

## Studies on seed germination and growth in weed species of rice field under salinity stress

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### Abstract

An investigation was made to see the salt tolerance of 10 weed species of rice. Properly dried and treated seeds of weed species were placed on 9 cm diameter petridishes lined with Whatman No. 1 filter paper under 6 salinity regimes, viz. 0 (control), 4, 8, 16, 24 and 32 dS m<sup>-1</sup>. The petri dishes were then kept in germinator at 25±1.0°C and 12 hr light. The number of germinated seeds were recorded daily. The final germination percentage, germination index (GI), seedling vigour index, mean germination time and time for 50% germination were estimated. Root and shoot lengths of the weed seedlings were measured at 20 days after salt application and relative growth values were calculated. Results revealed that salinity decreased final germination percentage, seed of germination as measured by GI, and shoot and root length in all the species. Germination of most of the weed seeds was completely arrested (0) at 32 dS m<sup>-1</sup> salinity except in *E. colona* (12%) and *C. iria* (13.9%). The species *C. iria*, *E. colona*, *J. linifolia* and *E. crusgalli* showed better germination (above 30%) upto 24 dS m<sup>-1</sup> salinity level and were regarded as salt-tolerant weed species. *J. linifolia*, *F. miliacea*, *L. chinensis* and *O. sativa* L. (weedy rice) were graded as moderately tolerant and *S. zeylanica*, *S. grosus* and *C. difformis* were regarded as least tolerant weed species.

### Key words

Rice-field weeds, Weed seed germination, Seedling growth, Germination index, Salinity stress

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### Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the world population and the global rice production is 550 million tons and 2.15 million tons is in Malaysia (Rao *et al.*, 2007). Weed is a serious pest for rice causing annual yield loss by 15-21% worldwide (Oerke *et al.*, 1994). They account annual rice yield loss by 30-40% in Sri Lanka (Abeysekera, 2001), 30-90% in India (Murkhopadhyay, 1995), 57-61% in Philippines (Mukherjee *et al.*, 2008), 10-35% in Malaysia (Karim *et al.*, 2004; Lo *et al.*, 1990). It

has been estimated that the yield losses of rice by grasses, broad-leaved and sedges were 41, 28 and 10%, respectively in Malaysia (Azmi, 1992). Recently reported that grain yield loss of rice in Malaysia about 42% in uncontrolled field by the infestation of *Fimbristylis miliacea*. Generally rice crop is sensitive to salinity. Predicted global climate changes will cause sea levels rising and it will not only increase the salinity level of the salt-affected lands, rather will also increase the total saline area. Salinity increases rapidly in such areas where the global temperature is high (Lund

and Curry, 2006). In fact, salinity is a major abiotic constraint for crop production worldwide which affect many important physiological processes (Munns and Tester, 2008). Germination and seedling growth of plant species are reduced in saline soils due to an external osmotic potential that prevents water uptake or due to the toxic effects of  $\text{Na}^+$  and  $\text{Cl}^-$  ions or both on the germinating seed (Murrillo-Amador et al., 2002). Weeds are life-long companions of rice crops in the field. In comparison to crops, weeds are generally more tolerant to the environmental stresses especially *Echinochloa oryzochloa* is relatively more tolerant to salt (Kim et al., 1999). Abogadallah et al. (2010) noted that seed germination of barnyard grass (*E. crusgalli*) is influenced due to salinity stress. Therefore, efforts are underway to develop salt tolerant rice cultivar for the cultivation in saline area. Weed management is very essential for successfully rice cultivation in the salt affected area. In coastal areas of Malaysia, salinity is a major obstacle towards satisfactory rice yield. Previously about 10 major weed species were identified in the rice field at the coastal area of Peninsular Malaysia by Hakim et al. (2010). Our present investigation was conducted with those 10 (10) major weed species. Although much research have been done for understanding the influences of saline habitats on seed germination, seedling growth, reproduction and population dynamics of crop plants (Khan et al., 2002), however, scant attention has been paid to the responses of weeds to salinity. It is necessary to identify the tolerance level of weed species at germination and early growth stages for successful weed management in the rice fields under saline environment. Therefore, this study was conducted to assess the seed germination and growth of 10 major weeds of rice field at different levels of salinity.

### Materials and Methods

The experiment was conducted in the laboratory of Weed Science, Department of Crop Science, University Putra Malaysia during the period from March to June 2009. Seeds of 10 major rice-field weed species namely *Leptochloa chinensis*, *Echinochloa crusgalli*, *Echinochloa colona*, *Jussia linifolia*, *Sphenodea zeylanica*, *Fimbristylis miliacea*, *Oryza sativa* L. (weedy rice), *Cyperus iria*, *Cyperus difformis* and *Scirpus grossus* were collected from the mature weed plants of rice fields of Peninsular Malaysia during February to July 2008. After sun drying for three days the seeds were kept in glass bottles at a temperature 45°C for 4 months. Forty healthy seeds of uniform size were selected for each treatment. Seeds were allowed to germinate on two sheets of filter paper (Whatman No. 1) in 9 cm diameter petridishes. Each filter paper was moistened with either 10 ml of water or salt solution of 5 different salinity levels viz. 0 (distilled water as a control), 4, 8, 16, 24 and 32  $\text{dS m}^{-1}$  NaCl and was then placed in seed germinator plate. Temperature in the germinator was maintained at  $25 \pm 1^\circ\text{C}$  with 12 hr light. The petridishes were arranged in completely randomized design (CRD) with 4 replications of each treatment. The number of germinated seeds was counted daily and was continued up to 20

days. Shoot and root length of seedlings were measured at 20 DAS (days after seeding). After final count, final germination % (FGP) and germination index (GI), which indicates the seed of germination, were calculated as described in the Association of Official Seed Analysis (AOSA, 1983). Mean germination time (MGT) was calculated as per Ellis and Robert, 1981. The time to 50% germination ( $T_{50}$ ) was calculated using the method of Farooq et al. (2005). The seedling vigor index was calculated according to the method of Abdul-Baki and Anderson, 1973. The relative growth value of different weed species was calculated by following the formula proposed by Ashraf and Waheed (1990).

This experiment was repeated two times. In the preliminary analysis two experiments data were considered together taking time as a factor but there was no significant difference between the means of two times data ( $F$  value = 0.1857). So, the pooled values are presented. Data were analyzed using analysis of variance (ANOVA) and means were separated by least significant difference (LSD) using statistical analysis system (SAS, Version 9.0).

### Results and Discussion

Final germination was affected even at 4  $\text{dS m}^{-1}$  with clear differences among the species and was inversely and linearly related to salt concentration (Fig. 1). At 4  $\text{dS m}^{-1}$  salinity, the most affected species was *S. zeylanica* (76% germination) and around 85% germinations were recorded in species *S. grossus*, *O. sativa* (weedy rice), *C. difformis* and *F. miliacea*. While rest of the species were least affected with more than 90% germination at this salinity level. With further increase in salinity levels, species differences were even clearer. Species *S. grossus*, *S. zeylanica* and *C. difformis* appeared to be the most salt sensitive with less than 65% germination at 8  $\text{dS m}^{-1}$ , less than 17% at 16  $\text{dS m}^{-1}$  and at 24  $\text{dS m}^{-1}$ , germination was completely inhibited in these species. While *C. iria*, *E. colona*, and *J. linifolia* appeared to the most salt tolerant achieving more than 80% germination at 8  $\text{dS m}^{-1}$ , over 60% at 16  $\text{dS m}^{-1}$  and over 30% germination at 24  $\text{dS m}^{-1}$ . At 32  $\text{dS m}^{-1}$  salinity however, though germination of *J. linifolia* was completely inhibited; species *C. iria* and *E. colona* still had about 12% germination. Salt tolerance of *L. chinensis*, *E. crusgalli*, *F. miliacea* and *O. sativa* (weedy rice) appeared to be at intermediate level. However, salinity reduced the germination rate to less than 50% at levels of salinity  $>16 \text{ dS m}^{-1}$ . Germination of *C. difformis* was affected more by increasing the salt concentration than the others species, *C. iria* and *F. miliacea*. These findings are supported by Chauhan and Johnson, 2009; Khan et al. (2006), Abogadallah et al. (2010) reported that among five, one genotype of barnyard grass (*E. crusgalli*) named "Fows A3", germinated in 300 mM NaCl (43%). Similar result was observed by Chauhan and Johnson (2009) that the seed germination of jungle rice (*E. colona*) decreased linearly with increasing the NaCl concentration. Percent seed germination of *E. crusgalli* reduced

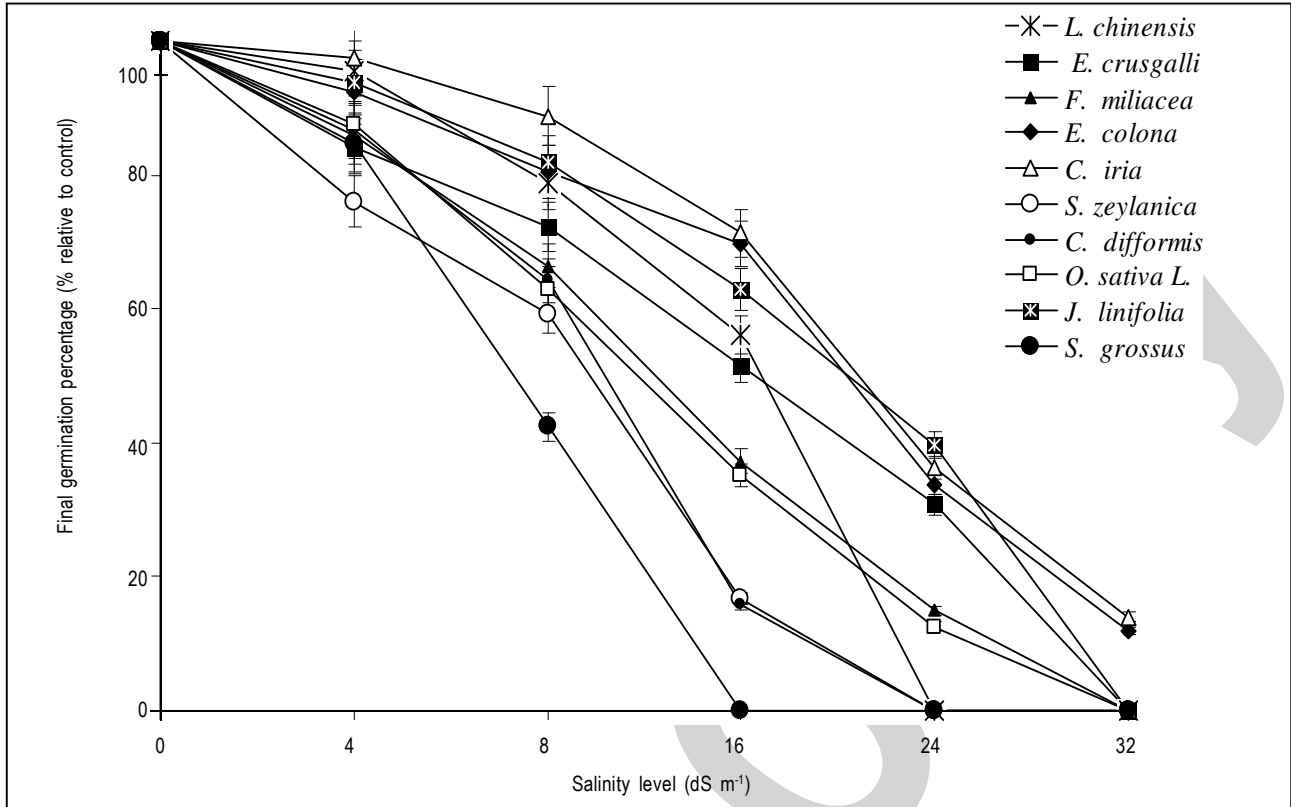


Fig. 1: Effect of salinity (NaCl) on final germination percentage of different weed species

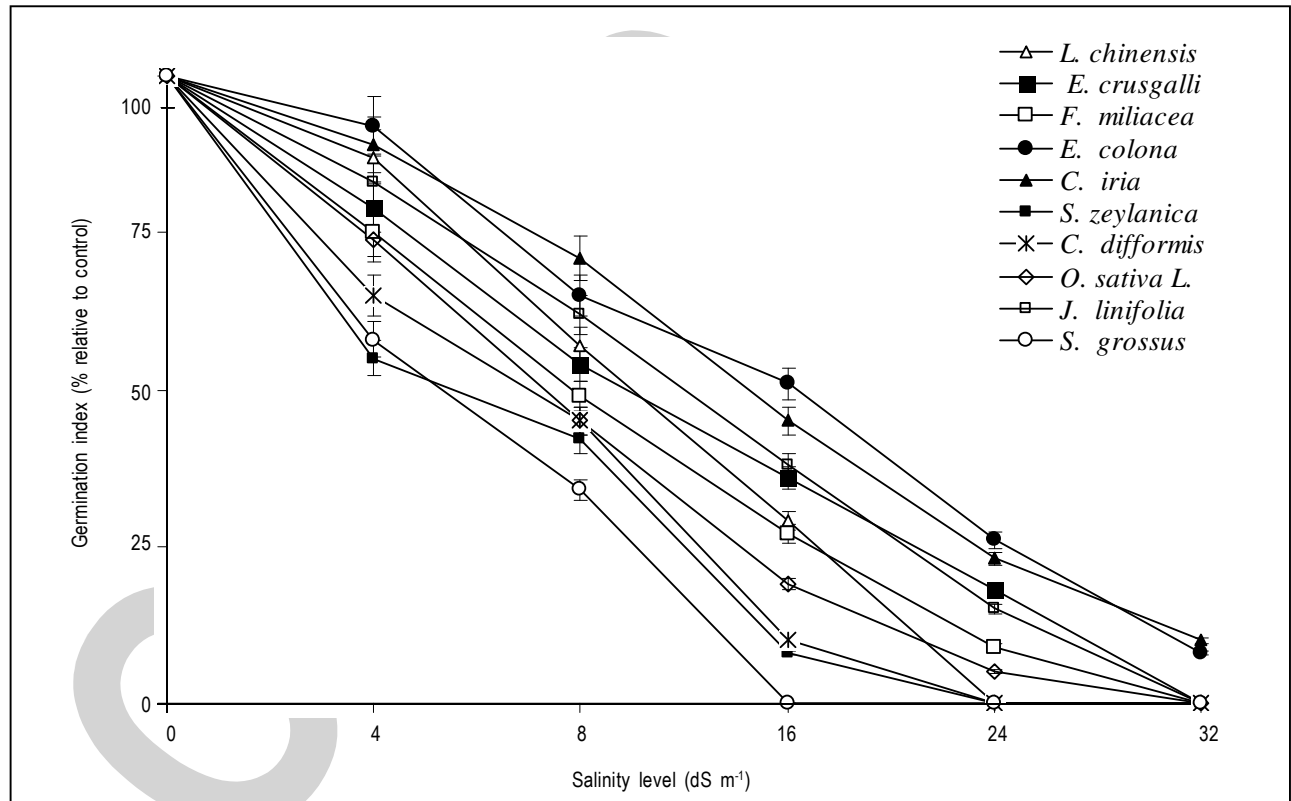


Fig. 2: Effect of salinity (NaCl) on germination index of different weed species

**Table - 1:** Effect of salinity (NaCl) on shoot length of different weed species

Species	Salinity level (dS m <sup>-1</sup> ) on shoot length (mm)					
	0	4	8	16	24	32
<i>L. chinensis</i>	7.7e f	5.3 e (69)	3.7 d (48)	2.3 e (30)	0.0 e	0.0 b
<i>E. crusgalli</i>	39.5 b	32.2 b (82)	24.0 b (61)	14.2 a (36)	7.0 a (18)	0.0 b
<i>F. miliacea</i>	8.8 e	7.1 d (81)	4.2 d (48)	2.5 e (29)	1.5 e (17)	0.0 b
<i>E. colona</i>	21.0 c	17.2 c (82)	13.2 c (63)	9.9 c (47)	5.9 b (28)	2.1 a (10)
<i>C. iria</i>	11.9 d	10.2 cd (86)	7.8 cd (66)	5.9 d (50)	3.8 c (32)	1.9 a (16)
<i>S. zeylanica</i>	12.3d	7.4 d (60)	4.0 d (33)	1.9 e (16)	0.0 e	0.0 b
<i>C. difformis</i>	11.2 d	7.2 d (63)	3.5 d (31)	2.2 e (20)	0.0 e	0.0 b
<i>O. sativa</i>	54.0 a	45.5 a (84)	27.7 a (51)	12.2 b (23)	6.5 a b (12)	0.0 b
<i>J. linifolia</i>	6.8 f	5.5 e (81)	3.8 d (56)	2.6 e (38)	1.7 d (25)	0.0 b
<i>S. grossus</i>	9.0 e	5.2 e (58)	2.5 e (28)	0.0 f	0.0 e	0.0 b

Means with the same letter in the columns do not differ significantly ( $p < 0.05$ ). Values within parenthesis indicate percent value relative to control

**Table - 2:** Effect of salinity (NaCl) on root length of different weed species

Species	Salinity level (dS m <sup>-1</sup> ) on root length (mm)					
	0	4	8	16	24	32
<i>L. chinensis</i>	10.5 e	7.0 d (67)	3.8 e (36)	1.7 e (16)	0.0 d	0.0
<i>E. crusgalli</i>	30.5 b	25.0 b (82)	15.0 b (49)	7.5 a (25)	2.7 a (9)	0.0
<i>F. miliacea</i>	7.6 f	3.8 g (50)	2.1 f (26)	1.3 e (17)	0.0 d	0.0
<i>E. colona</i>	18.6 c	16.2 c (87)	9.8 c (53)	6.4 b (34)	2.3 b (12)	0.0
<i>C. iria</i>	15.0 d	12.4 d (83)	6.8 d (45)	4.6 c (31)	1.9 c (13)	0.0
<i>S. zeylanica</i>	10.3 e	6.3 ef (61)	3.5 e (34)	1.2 e (12)	0.0 d	0.0
<i>C. difformis</i>	10.0 e	5.8 f (58)	2.4 f (24)	0.0 f	0.0 d	0.0
<i>O. sativa</i>	50.0 a	39.2 a (78)	20.2 a (40)	7.5 a (15)	0.0 d	0.0
<i>J. linifolia</i>	15.5 d	12.6 c (81)	7.7 d (50)	2.8 d (18)	0.0 d	0.0
<i>S. grossus</i>	7.5 f	3.6 g (48)	1.7 f (23)	0.0 f	0.0 d	0.0

Means with the same letter in the columns do not differ significantly ( $p < 0.05$ ). Values within parenthesis indicate percent value relative to control

**Table - 3:** Effect of salinity (NaCl) on time (days) to 50% germination of different weed species

Species	Salinity level (dS m <sup>-1</sup> ) on germination					
	0	4	8	16	24	32
<i>L. chinensis</i>	3.65 f	5.38 e	8.12 d	10.85 c	0.0 c	0.0 c
<i>E. crusgalli</i>	5.21 e	8.77 c	9.54 c	11.32 c	13.00 b	0.0 c
<i>F. miliacea</i>	8.98 b	10.13 b	11.96 b	13.28 b	15.19 a	0.0 c
<i>E. colona</i>	5.81 de	6.81 d	8.08 d	10.05 c	12.73 b	14.15 b
<i>C. iria</i>	3.90 f	4.79 e	7.06 d	10.29 c	13.13 b	16.00 a
<i>S. zeylanica</i>	10.08 a	13.29 a	15.09 a	16.23 a	0.0 c	0.0 c
<i>C. difformis</i>	6.94 c	8.42 c	10.14 c	12.93 b	0.0 c	0.0 c
<i>O. sativa</i>	6.22 cd	8.06 c	9.45 c	10.68 c	14.88 a	0.0 c
<i>J. linifolia</i>	5.04 e	6.58 d	8.09 d	10.69 c	13.45 b	0.0 c
<i>S. grossus</i>	8.57 b	10.51 b	11.79 b	0.0 d	0.0 c	0.0 c

Means with the same letter in the columns do not differ significantly ( $p < 0.05$ )

significantly with increasing the salt concentration of NaCl and no seed germinated at higher salinity levels of 2% NaCl (Rahman and Ungar, 1990). Chauhan *et al.* (2006) observed that the germination percentage of annual sowthistle seeds was significantly decreased with increasing the salinity level and germination completely inhibited at 320 mM NaCl concentration.

At salinity levels of 4 and 8 dS m<sup>-1</sup>, *C. iria*, *J. linifolia* and *E. colona* showed higher germination index (GI) followed by *L. chinensis* and *E. crusgalli* and the lowest performance was recorded in *S. grossus* (Fig. 2). As salinity increased to 24 dS m<sup>-1</sup> *C. iria*, *E. colona*, *E. crusgalli* and *J. linifolia* was the best performer but at 32 dSm<sup>-1</sup> level only two species viz. *C. iria* and *E. colona* showed

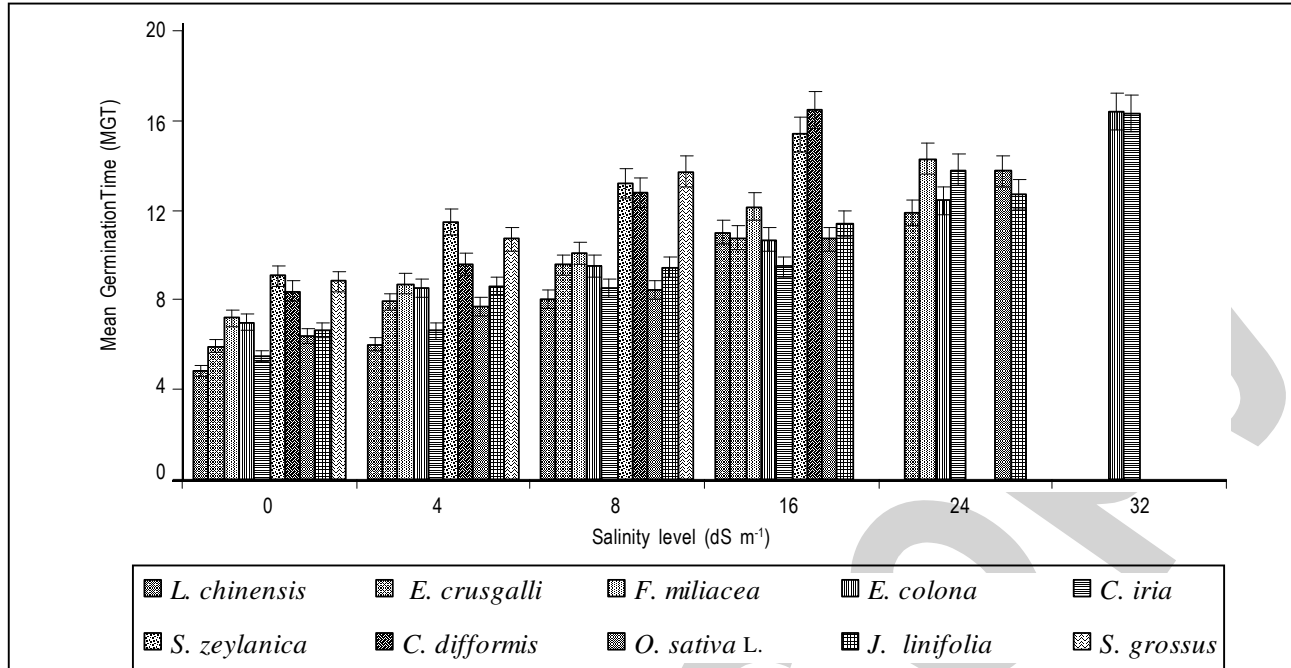


Fig. 3: Effect of salinity (NaCl) on mean germination time (days) of different weed species

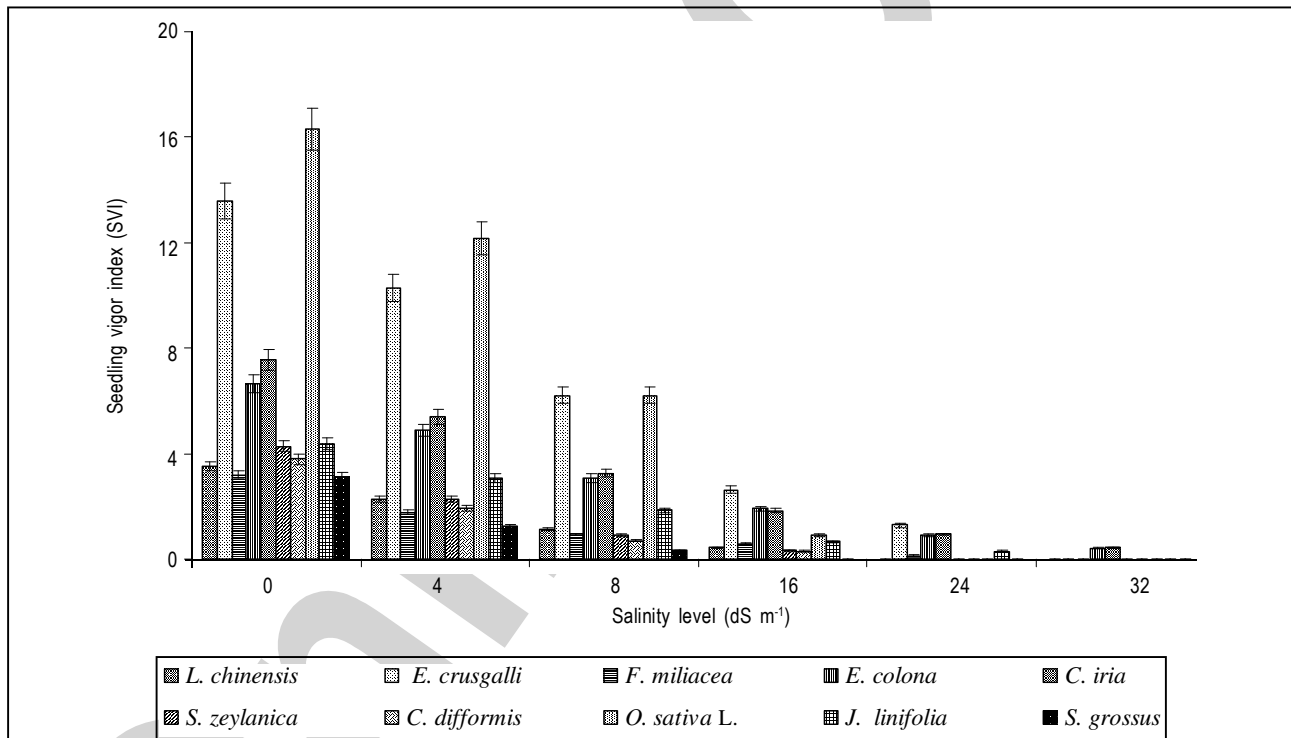


Fig. 4: Effect of salinity (NaCl) on seedling vigor index of different weed species

better GI value compared to others (Fig. 2). The results of our study supported by Carpici *et al.* (2009) and observed that increasing salinity levels linearly decreased germination index in all of the cultivars of maize (*Zea mays L.*). Khan *et al.* (1997) found that the decreasing trend of GI value with the increase in salinity. The

germination seed of common ragweed was significantly decreased with increasing the salt concentration (Ditommaso, 2004). Rejili *et al.* (2009) also reported that the germination index of *Lotus creticus (L.)* decreased significantly due to increase in salinity levels. Germination index of chickpea (*Cicer arietinum L.*) was significantly

affected by NaCl and seed size. Small seeds of all cultivars of chickpea showed the highest GI values at all NaCl concentrations, while GI gradually decreased by the increasing of NaCl concentration (Kaya et al., 2008).

Increase of mean germination time (MGT) indicates that germination is delayed comparatively. In this study MGT increased with increasing salinity levels in all species (Fig. 3). At 8 dS m<sup>-1</sup> salinity, the higher value of MGT was found in *S. grossus*, followed by *S. zeylanica* and *C. difformis*, proving the longest delay in germination. At these levels *L. chinensis*, *C. iria* and *O. sativa* (weedy rice) showed better performance followed by *J. linifolia*, *E. colona*, *E. crusgalli* and *F. miliacea*. At 16 dS m<sup>-1</sup>, *C. iria*, *E. colona*, *O. sativa* (weedy rice) and *E. crusgalli* appeared with the lowest MGT value but *S. grossus* resulted in no germination. Species *L. chinensis*, *S. grossus*, *S. zeylanica*, and *C. difformis* did not germinate at 24 dS m<sup>-1</sup> salinity level. Most species showed no germination at 32 dS m<sup>-1</sup> except *C. iria*, and *E. colona* (Fig. 3). Salinity  $\leq 4$  dS m<sup>-1</sup> resulted in a delay of 1-2 days, whereas higher salinity levels delayed germination by more than a week. The results of our study are in agreement with the observations of Kaya et al. (2008) in chickpea. The increase in MGT with the increasing NaCl stress was also supported by Kaya et al. (2009). Similar result was also observed by Redondo-Gomez et al. (2008), where MGT of *Limonium emarginatum* was higher in salt stress condition.

In general, increasing trend of the time to 50% germination was observed with increasing salinity levels in all species. At extreme level of salinity (32 dS m<sup>-1</sup>), only two species *E. colona* and *C. iria* were found to germinate after a long time of 14-16 days. The second extreme salinity (24 dS m<sup>-1</sup>) fully arrested the germination of 4 species viz. *L. chinensis*, *S. zeylanica*, *C. difformis* and *S. grossus*. The other 6 species viz. *E. crusgalli*, *E. colona*, *C. iria*, *O. sativa*, *J. linifolia* and *F. miliacea* were able to germinate after 12-15 days (Table 3). The longest time was required for the germination by the species *S. zeylanica* under all salinity levels. The shortest time was recorded for *C. iria* (4.79 days) followed by *L. chinensis* (5.38 days) under 4 dS m<sup>-1</sup>. However, under the salinity level of 8 dS m<sup>-1</sup> the trend was somehow changed i.e. *L. chinensis*, *E. colona*, *C. iria* and *J. linifolia* were statistically similar in germination seed and those values were significantly different with other species. Farooq et al. (2006) observed that when rice seeds were treated with ethanol (1 and 5%) solutions less time was required to give 50% germination. Salinity affected to time 50% germination of citrus (Zerki, 1993).

Different levels of salinity significantly affected the seedling vigor index (SVI) in all species. At the high level of salinity (32 dS m<sup>-1</sup>) the SVI of the all the species except *C. iria* and *E. colona* was zero (Fig. 4). Species *S. grossus*, *O. sativa* L., *S. zeylanica* and *C. difformis* were more seriously affected. *E. crusgalli*, *J. linifolia*, *F. miliacea* and *L. chinensis* were moderately affected and *E. colona*

and *C. iria* were least affected at different levels of salt stress. Seedling vigor index of maize was also significantly affected under different salt stresses (Janmohammadi et al., 2008). Similar result was found and noted by Djanaguairaman et al. (2003) that seedling vigor index significantly showed decreasing trend with increasing the salt concentration.

Shoot length of all the weed species declined in general at all the salt treatments as compared to control (Table 1). However, species *C. iria*, *E. crusgalli*, *E. colona*, *J. linifolia*, *F. miliacea* and *Oryza sativa* L. (weedy rice) exhibited lower reduction in shoot length (<20%) than other species at 4 dS m<sup>-1</sup>. At salinity levels upto 16 dS m<sup>-1</sup> the species *E. colona* and *C. iria* had produced significantly higher shoot length compared to others and lowest length was recorded in *S. grossus*. At the salinity level of 24 dS m<sup>-1</sup> and above, the species *L. chinensis*, *C. difformis*, *S. zeylanica* and *S. grossus* showed drastic reduction in shoot length. At this level, the highest shoot length was recorded in *C. iria* followed by *E. colona*, *J. linifolia*, *E. crusgalli*, *F. miliacea* and *O. sativa* (Table 1). At 32 dS m<sup>-1</sup>, the differences in shoot lengths of most species were obscure due to serious reduction except in *E. colona* and *C. iria*. The reduction in seedling height of many crop plants grown under saline environment is a common phenomenon (Javed and Khan, 1995). Our result supported by Rahman and Ungar (1990) and noted that the seedling length of *Echinochloa crusgalli* significantly decreased due to increased salt concentration of NaCl. Okcu et al. (2005) reported that the shoot and root lengths of pea decreased with the increased salt concentrations. Similar results were also observed and noted by Atak et al. (2008) that shoot length of dry green pea declined with the increase of NaCl. Kaya et al. (2008) observed the similar result that the shoot length of chickpea (*Cicer arietinum* L.) was significantly influenced by NaCl and severely affected by with complete inhibition of shoots at 16.3 dS m<sup>-1</sup> NaCl stress in all cultivars.

Root lengths were more affected with increased salinity levels compared to shoot and salt effects on root were more prominent than shoot at each specific salinity level (Table 2). At 16 dS m<sup>-1</sup>, *C. difformis* and *S. grossus* showed serious reduction in root length however, species *E. colona*, *C. iria* and *E. crusgalli* showed better performance followed by *J. linifolia*, *L. chinensis* and *O. sativa* (weedy rice). At all salinity level up to 24 dS m<sup>-1</sup>, *E. colona*, *C. iria* and *E. crusgalli* produced significantly longer roots compared to other species. At 32 dS m<sup>-1</sup>, root growth was completely arrested for all species (Table 2). Soltani et al. (2002) observed that root length was diminished by increasing NaCl concentration. The gradual decrease in root length with the increase in salinity might probably be due to more inhibitory effect of NaCl to root growth compared to that of shoot growth (Rahman et al., 2001). Salt concentration caused corresponding decrease in root length in all cultivars of chickpea and the longest roots were obtained from small seeds in each cultivar under all salt stress conditions (Kaya et al., 2008). Similar result was observed and noted by Momayezi et al. (2009) that the

root length of all rice genotypes was reduced with increasing salt concentration.

Increasing salt level had detrimental effects on germination percentage, seed of germination, mean germination time and relative shoot and root length of different weed species under present investigation. The weed species of *C. iria*, *E. colona* and *E. crusgalli* showed best performance and *J. linifolia*, *L. chinensis* and *O. sativa* L. (weedy rice) proved moderate up to 24 dS m<sup>-1</sup> while *S. grossus*, *F. miliacea*, *C. difformis* and *S. zeylanica* were more sensitive to  $\geq 16$  dS m<sup>-1</sup>.

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