for 50% inhibition of the maximum seedling emergence and e indicates the slope. A t-test was used to assess significant difference between light and dark treatment in the light experiment.

Results

Effect of temperature

Seed germination differed among incubated temperature regimes for both species (Fig. 1). *Emex australis* and *E. spinosa* germinated at temperatures from 15/10 to 25/15°C (day/night). Highest germination was occurred at 20/12°C in both species. However, germination of *E. spinosa* was greater at this temperature when compared with *E. australis* (Fig. 1). No germination was observed at 10/5 and 35/25°C in both species, whereas 7% and 3% occurred at 30/20°C in *E. australis* and *E. spinosa* respectively. *Emex spinosa* took significantly longer (5.25 day) at 25/15°C and least time (3.25 day) to start germination at 20/12°C (Table 1). Minimum time to obtain 50% germination was taken at 20/12°C. GI decreased and MGT increased at above or below 20/12°C. Time to start *E. australis* seed germination was least at 25/15°C (Table 2). Time to achieve 50% germination decreased with increase in temperature, and the lowest was recorded at 30/20°C. Minimum MGT was observed at 25/15°C, whereas maximum GI was recorded at 15/10°C (day/night) temperature.

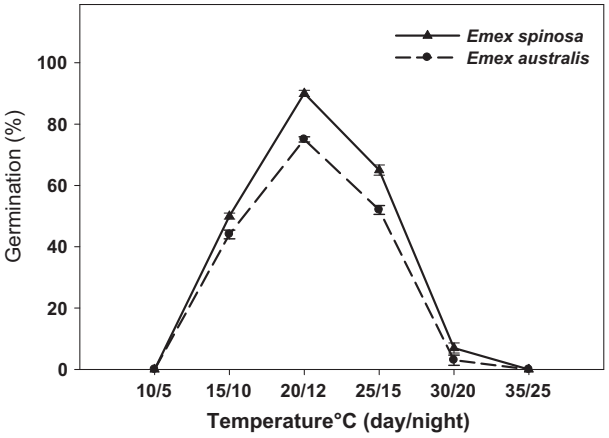

Fig. 1 Effect of alternate day/night temperature on germination of *Emex spinosa* and *Emex australis*. Vertical bars represent ± standard error of the mean.

Effect of light

When *E. spinosa* and *E. australis* seed were exposed to continuous darkness at 20/12°C day/night temperature, germination was 58% and 62%, respectively, which was significantly lower ($P < 0.05$) than those under 10 or 24 h photoperiod (Fig. 2). Maximum germination occurred at 10 h photoperiod in both species. Germination of both species was stimulated by light, but the 10 h photoperiod significantly stimulated germination when compared with continuous light. In both species, there was no difference in time to start germination with tested light condition (Tables 1 and 2). *Emex spinosa* seed showed minimum T_{50} and MGT with 10 h photoperiod when compared with continuous darkness or light (Table 1). In *E. australis* seed, MGT

Table 1 Effect of environmental factors on the germination or emergence parameters of *Emex spinosa*

Treatments		Time to start Germination or emergence (days) ± SE	T_{50} or E_{50} (days) ± SE	MGT or MET (days) ± SE	GI or EI ± SE
Temperature (°C)	10/5°C	NG	NG	NG	NG
	15/10°C	3.88bc ± 0.27	4.75a ± 0.22	5.51bc ± 0.20	11b ± 0.35
	20/12°C	3.25c ± 0.22	3.5b ± 0.25	4.75c ± 0.41	13.3a ± 0.41
	25/15°C	5.25a ± 0.22	5.5a ± 0.25	5.75b ± 0.22	8.75c ± 0.22
	30/20°C	4.25b ± 0.22	5.25a ± 0.22	6.75a ± 0.30	7.25d ± 0.22
	35/25°C	NG	NG	NG	NG
	LSD at $P \leq 0.05$	0.82	0.83	1.00	1.11
	$SED_{(d.f. = 12)}$	0.38	0.39	0.45	0.51
Light	Dark	3.75 ± 0.22	3.75b ± 0.22	5.88a ± 0.11	10.5b ± 0.56
	10 h light	3.50 ± 0.25	3.75b ± 0.20	4.68b ± 0.20	13.3a ± 0.22
	24 h light	3.50 ± 0.25	4.75a ± 0.22	5.00ab ± 0.35	12.3ab ± 0.54
	LSD at $P \leq 0.05$	NS	0.80	0.90	1.89
	$SED_{(d.f. = 9)}$	0.39	0.35	0.40	0.83
pH	Control	3.00 ± 0.00	3.42 ± 0.05	4.35b ± 0.03	11.20 ± 0.52
	6	3.00 ± 0.00	3.60 ± 0.06	4.71ab ± 0.18	11.47 ± 0.85
	7	3.00 ± 0.00	3.72 ± 0.14	4.61ab ± 0.16	10.25 ± 1.23
	8	3.00 ± 0.00	3.60 ± 0.07	4.55ab ± 0.11	10.69 ± 1.22
	9	3.00 ± 0.00	3.73 ± 0.17	4.87a ± 0.15	11.78 ± 1.11
	LSD at $P \leq 0.05$	NS	NS	0.420	NS
	$SED_{(d.f. = 15)}$	0.00	0.16	0.62	1.39
NaCl	Control	2.3 ± 0.00	2.74b ± 0.02	4.64c ± 0.01	18.62ab ± 0.36
	50 mM	2.3 ± 0.00	2.87b ± 0.07	4.69bc ± 0.02	16.79b ± 0.56
	100 mM	2.3 ± 0.00	2.71b ± 0.13	5.15ab ± 0.02	18.93a ± 1.16
	150 mM	2.3 ± 0.00	3.06b ± 0.13	5.29a ± 0.25	9.37c ± 0.53
	200 mM	2.3 ± 0.00	4.00a ± 0.42	5.28a ± 0.24	3.08d ± 0.27
	250 mM	NG	NG	NG	NG
	LSD at $P \leq 0.05$	NS	0.60	0.47	1.98
	$SED_{(d.f. = 15)}$	0.00	0.28	0.70	0.92
Osmotic potential	Control	3.00b ± 0.00	3.20 ± 0.08	4.72ab ± 0.03	18.15a ± 0.51
	−0.2 MPa	3.00b ± 0.00	3.23 ± 0.11	5.91a ± 0.02	15.70a ± 1.90
	−0.4 MPa	3.25b ± 0.19	3.29 ± 0.21	6.53a ± 0.23	8.70b ± 2.63
	−0.6 MPa	3.96a ± 0.22	3.98 ± 0.02	3.53b ± 1.18	0.94c ± 0.49
	−0.8 MPa	NG	NG	NG	NG
	−1.0 MPa	NG	NG	NG	NG
	LSD at $P \leq 0.05$	0.47	NS	1.86	5.11
	$SED_{(d.f. = 12)}$	0.68	0.26	0.59	2.34
Burial depth	0 cm	6.50d ± 0.22	9.68b ± 0.08	7.75c ± 0.15	6.96a ± 0.52
	2 cm	6.75d ± 0.19	8.99c ± 0.03	7.74c ± 0.12	5.57b ± 0.32
	4 cm	7.25cd ± 0.22	9.08c ± 0.06	7.84c ± 0.11	3.70c ± 0.22
	6 cm	8.00bc ± 0.32	8.72c ± 0.09	7.60c ± 0.08	3.21c ± 0.61
	8 cm	8.50b ± 0.22	9.64b ± 0.33	8.46b ± 0.15	3.06c ± 0.59
	10 cm	13.75a ± 0.19	15.00a ± 0.16	13.67a ± 0.09	0.99d ± 0.07
	LSD at $P \leq 0.05$	0.88	0.48	0.36	1.30
	$SED_{(d.f. = 18)}$	0.41	0.22	0.54	0.62

T_{50} or E_{50}, time to obtain 50% germination or emergence; MGT or MET, mean germination or emergence time; GI, germination index; EI, emergence index; SE, standard error of the mean; NG, no germination; SED, standard error of the difference.
The values followed by different letters were significantly different at $P \leq 0.05$.

was lowest and GI was highest under 10 h photoperiod when compared with tested light conditions (Table 2).

Effect of pH

Germination of the species occurred with buffer solutions pH 6–9. Compared with distilled water (control), no treatment significantly decreased the germination (Fig. 3A). Time to onset of germination (T_{50}) and GI were not affected by pH buffer solutions (Table 1). However, pH 9 buffer increased the MGT of *E. spinosa* seed compared with the control. Compared with distilled water (control), there was no significant difference in *E. australis* germination at pH 6, 7 and 8. However, pH 9 buffer reduced the germination to 64%

Table 2 Effect of environmental factors on the germination or emergence parameters of *Emex australis*

Treatments		Time to start Germination or emergence (days) ± SE	T_{50} or E_{50} (days) ± SE	MGT or MET (days) ± SE	GI or EI ± SE
Temperature (°C)	10/5°C	NG	NG	NG	NG
	15/10°C	5.50a ± 0.25	6.25ab ± 0.22	8.25a ± 0.22	13.5a ± 0.56
	20/12°C	4.50b ± 0.25	6.75a ± 0.22	9.25a ± 0.22	10.8b ± 041
	25/15°C	4.13b ± 0.11	5.5bc ± 0.25	5.25b ± 0.41	5.25c ± 0.41
	30/20°C	5.50a ± 0.30	5.25c ± 0.22	5.50b ± 0.25	4.25c ± 0.22
	35/25°C	NG	NG	NG	NG
	LSD at $P \leq 0.05$	0.80	0.88	1.02	1.50
	$SED_{(d.f. = 12)}$	0.36	0.37	0.47	0.69
Light	Dark	4.50 ± 0.25	5.25 ± 0.22	9.25a ± 0.22	8.75b ± 0.22
	10 h	3.75 ± 0.22	5.50 ± 0.25	8.50ab ± 0.25	13.00a ± 0.35
	24 h	4.00 ± 0.35	5.50 ± 0.25	8.25b ± 0.22	12.5a ± 0.25
	LSD at $P \leq 0.05$	NS	NS	0.39	1.03
	$SED_{(d.f. = 9)}$	0.46	0.39	0.37	0.46
pH	Control	5.25a ± 0.19	6.78 ± 0.13	9.40 ± 0.17	11.64bc ± 0.54
	6	4.75ab ± 0.19	6.44 ± 0.32	9.31 ± 0.06	13.10ab ± 0.64
	7	4.50b ± 0.22	6.09 ± 0.13	8.92 ± 0.14	14.20a ± 0.69
	8	4.75ab ± 0.19	6.18 ± 0.55	9.33 ± 0.23	13.52a ± 0.38
	9	5.00ab ± 0.00	6.30 ± 0.35	9.07 ± 0.18	10.22c ± 0.59
	LSD at $P \leq 0.05$	0.70	NS	NS	1.70
	$SED_{(d.f. = 15)}$	0.33	0.47	0.23	0.80
NaCl	Control	4.50c ± 0.22	6.52b ± 0.23	9.16ab ± 0.13	12.60a ± 0.83
	50 mM	5.00bc ± 0.00	6.40b ± 0.16	8.43b ± 0.12	8.24b ± 0.96
	100 mM	5.75b ± 0.19	7.05ab ± 0.46	8.61b ± 0.31	3.48c ± 0.53
	150 mM	6.75a ± 0.37	8.17a ± 0.51	9.88a ± 0.45	2.11c ± 0.25
	200 mM	NG	NG	NG	NG
	250 mM	NG	NG	NG	NG
	LSD at $P \leq 0.05$	0.94	1.14	0.88	2.15
	$SED_{(d.f. = 12)}$	0.44	0.52	0.41	0.99
Osmotic potential	Control	4.50c ± 0.22	6.26c ± 0.13	9.27c ± 0.03	13.62a ± 0.73
	−0.2 MPa	5.75b ± 0.19	7.21b ± 0.07	9.91b ± 0.17	7.49b ± 0.69
	−0.4 MPa	7.75a ± 0.19	8.53a ± 0.38	11.38a ± 0.16	3.02c ± 0.23
	−0.6 MPa	NG	NG	NG	NG
	−0.8 MPa	NG	NG	NG	NG
	−1.0 MPa	NG	NG	NG	NG
	LSD at $P \leq 0.05$	0.84	0.75	0.44	1.90
	$SED_{(d.f. = 9)}$	0.37	0.33	0.20	0.84
Burial depth	0 cm	9.00b ± 0.32	11.82d ± 0.18	10.34d ± 0.21	4.05a ± 0.31
	2 cm	9.00b ± 0.32	11.77d ± 0.06	10.39d ± 0.14	4.18a ± 0.16
	4 cm	9.25b ± 0.19	11.77d ± 0.07	10.31d ± 0.14	4.05a ± 0.13
	6 cm	9.50b ± 0.22	13.15c ± 0.16	11.16c ± 0.16	4.32a ± 0.29
	8 cm	9.50b ± 0.22	13.75b ± 0.10	12.50b ± 0.10	2.46b ± 0.22
	10 cm	14.00a ± 0.32	14.70a ± 0.25	13.67a ± 0.21	1.63c ± 0.22
	LSD at $P \leq 0.05$	1.03	0.45	0.50	0.69
	$SED_{(d.f. = 18)}$	0.50	0.22	0.24	0.33

T_{50} or E_{50}, time to obtain 50% germination or emergence; MGT or MET, mean germination or emergence time; GI, germination index; EI, emergence index; SE, Standard error of the mean; NG, no germination; SED, standard error of the difference.
The values followed by different letters were significantly different at $P \leq 0.05$.

(Fig. 3B). Increase in pH did not affect T_{50} and MGT compared with the control, but GI decreased at pH 9 compared with the control or pH 7 (Table 2).

Effect of salt stress

A three-parameter logistic model $\{G\ (\%) = 89/[1 + (x/153)^{5.1}],\ R^2 = 0.988\}$ was fitted to the germination (%) of *E. spinosa* obtained at different NaCl concentrations (Fig. 4A). Germination of *E. spinosa* seed was tolerant up to 50 mM NaCl concentration. Germination was 87% at 50 mM NaCl and some germination (21%) occurred even at 200 mM NaCl. However, germination was completely inhibited at 250 mM NaCl. *Emex spinosa* germination was half of maximum germination with a NaCl concentration of 153 mM. When non-ger-

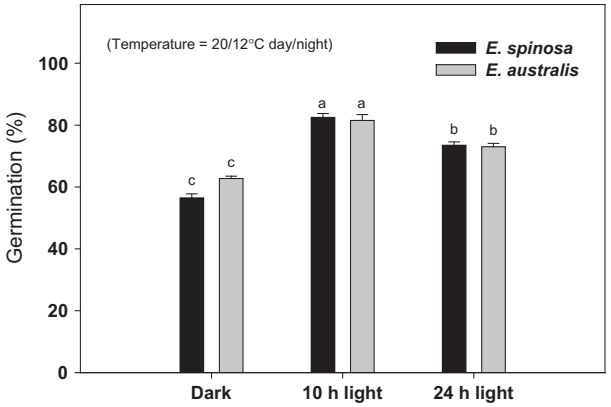

Fig. 2 Effect of light in a 10 h photoperiod and continuous dark on germination of *Emex spinosa* and *Emex australis*. Vertical bars represent ± standard error of the mean. The bars with different letters are significant different at $P > 0.05$ in each species.

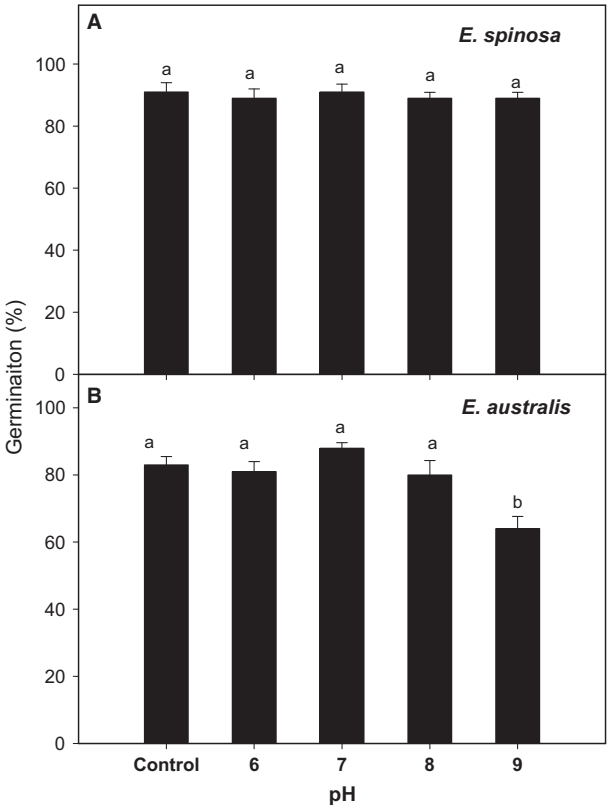

Fig. 3 Effect of pH on seed germination of *Emex spinosa* (A) and *Emex australis* (B). Vertical bars represent ± standard error of the mean. The bars with different letters are significant different at $P > 0.05$ in each species.

minated seeds of *E. spinosa* were removed form 250 mM NaCl and placed in distilled water, germination was 78%, indicating that the saline solution had not adversely affected seed viability. All concentrations of NaCl did not affect the time to start germination and MGT (Table 1). Salt concentration at 200 mM NaCl resulted in a significantly higher T_{50} as compared

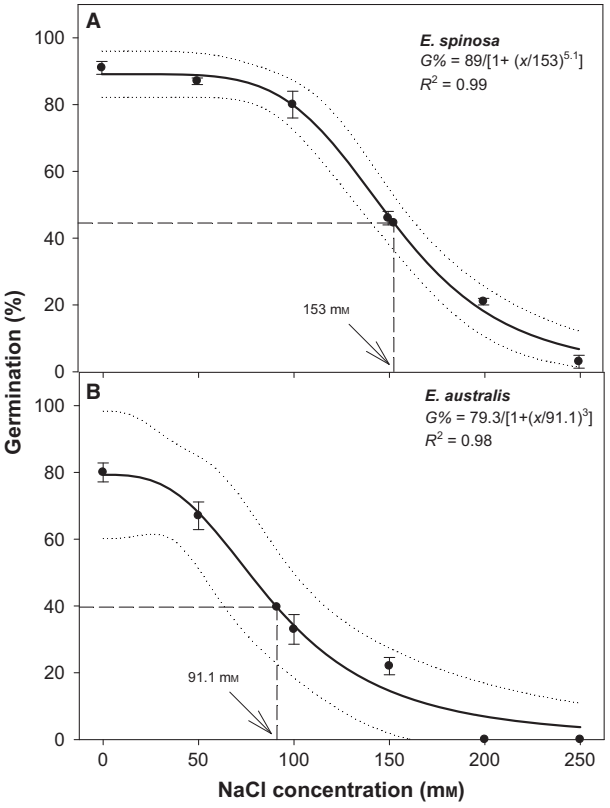

Fig. 4 Effect of NaCl concentration on seed germination of *Emex spinosa* (A) and *Emex australis* (B). The bold lines represent a three-parameter logistic model fitted to the data of both species and dotted lines show 95% confidence intervals. Vertical dash line represents *X*-axis value at 50% of maximum germination. Vertical bars represent ± standard error of the mean.

with 0–150 mM NaCl concentrations. GI was reduced significantly with increase in the salt concentration.

Germination of *E. australis* was steadily decreased with increase in NaCl concentration and completely inhibited at 200 mM (Fig. 4B). According to fitted model $\{G\ (\%) = 79.3/[1 + (x/91.1)^3],\ R^2 = 0.98\}$, 91.1 mM NaCl concentration was required to inhibit 50% of maximum germination of *E. australis* (Fig. 4B). When non-germinated seeds of *E. australis* were remove form 200 and 250 mM NaCl concentration and placed in distilled water, germination was 74%, indicating that the saline solution had not adversely affected seed viability. NaCl concentration of 150 mM significantly increased the time to start germination (6.75 day) compared with 50 mM (5.00 day), 100 mM (5.75 day) or control (4.5 day) (Table 2). Maximum time to obtain 50% germination (8.17 day) was observed with 150 mM NaCl concentration. MGT at 150 mM NaCl concentration was decreased when compared with 50 or 100 mM NaCl concentration. GI at 100 and 150 mM NaCl concentrations was significantly reduced when compared with control or 50 mM.

Effect of osmotic stress

A three-parameter logistic model {G (%) = 92/[1 + x/−0.33]$^{3.1}$, R^2 = 0.98} was fitted to germination % of *E. spinosa* obtained at different osmotic potential. Germination of *E. spinosa* significantly decreased from 98% to 10% as osmotic stress increased from 0 to −0.6 MPa (Fig. 5A). The osmotic potential required for 50% inhibition of the maximum germination of *E. spinosa* was −0.33 MPa. Germination was completely inhibited at osmotic potential of −0.8 MPa or greater. An osmotic potential of −0.6 MPa significantly increased the time to start germination (3.94 day) when compared with 0 to −0.4 MPa (3.00–3.25 day). There was no difference in T_{50} from 0 to −0.6 MPa (Table 1). Increase in osmotic stress from 0 to −0.4 MPa significantly increased the MGT and decreased the GI of *E. spinosa* seed. However, osmotic potential of −0.2 MPa gave similar GI to the control.

Germination of *E. australis* decreased from 84% to 28% as osmotic stress increased from 0 to −0.4 MPa and germination was completely inhibited at osmotic potential of −0.6 MPa. A three-parameter logistic model showed that osmotic potential for 50% inhibition of the maximum germination was −0.26 MPa (Fig. 5B). Maximum time to start germination (7.75 day), T_{50} (8.5 day) and MGT (11.38 day) was recorded at −0.4 MPa osmotic potential (Table 2). GI significantly decreased with increase in osmotic stress. *Emex australis* is more sensitive to osmotic stress in comparison with *E. spinosa*.

Effect of burial depth on seedling emergence

A three-parameter logistic model {E (%) = 72.2/[1 + (x/8.37)$^{3.09}$], R^2 = 0.97} was fitted to the seedling emergence data of *E. spinosa* from 0 to 10 cm burial depth. Emergence was 71% in seed that placed on soil surface which was similar to the seed that buried at 2 cm depth. The emergence after this point progressively decreased as depth increased (Fig. 6A). The fitted model estimated that 8.37 cm depth was required to inhibit 50% seedling emergence of the maximum seedling emergence. Maximum time to start emergence

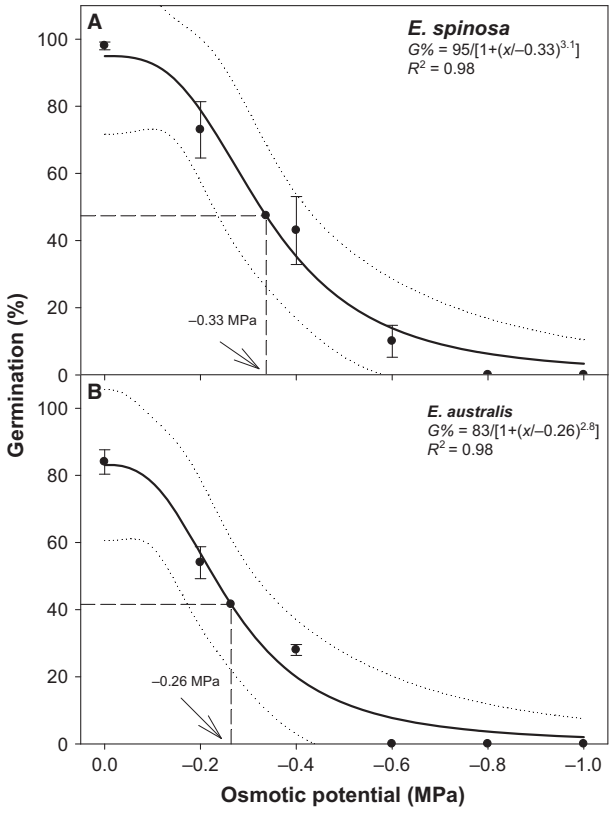

Fig. 5 Effect of osmotic potential on seed germination of *Emex spinosa* (A) and *Emex australis* (B). The bold lines represent a three-parameter logistic model fitted to the data of both species and dotted lines show 95% confidence intervals. Vertical dash line represents *X*-axis value at 50% of maximum germination. Vertical bars represent ± standard error of the mean.

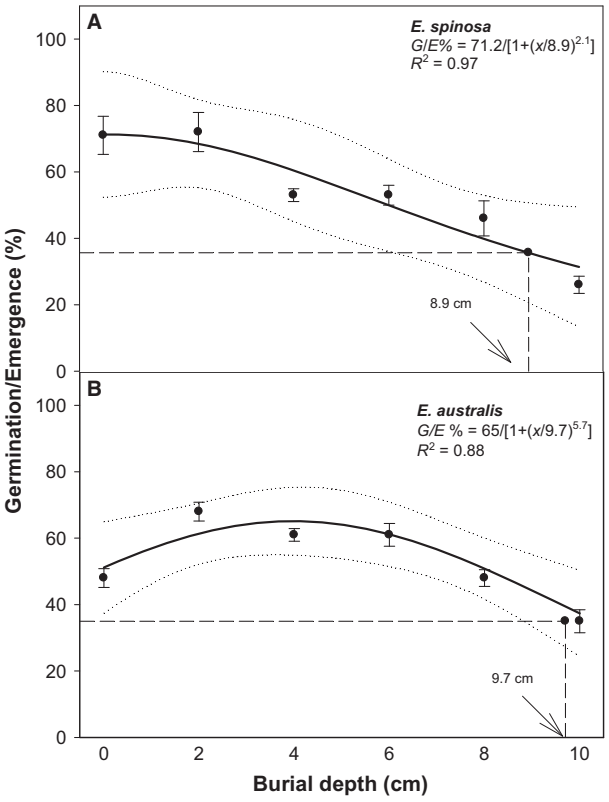

Fig. 6 Effect of burial depth on seedling emergence of *Emex spinosa* (A) and *Emex australis* (B). The bold lines represent a three-parameter logistic model fitted to the data of both the species. Dotted lines show 95% confidence intervals. Vertical dash line represents *X*-axis value at 50% of maximum emergence. Vertical bars represent ± standard error of the mean.

(13.75 day) was recorded with 10 cm burial depth and significantly decreased with decreased in burial depth up to 4 cm (Table 1). However, there was no significant difference in time to start emergence among burial depth from 0 to 4 cm. After 6 cm burial depth, T_{50} and MET progressively increased with as burial depth increased. EI decreased with increasing depth of burial.

A three-parameter logistics model $\{E\ (\%) = 67.3/[1 + (x/9.7)^{5.7}],\ R^2 = 0.88\}$ was fitted to the seedling emergence of *E. australis* at burial depths of 0–10 cm (Fig. 6B). Seed placed at the soil surface (0 cm) showed lower emergence as compared with 2 cm depth. Seedling emergence increased from 48% to 68% as burial depth increased from 0 to 2 cm; after this depth, the seedling emergence decreased as burial depth increased. According to fitted model, depth for 50% inhibition of the maximum seedling emergence was 9.7 cm. *Emex australis* seed placed at 8 or 10 cm took maximum time to emerge as compared with all other depths. Burial depth 6 cm or greater significantly increased the E_{50} and MET. Whereas, burial depth of 8 cm or greater significantly reduced the EI of *E. australis*. Seedling emergence of *E. spinosa* from the seed placed on soil surface was greater than that of *E. australis* (Fig. 6A,B), which can lead to survival of *E. spinosa* in no-till farming system.

Discussion

Emex australis and *E. spinosa* are weeds of winter crops and problematic in wheat. In wheat, both weeds germinate in November and December and attain maturity in April and May (Marshall & Weiss, 1982; Javaid & Tanveer, 2013). Our data suggested that the weed species are capable of emerging in a wide range of environmental conditions. *Emex spinosa* and *E. australis* have maximum germination at 20/12°C; however, considerable germination also occurs below or above this temperature with low germination parameters (time to start germination/emergence, time taken to 50% germination/emergence, mean germination/emergence time and germination/EI). These results showed that seed germination and germination parameters of both species are affected by warmer temperature (>30°C) which leads to low degree of success of the species. However, increase in temperature from 10/15 to 20/12°C day/night stimulated germination of both species (Fig. 1). No germination occurred at 35/25°C, indicating that these species act as winter annual weeds that germinate only at temperatures ranging from 15/10 to 30/20°C. Considering average soil temperature (20°C) in November and December in southern Punjab, Pakistan, temperature would not be a limiting factor for germination of both species. Similarly, Weiss (1980) reported that germination of aerial achenes of *E. spinosa* was temperature dependent, and optimum germination was recorded between 18/13 and 27/22°C. Benvenuti *et al*. (2001) have reported that thermal optima for germination of a Polygonaceae member *Rumex obtusifolius* L. were between 20 and 25°C in light or dark.

Maximum germination of the weeds occurred under light conditions. The high level of germination in the absence of light indicates that both species can germinate without light trigger, although light may improve germination. These results indicate that *E. australis* and *E. spinosa* may germinate in soil and under plant canopy and litter shade. Similar to our results, Evenari *et al*. (1977) reported that *E. spinosa* seeds have higher germinability in light than dark at 25°C. Similarly, Weiss (1980) compared the germination of aerial and subterranean achenes of *E. spinosa* under light and dark conditions and found that aerial achenes had a strong light requirement for germination, but there was no significant difference in subterranean achenes between light and dark treatments.

Both weeds can germinate over a wide range of pH (6–9), which covers the pH range of the most Pakistani soils, but pH 9 depressed germination parameters, and *E. australis* is more sensitive as compared with *E. spinosa* at pH 9. The ability of these species to germinate over a wide range of pH indicates that they are able to adapt to wide range of soil conditions. This characteristic is common for invasive weed species (Watanabe *et al*., 2002) and it will aid *E. spinosa* and *E. australis* ability to invade diverse habitats. A number of previous studies indicated that the germination of weed species was not affected by pH levels from 4 to 9 (Chachalis *et al*., 2008). Our data suggested that *E. spinosa* was fairly tolerant to salinity as compared with *E. australis*. Salinity might negatively affect some important physiological processes in plants. Additionally, sodium ions can alter soil structure and fertility by replacing calcium and magnesium in the anion exchange process, and this leads to nutrient and water stress (DiTommaso, 2004). Ability to germinate at high pH and salinity level will enable these weed to spread in lowland areas of Pakistan. This could be an important attribute of weed that enables it to colonise saline areas. In a way similar to *E. spinosa* and *E. australis*, seed of *Mimosa invisa* Mart. (giantsensitive plant) (Chauhan & Johnson, 2008) and *Brassica tournefortii* Gouan (African mustard) (Chauhan *et al*., 2006b) germinated at high concentration of NaCl.

Water stress is an important factor limiting seed germination and depressing germination parameters of both the species. These results help to explain the association between rain or water availability and

germination of the *Emex* species. Our finding also indicated that germination parameters of both species declined with increase in osmotic stress, which may lead to weaker seedlings. *Emex australis* weed spread may be restricted to well-drained soil due to its inability to germinate under low soil moisture condition. However, *E. spinosa* may spread in areas with low moisture conditions, because this species exhibited potential to tolerate low water potential. Measures should be taken to control the weed after irrigation or rain in winter crops. Several weed species such as *Eupatorium adenophorum* Spreng. (crofton weed) (Lu *et al*., 2006), *Caperonia palustris* L. (texasweed) (Koger *et al*., 2004), *Synedrella nodiflora* Gaertn. (synedrella) (Chauhan & Johnson, 2009) and *Scoparia dulcis* L. (goat weed) (Jain & Singh, 1989) are sensitive to osmotic stress. In contrast, other weed species such as *Hibiscus trionum* L. (venice mallow) (Chachalis *et al*., 2008), *Rapistrum rugosum* L. (turnipweed) (Chauhan *et al*., 2006a) and *Solanum sarrachoides* Sendtn. (hairy nightshade) (Zhou *et al*., 2005) exhibited potential to tolerate low water potential.

Seedling emergence of *E. spinosa* was adversely affected with increasing burial depth from 0 cm to onward. Highest germination of *E. spinosa* at soil surface indicates that this species can germinate under limited moisture conditions, corroborated by the fact that *E. spinosa* was less sensitive to water stress compared with *E. australis*. *Emex spinosa* is likely to be favoured in no-till systems or under deep tillage conditions, because considerable germination or emergence of the weed occurred when seeds were placed on the soil surface, as well as seeds buried at 8 cm. Seedling emergence of *E. australis* increased when burial depth increased from 0 to 2 cm and then declined with increasing burial depth (Fig. 6). *Emex australis* seed placed at the soil surface showed lower germination when compared with emergence at 2 cm depth. This might be due to lack of moisture at the soil surface. These results are in line with those of Andersson *et al*. (2002), who reported that seeds of four *Bromus* species sown on the soil surface had delayed and reduced germination compared with emergence of buried seeds, and germination was slightly inhibited by light. However, considerable emergence of both species occurred even at a burial depth of 10 cm. Thus, they will be difficult to manage by burying the seed using deep tillage. Our results supported the findings of Baskin and Baskin (1998), who reported that larger weed seeds with greater carbohydrates reserves can germinate from greater depths. It has been reported that considerable seedling emergence of some weed species may occur from 8 cm (Baskin & Baskin, 1998). In contrast, seedling emergence of many weed species is adversely affected with increasing the depth of burial (Benvenuti, 2003). Deep tillage that will bury the seed below 6 cm or greater is a possible weed management option for farmers.

Emex australis took more time to start germination or emerge compared with *E. spinosa*, even in control treatments (Tables 1 and 2). Germination or emergence % and GI or EI of *E. spinosa* in all the experiments are greater than those of *E. australis*. Weiss and Julien (1975) reported that *E. spinosa* is more erect, has small and more seeds than *E. australis* and may become a more serious weed than *E. australis*. Our data suggested that *E. australis* may not be considered as much of a problem as *E. spinosa* in Punjab, Pakistan. However, both weeds have a capacity to disperse and establish in a range of conditions and thus are likely to be successful weeds.

Acknowledgements

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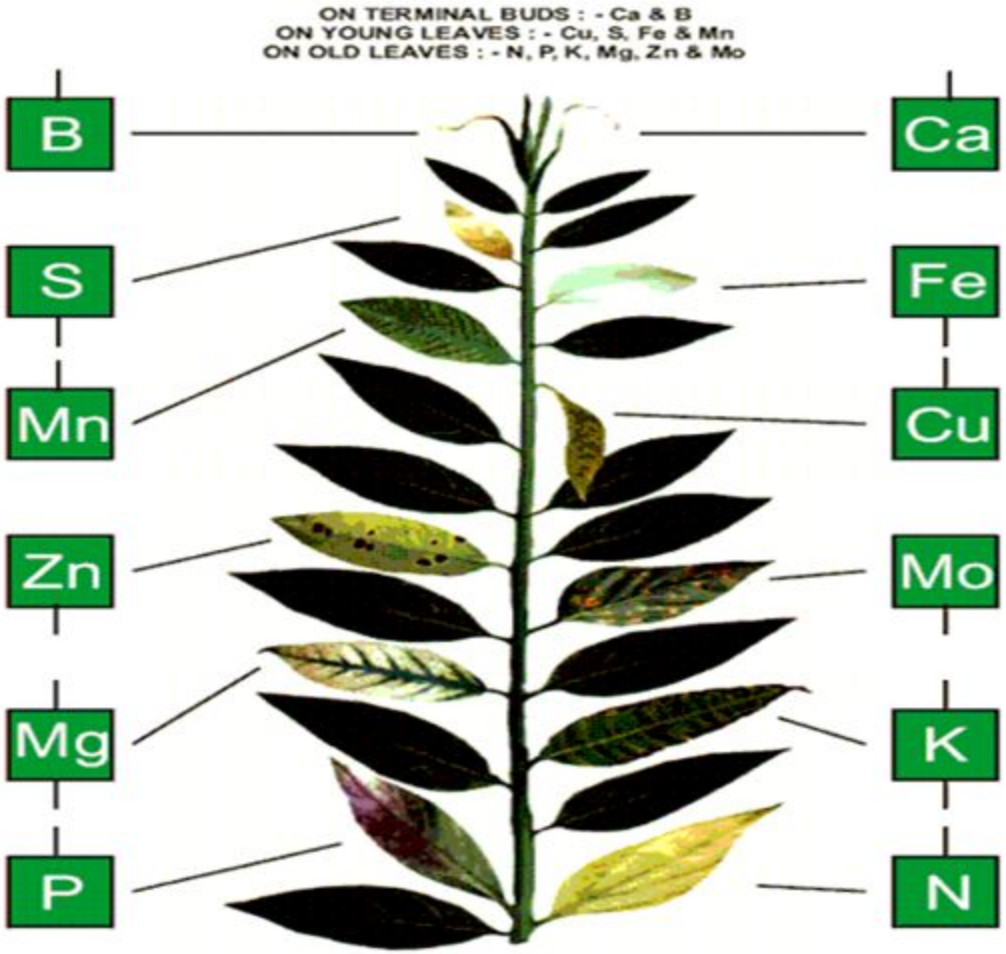
“NUTRIENTS STRESS ON CROP PLANTS”

Nutrients Deficiency Symptoms on Leaves

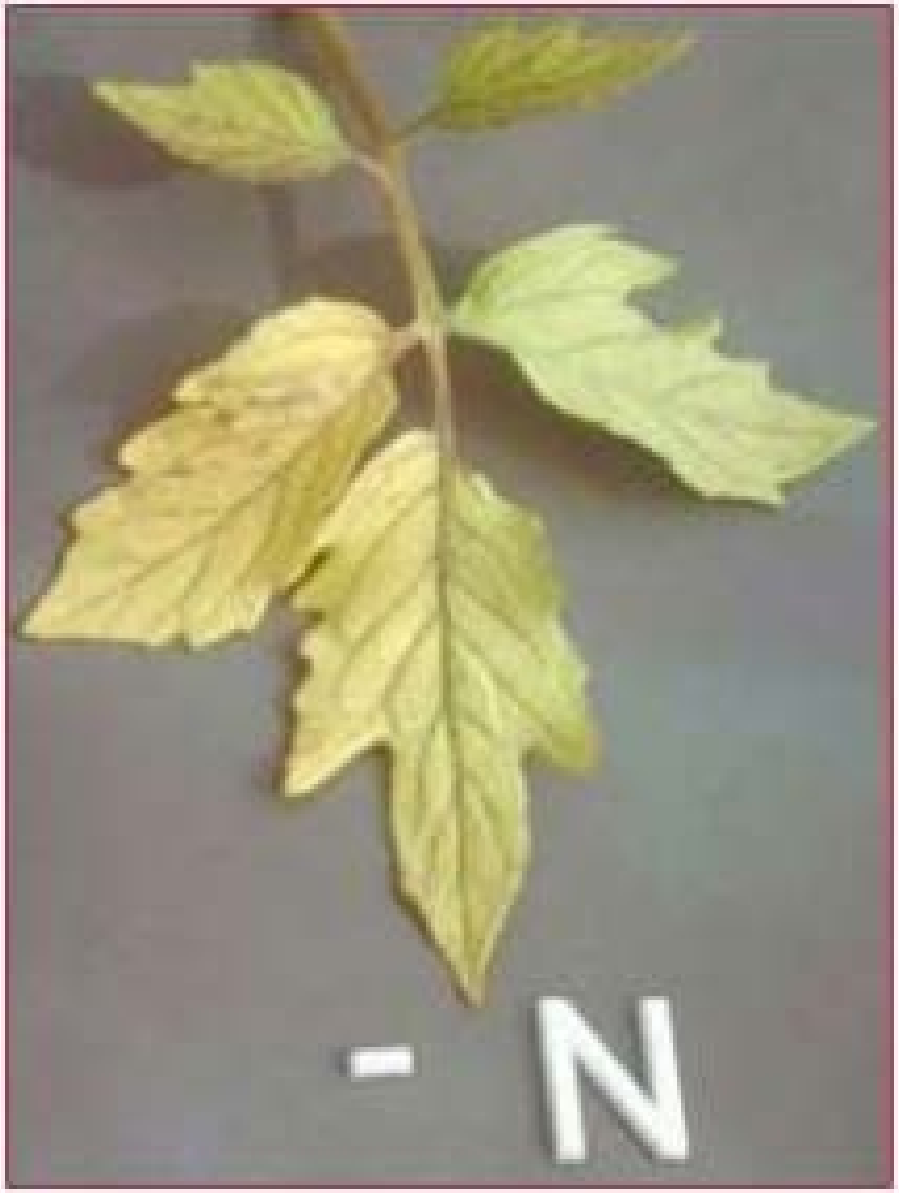
The most common symptoms of nutrient deficiency are stunted growth and leaf discoloration. The position of the symptoms (distal, basal or intermediate) depends on the mobility of the nutrient inside the plant (young leaves competing with oldest leaves)



Nutrient deficiency symptoms in plants

NITROGEN:

- Chlorotic symptoms shown on leaves are direct source result of N deficiency.
- Older leaves become uniformly yellow (chlorotic).
- Branching is reduced resulting in short spindly plants.



Nitrogen deficient tomato leaf

Healthy tomato leaf

PHOSPHORUS:

- Typical symptom is necrotic spots on leaves.
- Plant are dwarf or stunted is the major visual symptom.
- Sever deficiency conditions there is also tendency for leaves to develop a blue-gray luster.

Phosphorus deficient tomato leaf

POTASSIUNM:

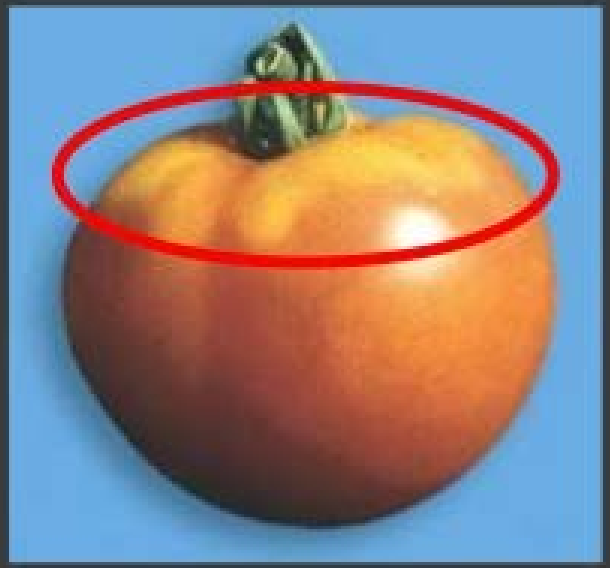
- Leaves show marginal necrosis (tip burn).
- The onset of K deficiency is generally characterized by a marginal chlorosis.
- The leaves show more advanced deficiency status, with necrosis in the interveinal spaces between the main veins along with interveinal chlorosis.

Because potassium is very mobile within the plant, symptoms only develop on young leaves in the case of extreme deficiency.

Potassium deficient tomato leaf

Initial symptoms on tomato leaf

Typical potassium (K) deficiency of fruit is characterized by color development disorders, including greenback, blotch ripening and boxy fruit .



CALCIUM:

- Calcium deficient leaves show necrosis around the base of leaves.
- Classic symptoms of C deficiency includes blossom end rot (BER) burning of the end part of tomato fruit.
- The blossom end area darkens & flattens out, then appearing leathery & dark brown, and finally it collapses & secondary pathogen take over the fruit.

Plants under chronic calcium deficiency have a much greater tendency to wilt than non-stressed plants.

Calcium-deficient leaves

Blossom-end rot

MAGNESIUM:

- leaves show advanced interveinal chlorosis, with necrosis developing in the high chlorotic tissue.
- In its advanced form, Magnesium deficiency may superficially resembles potassium deficiency.
- Symptoms generally starts with mottled chlorotic areas developing in the interveinal tissues.

Magnesium deficient tomato leaf

Healthy tomato leaf

SULPHUR:

- The veins & petioles exhibit a very distinct reddish color.
- Visual symptom of S deficiency are very similar to the chlorosis found in nitrogen deficiency.
- However, in Sulphur deficiency the yellowing is much more uniform over the entire plant including young leaves.

With advanced sulfur deficiency brown lesions and/or necrotic spots often develop along the petiole, and the leaves tend to become more erect and often twisted and brittle

Manganese

- These leaves shows interveinal chloroses develop under limited supply of mn.
- The early stages of chloroses induce by manganese deficiency are somewhat similar to iron deficiency.
- As the stress increase, the leaves take on a gray metallic sheen and develop dark freckled and necrotic area around the veins.

A purplish luster may also develop on the upper surface of the leaves.

Iron

- The iron deficient leaves show intense chlorosis at the base of the leaves with some green netting.
- The most common symptom for iron deficiency start out as an interveinal chlorosis of the youngest leaves , evolves into an overall chlorosis , and ends as a totally bleached leaf.
- The bleached areas often develop necrotic spots.
- Up until the time the leaves become almost completely white they will recover upon application of iron.

Iron deficiency is strongly associated with calcareous soils and anaerobic conditions, and it is often induced by an excess of heavy metals

Copper

- The copper deficient leaves are curled and petioles bend downward.
- Copper deficiency may be expressed as light overall chlorosis along with the permanent loss of turgor in the young leaves.
- Recently matured leaves show netted , green veining with areas bleached to a whitish gray.

Some leaves develop sunken necrotic spots and have a tendency to bend downward.

Zinc

- This leaf's show an advanced case of interveinal necrosis.
- In the early stages of zinc deficiency the younger leaves become yellow and pitting develop in the interveinal upper surface of the mature leaves.
- As the deficiency progresses these symptom develop into an intense interveinal necrosis but the main vein remain green , as in the symptom of the recovering iron deficiency.

Zinc deficiency

Thank You