

PHYTOTOXIC EFFECTS OF *Calotropis procera* EXTRACT ON GERMINATION AND SEEDLING VIGOR OF WHEAT

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ABSTRACT

Numerous plants are recognized for their phytotoxic and inhibitory influence on growth of cultivated crops. This study was conducted to probe the allelopathic effect of *Calotropis procera* water extract on the seed germination and seedling vigor of wheat. *C. procera* extract with 1:20 w/v concentration was applied on three different germination media (blotter paper, sand and soil) in addition to wheat seed soaking in *C. procera* extract. Seed germination percentage and seed germination index were significantly reduced up to 48.30% and 33.27%, respectively. Whereas, mean germination time and time to 50% germination were significantly increased up to 69.56% and 82.48%, respectively with the extract of *C. procera*, as compared to distilled water (control). A significant reduction of 133%, 222% and 80.77% in root, shoot length and seedling biomass were observed by applying *C. procera* extract. Seedling vigor index was also declined up to 87.23% with *C. procera* extract irrespective of germination media. Application of *C. procera* extract on soil media had a greater inhibitory effect than that of other germination media or seed soaking. It is concluded that presence of *C. procera* in wheat field may retard the germination and seedling vigor of wheat plants.

Key words: Allelopathy, *Calotropis procera*, extract, germination, phytotoxicity, seedling vigor, wheat.

INTRODUCTION

Calotropis procera plant is generally known as milkweed, calotropis, rubber bush or kapock tree, sodom apple (Kareem *et al.*, 2008) and locally known as "Aak" in Pakistan. It is a member of family Asclepiadaceae (Parihar *et al.*, 2011) whose members are distributed throughout the world in tropical and sub-tropical regions. It is abundant in warm climate areas having dry, sandy and alkaline soils. It is mostly noted in waste and fallow lands along roads, streets, residential colony parks, sand dunes as well as in crop fields as weed (Sastri and Kavathekar, 1990). *Calotropis procera* has got perennial

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growth habit with tall and erect stem having large number of branches thus assuming the shape of shrub or sometimes small trees which can grow up to 2-3 m height. Mode of propagation is mainly the seed which are transported by wind and water while asexual propagation at localized scale also occurs through suckers from the roots (Parsons and Cuthbertson, 1992). In Pakistan, it is considered to be among dominant plant of natural flora (Qureshi et al., 2009).

Many plants are known to be phytotoxic in nature as they produce and release numerous allelochemicals into the environment. These allelochemicals are actually secondary metabolites and are phytotoxic (Farooq et al., 2011). Phytotoxic impact of these anxious compounds on other plants is usually dominant at early growth stages, causing inhibition of seed germination and seedling growth (Farooq et al., 2008; Jabran et al., 2010a). Allelochemicals disrupts the various physiological and metabolic functions of plants such as photosynthesis, nutrient and water uptake, DNA synthesis and respiration (Einhelling, 2002). Allelopathic potential of many crop plant and weeds have been investigated against different crops (Kato-Noguchi and Tanaka, 2006; Farooq et al., 2008; Jabran et al., 2010a). These plants release different types of water soluble phytotoxins in their surrounding environment and in soil thereby inhibiting the germination and growth of different crops (Kadioglue et al., 2005; Singh et al., 2005; Batish et al., 2007). These allelochemicals can be used as potential source for natural herbicides, pharmaceuticals and biological control agents (Hirai, 2003; Cheema et al., 2004; Macias et al., 2007; Norton et al., 2008; Jabran et al., 2008; 2010b; Razzaq et al., 2010; 2012).

Calotropis procera has largely been studied for its pharmacological (Nenaah and Ahmed, 2011), insecticidal (Begum et al., 2010) and anti-phytopathogenic (Kareem et al., 2008) properties and has extensively been used for the control of many plants (Ahmad and Khan, 2004), animals (Iqbal et al., 2005; Choedon et al., 2006), and diseases as well (Das et al., 2008; Shahia et al., 2010). However, previous studies revealed that limited number of investigations have been carried out to assess the phytotoxic and allelopathic effects of this plant in various crops (Kayode, 2004; Samreen et al., 2009). But no work has been done on the phytotoxic effect of *C. procera* on wheat.

Its widespread and persistent occurrence near barley, oat, rice, sorghum, maize, cotton, sugarcane fields and especially around wheat crop fields makes it suspicious to cause some adverse effect on these crops through allelopathic interaction. Moreover, it may invade our wheat fields in the near future as did the other weeds. Therefore there is always a threat that it may become a major weed of our cropping system. Keeping in view these facts, a study was planned to evaluate

the phytotoxic effect of *C. procera* on germination and seedling vigor of wheat (*Triticum aestivum* L.).

MATERIAL AND METHODS

This study was conducted at room temperature during winter season in the Laboratory, Department of Agronomy, University College of Agriculture University of Sargodha, Pakistan during 2010-11.

Collection of *C. procera* plants and preparation of extracts from leaves

Calotropis procera plants were collected from cropped area of the experimental research station of University College of Agriculture, University of Sargodha, Pakistan. The plants were uprooted and dried under shade. Their leaves were chopped into small pieces with the help of scissors. Dried and chopped leaves of *C. procera* were weighed and immersed in tap water at the ratio of 1:20 (w/v) at room temperature for 48 h. The water extract was collected in bottles and tagged by filtering through 10- and 60-mesh sieves.

Effect of *C. procera* extract on germination capacity and germination indices of wheat

Fifty wheat seeds were sown in 9 cm diameter petri-plates on three different germination media viz., blotter paper (BP), sand (Sa), and soil (So) and were soaked in (Sk) in *C. procera* extract overnight. As positive controls of all these treatments, seeds were also soaked in equal quantities of distilled water. Experiment was laid out in completely randomized design (CRD) with split plot arrangement and each treatment was replicated four times. Seeds of wheat variety Saher-2006 were used in this study.

Petri dishes were placed in laboratory at room temperature of 20 ± 2 °C for 15 days. The petri dishes were observed daily up to 15 days. Germinated seeds were counted daily according to the seedling evaluating procedure in the (AOSA, 1990). The seeds were considered as germinated seeds when the radical size was 2 mm. The numbers of germinated seeds were recorded after 24 h, and these seeds were discarded. Fifteen days after sowing, the germination percentage was calculated using the formula (Germinated seeds / Total seeds x 100) for each replication of the treatment. Mean germination time (MGT) was calculated according to the equation of (Moradi *et al.*, 2008).

$$\text{MGT} = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds, which were germinated on day D, and D is the number of days counted from the beginning of germination. The germination index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) by following formula:

$$GI = \frac{\text{No of germinate or emerged seeds}}{\text{Days of first count}} + \dots + \frac{\text{No of germinated or emerged seeds}}{\text{Days of final t count}}$$

The time to 50% germination (T_{50}) was calculated according to the following formula of (Coolbear et al., 1984) modified by (Farooq et al., 2005).

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

Where N is the final number of germination and n_i , n_j cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

Effect of *C. procera* extract on seedling dry weight, root shoot lengths and seedling vigor index

After 15 days of germination, seedlings were uprooted and thoroughly washed with water. The seedlings root shoot length were measured in cm and were weighed separately after drying them in oven at 65 °C for 48 h until constant dry weight was obtained. Seedling vigor index (SVI) was calculated according to the formula described by Orchard (1977):

Seedling vigor index (SVI) = [seedling length (cm) × germination percentage]

Statistical analysis

The data collected was subjected to analysis of variance (ANOVA) and significant difference among treatment mean was identified by using Fisher's at 5 % probability level (Steel et al., 1997).

RESULTS AND DISCUSSION

Calotropis procera extracts were found to be phytotoxic and decreased the germination traits and growth of wheat compared with control (Table-1&2). Germination percentage was significantly reduced with *C. procera* extract when compared with distilled water (Table-1). It also differed significantly by growing seed in various germination media as well as soaked in *C. procera* extract. Significantly lowest germination percentage was recorded in soil medium (12.5%) followed by sand medium (26.5%). This may be due to presence of phytotoxins leachates present in the water extract of *C. procera*. This finding supported the results of Tanveer et al. (2010); they found that water extract of *Euphorbia helioscopia* L. reduced the germination percentage, seedling emergence and seedling vigor index in wheat, chickpea and lentil crops. Regarding treatment and treatment mode (T x M) interactions, the lowest germination percentage (7.0%) was

found with the *C. procera* extract applied to the soil which remained significantly at par with that observed in petri plate where *C. procera* extract was applied to sand. The highest germination percentage (60%) was noted in petri plate in which seed soaked with *C. procera* extract.

Germination index indicates the speed of germination therefore it is an important parameter of seedling vigor. *Calotropis procera* extract significantly reduced germination index of wheat seeds up to 33.27% (Table-1). It also varied significantly with the germination media or soaked seed and significantly lowest germination index (1.48) was recorded in soil medium followed by sand medium (1.51). Among the entire T x M interactions, the minimum germination index (0.54) was calculated in petri plate where *C. procera* extract was applied to the soil medium which was statistically similar to that observed in sand (1.65) where *C. procera* extract was applied. This delay in seed germination and reduction in germination index may reflect a presence of water soluble inhibitors in *C. procera* extract. This finding is in line with the findings of Stavrianiakou *et al.* (2004), Dongre and Yadav (2005) and Kadioglue *et al.* (2005) who revealed suppression in the germination rate and final germination of different crops with water extract of different broad and narrow leaf weeds. The highest value of germination index was found in petri plate where wheat seeds were sown on blotter paper and distilled water was applied to it.

Mean germination time (MGT) and time to 50% germination (T_{50}) are important parameters of seedling vigor which indicate speed of germination. Their greater values represent lower seedling vigor. Data presented in table 1 showed that in all treatment mode, *C. procera* extract significantly prolonged MGT and T_{50} in wheat up to 69.56% and 82.48%, respectively compared with distilled water. However non-significant differences in MGT and T_{50} were observed among various germination media used. Wheat seed in all the *C. procera* extract-treated materials including blotter paper, sand, soil and seed significantly increased MGT and T_{50} up to 13 days and 12.5 days, respectively compared with their counterparts supplied with distilled water. These results are similar to those of Al-Zahrani and Al-Robai (2007) and Oudhia (2001) who found 5.25% and 26.6% reduction in germination percentage of wheat by applying 60% leaf extract and 1:10 w/v (roots: water) root leachates of *C. procera* to wheat seeds. Up to a 68.0% reduction in germination percentage has also been observed in sunflower seeds treated with 10% w/v extract of *C. procera* leaves (MacDonald and Omonhinmin, 1998).

Data related to root and shoot length are presented in table 2 which indicates that *Calotropis procera* extract significantly reduced both the root length and shoot length up to 133% and 222%

respectively when compared with distilled water. Treated material means showed that irrespective of media used for germination, significantly greater root and shoot lengths were produced by seedlings grown in soil and sand with 4.88 cm and 4.48 cm; 5.32 cm, and 3.93 cm values, respectively compared with those grown on other media. Regarding T x M interactions, blotter paper seedlings treated with *C. procera* extract gave lowest root length (0.920 cm) and shoot length (0.867 cm) which was statistically similar to that obtained by seeds soaked in *C. procera* extract before sowing. Maximum root length (7.100 cm) and shoot length (8.233 cm) were attained by seedlings grown in soil and supplied with distilled water. Agarwal et al. (2002), Stavrianiakou et al. (2004), and Dongre and Yadav (2005) observed inhibition in the root and shoot length in wheat, pea and lentil with water extracts of various weeds. Al-Zahrani and Al-Robai (2007) also noticed from 19.4% to 90.8% and 2.2% to 88.8% reduction in radicle and plumule lengths respectively by supplying wheat seedlings with 5 to 60 % concentrated extracts of *C. procera* leaves. Data regarding seedling dry weight are given in table 2 which revealed that irrespective of germination media, significant reduction in seedling biomass (0.052 g) was noted by applying *C. procera* extract. Non-significant difference in this parameter can be observed among media used as shown by their means across treatments. However in all the germination media, soaked seeds with *C. procera* extract produced significantly lower biomass ranging from 0.037 g to 0.077 g compared with those of distilled water that ranging from 0.197 g to 0.297 g. This may be due to phytotoxic effect of proteases enzymes present in *C. procera* extract (Singh et al., 2010). The regression analyses of seedling dry weight with germination percentage (GP), germination index (GI), mean germination time (MGT), time to 50% germination (T_{50}), root length, shoot length, and seedling vigor index (SVI) have been depicted in figures 1, 2, 3, 4, 5, 6, and 7 respectively which revealed that seedling dry weight had a strong positive relationship with germination percentage ($R^2=0.066$), germination index ($R^2=0.039$), root length ($R^2=0.502$), shoot length ($R^2=0.742$), and seedling vigor index ($R^2=0.640$) whereas had a strong negative relationship with mean germination time ($R^2=0.935$) and time to 50% germination ($R^2= 0.928$). Data shown in table 2 revealed that seedling vigor index SVI was significantly declined up to 130% by the application of *C. procera* extract irrespective of germination media. Seedlings grown in soil when *C. procera* extract applied showed the lowest value of SVI (87.23) which was significantly different from all other combinations. Whereas the highest SVI (310.0) could be observed in treatment where seed was sown in soil and distilled water was applied to the seedlings.

Table-1. Effect of *Calotropis procera* extract on germination percentage, germination index, mean germination time and time to 50% germination of wheat.

Treatments (T)	Treatment Mode (M)				Means
	Blotter Paper (BP)	Sand (Sa)	Soil (So)	Soaked seed (Sk)	
Germination %age					
<i>Calotropis procera</i> Extract (E)	45.00 ab	18.00 cd	7.000 d	47.00 ab	29.25 b
Distilled Water (W)	58.50 a	31.50 bc	23.50 c	60.00 a	43.38 a
Means	51.75 a	26.50 b	12.50 c	53.50 a	
Germination index (GI)					
<i>Calotropis procera</i> Extract (E)	5.80 b	1.65 de	0.54 e	6.28 b	3.57 b
Distilled Water (W)	7.95 a	3.25 c	2.42 cd	7.78 a	5.35 a
Means	6.88 a	1.52 b	1.48 c	7.03 a	
Mean germination time (MGT)					
<i>Calotropis procera</i> Extract (E)	13.00 a	13.00 a	13.00 a	13.00 a	13.00 a
Distilled Water (W)	7.77 b	7.40 b	7.67 b	7.83 b	7.67 b
Means	10.38 a	10.20 a	10.33 a	10.42 a	
Time to 50% germination (T ₅₀)					
<i>Calotropis procera</i> Extract (E)	12.50 a	12.50 a	12.50 a	12.50 a	12.50 a
Distilled Water (W)	7.07 b	6.67 b	6.70 b	6.97 b	6.85 b
Means	9.78 a	9.58 a	9.60 a	9.73 a	

Means not sharing same letter are significantly different at 5 % probability level.

Table-2. Effect of *Calotropis procera* extract on root length, shoot length, seedling dry weight and seedling vigor index of wheat.

Treatments (T)	Treatment Mode (M)				Means
	Blotter Paper (BP)	Sand (Sa)	Soil (So)	Soaked Seed (Sk)	
Root length (cm)					
<i>Calotropis procera</i> Extract (E)	0.92 d	3.46 c	2.66 c	0.94 d	1.99 b
Distilled Water (W)	2.50 c	5.50 b	7.10 a	3.53 c	4.66 a
Means	1.71 b	4.48 a	4.88 a	2.23 b	
Shoot length (cm)					
<i>Calotropis procera</i> Extract (E)	0.87 e	2.50 cd	2.40 d	1.30 de	1.72 b
Distilled Water (W)	4.67 b	5.37 b	8.23 a	3.93 bc	5.55 a
Means	2.78 c	3.93 b	5.32 a	2.62 c	
Seedling dry weight (g)					
<i>Calotropis procera</i> Extract (E)	0.04 c	0.08 c	0.05 c	0.04 c	0.05 b
Distilled Water (W)	0.29 a	0.30 a	0.27 a	0.20 b	0.26 a
Means	0.17 ab	0.18 a	0.16 ab	0.13 b	
Seedling vigor index (SVI)					
<i>Calotropis procera</i> Extract (E)	94.77 d	165.9 c	36.80 e	87.23 d	96.17 b
Distilled Water (W)	158.3 c	310.0 a	275.2 b	143.4 c	221.73 a
Means	126.5 c	237.9 a	156.0 b	115.3 c	

Means not sharing same letter are significantly different at 5 % probability level

Samreen et al. (2009) also reported 50-83% and 45-58% reduction in seedling dry weight of pearl millet (*Pennisetum americanum*) by applying *C. procera* extract of various concentrations prepared from its leaves and stem, respectively.

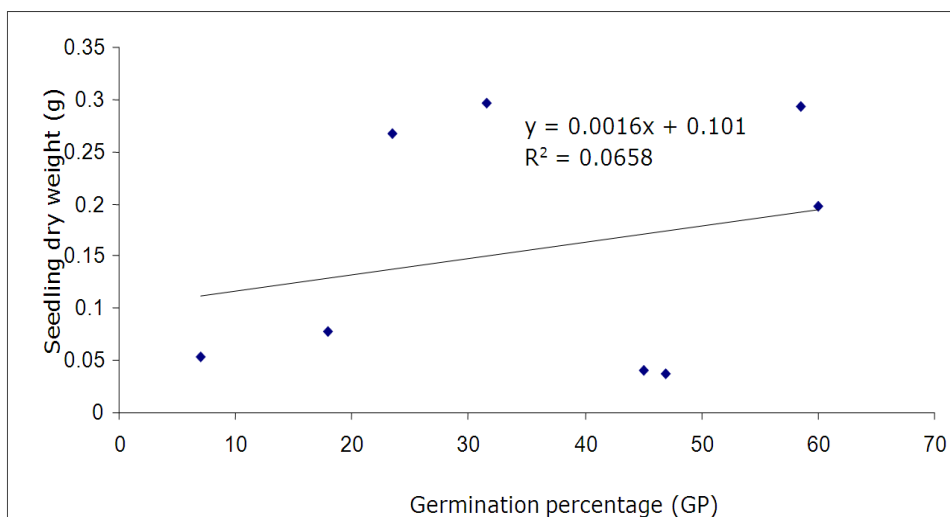


Figure 1. Regression analysis of seedling dry weight (g) as affected by germination percentage (GP).

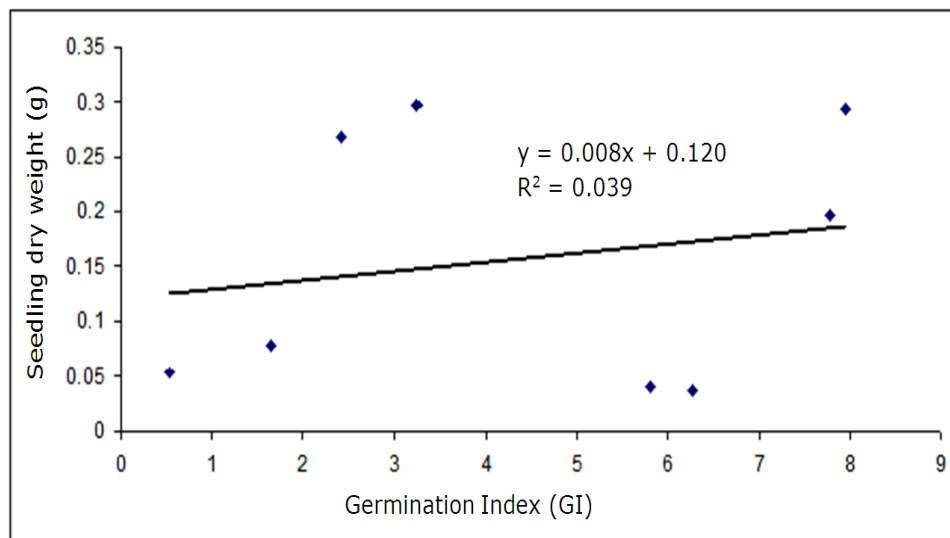


Figure 2. Regression analysis of seedling dry weight (g) as affected by germination index (GI).

CONCLUSION

In conclusion, the extract of the weed *Calotropis procera* inhibited the germination and seedling growth of wheat due to its phytotoxic effects. Hence, if present in field, this weed can disturb the stand establishment of wheat crop. There is a need to take a serious notice of the presence of this weed in the wheat crop fields and nearby places. Further research can explore the allelochemicals present in *C. procera* as well as the complex allelopathic mechanisms through which this phytotoxic plant disturb the neighboring plants.

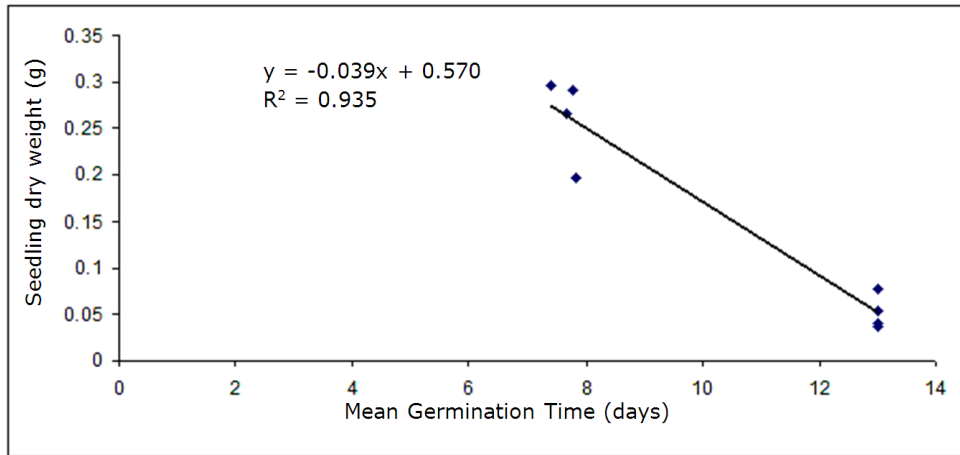


Figure 3. Regression analysis of seedling dry weight (g) as affected by mean germination time (days).

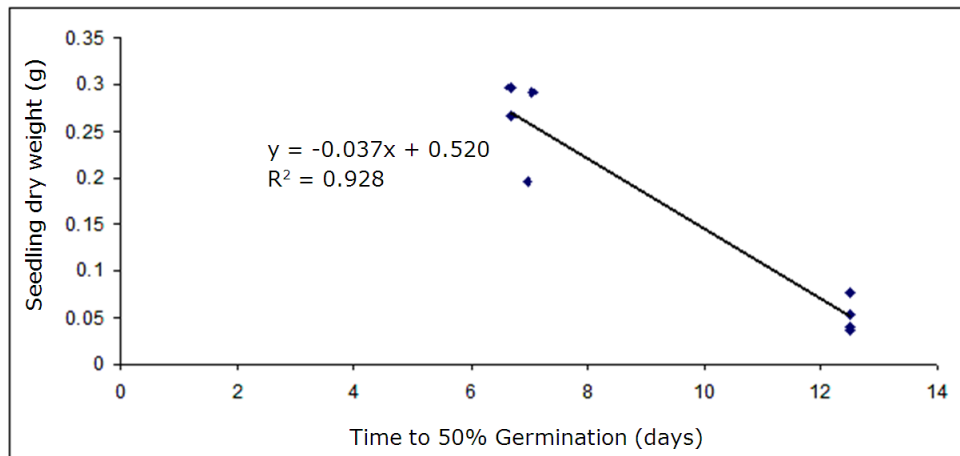


Figure 4. Regression analysis of seedling dry weight (g) as affected by time to 50% germination (days).

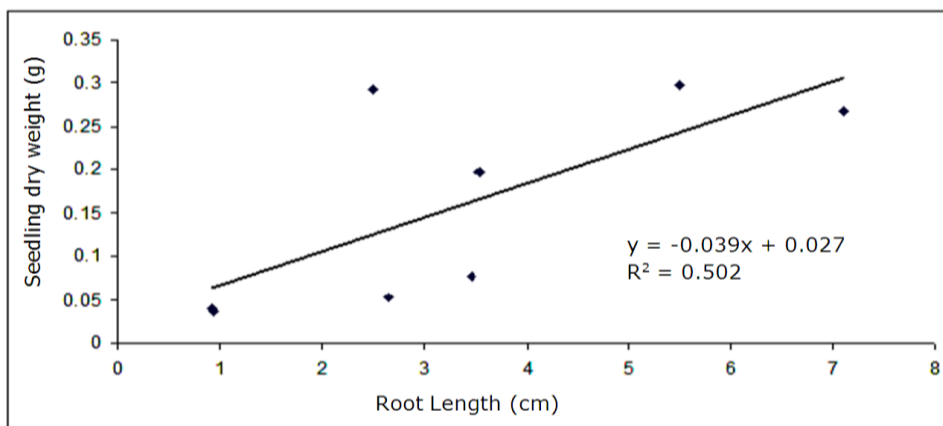


Figure 5. Regression analysis of seedling dry weight (g) as affected by root length (cm).

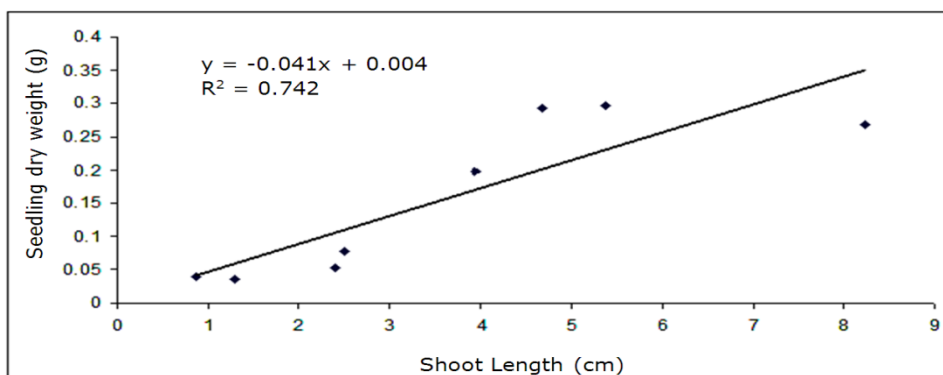


Figure 6. Regression analysis of seedling dry weight (g) as affected by shoot length (cm).

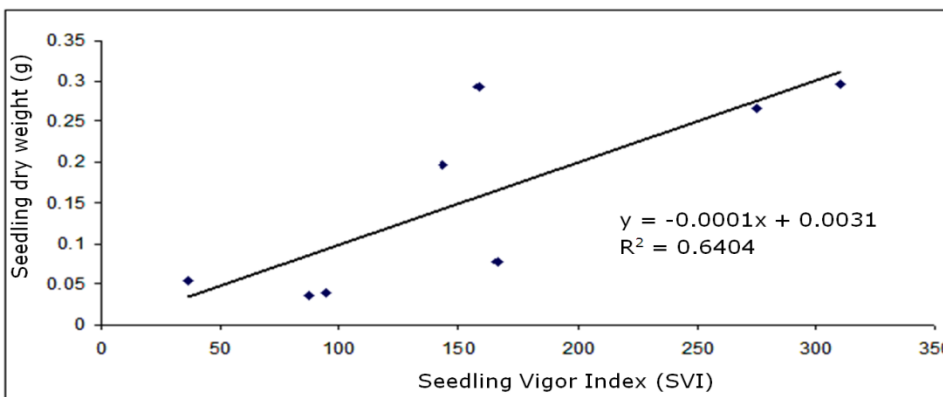


Figure 7. Regression analysis of seedling dry weight (g) as affected by seedling vigor index (SVI).

REFERENCES CITED

- Agarwal, A.R., A. Gahlot, R. Verma and P.B. Rao. 2002. Effect of weed extracts on seedling growth of some varieties of wheat. J. Environ. Biol. 23: 19-23.
- Ahmad, S.F. and T. A. Khan. 2004. Management of Root-Knot Nematode, *Meloidogyne Incognita*, by integration of *Paecilomyces Lilacinus* with organic materials in Chillli. Archives Phytopathol. Pl. Protect. 37(1): 35-40.
- Al-Zahrani, H.S. and S. A. Al-Robai. 2007. Allelopathic effect of *Calotropis procera* leaves extract on seed germination of some plants. JKAUA: Sci. 19: 115-126.
- Association of Official Seed Analysts (AOSA). 1983. Seed vigor testing handbook. Contribution No. 32 to the handbook on Seed Testing.
- Association of Official Seed Analysts (AOSA). 1990. Rules for testing seeds. J. Seed Technol. 12: 1-112.
- Batish, D.R., K. Lavanya, H.P. Singh and R.K. Kohli. 2007. Root mediated allelopathic interference of Nettle-leaved Goosefoot (*Chenopodium murale*) on wheat (*Triticum aestivum* L.). J. Agron. Crop Sci. 193: 37-44.
- Begum, N., B. Sharma and R. V. Pandey. 2010. Evaluation of insecticidal efficacy of *Calotropis procera* and *Annona squamosa* ethanol extracts against *Musca domestica*. J. Biofertil. Biopestici. 1(1): 1-6.
- Cheema, Z.A., A. Khaliq and S. Saeed. 2004. Weed control in maize (*Zea mays* L.) through sorghum allelopathy. J. Sustain. Agric. 23: 73-86.
- Choedon, T., G. Mathan, S. Arya, V. L. Kumar and V. Kumar. 2006. Anticancer and cytotoxic properties of the latex of *Calotropis procera* in a transgenic mouse model of hepatocellular carcinoma. World J. Gastroenterol. 12(16): 2517-2522.
- Coolbear, P., A. Francis and D Grierson. 1984. The effect of low temperature pre-sowing treatment under the germination performance and membrane integrity of artificially aged tomato seeds. J. Exp. Bot. 35: 1609-1617.
- Das, B.C., P. K. Sarker and M. M. Rahman. 2008. Aphidicidal activity of some indigenous plant extracts against bean aphid *Aphis craccivora* Koch (Homoptera: Aphididae). J. Pest Sci. 81(3): 153-159.
- Dongre, P.N. and B. Yadav. 2005. Inhibitory allelopathic effect of weed leaf leachates on seed germination of pea (*Pisum sativum* L.) Crop Res. Hisar, 29: 458-461.
- Einhelling, F.A. 2002. The physiology of allelochemicals action: clues and views. In: Allelopathy from Molecules to Ecosystem. (Eds.,

- M. J. Reigosa & N. Pedrol), Science Publishers, Enfield, New Hampshire, USA.
- Farooq, M., K. Jabran, H. Rehman and M. Hussain. 2008. Allelopathic effects of rice on seedling development in wheat, oat, barley and berseem. *Allelopath. J.* 22: 385-390.
- Farooq, M., K. Jabran, Z.A. Cheema, A. Wahid and H.M. Siddique. 2011. The role of allelopathy in agricultural pest management. *Pest Manag. Sci.* 67: 493-506.
- Farooq, M., S. M. A. Basra, K. Hafeez and N. Ahmad. 2005. Thermal hardening: a new seed vigor enhancement tool in rice. *Acta Botanica Sinica*, 47: 187-193.
- Hirai, N. 2003. Application of allelochemicals to agriculture. *Biol. Sci. Space* 17:4-5.
- Iqbal, Z., M. Lateef, A. Jabbar, G. Muhammad, M. N. Khan. 2005. Anthelmintic activity of *Calotropis procera* (Ait.) Ait. F. flowers in sheep. *J. Ethnopharmacol.* 102(2): 256-261.
- Jabran, K., M. Farooq, M. Hussain, H. Rehman and M.A. Ali. 2010a. Wild oat (*Avena fatua* L.) and canary grass (*Phalaris minor* Ritz.) management through allelopathy. *J. Plant Protec. Res.* 50: 32-35.
- Jabran, K., Z.A. Cheema, M. Farooq and M. Hussain. 2010b. Lower doses of pendimethalin mixed with allelopathic crop water extracts for weed management in canola (*Brassica napus* L.). *Int. J. Agric. Biol.* 12: 335-340.
- Jabran, K., Z.A. Cheema, M. Farooq, S.M.A. Basra, M. Hussain and H. Rehman. 2008. Tank mixing of allelopathic crop water extracts with pendimethalin helps in the management of weeds in canola (*Brassica napus*) field. *Int. J. Agric. Biol.* 10: 293-296.
- Kadioglue, I., Y. Yanar and U. Asav. 2005. Allelopathic effect of weed leachates against seed germination of some plants. *J. Environ Biol.* 26: 169-173.
- Kareem, S.O., I. Akpan and O.P. Ojo. 2008. Antimicrobial activities of *Calotropis procera* on selected pathogenic microorganisms. *Afr. J. Biomedic. Res.* 11: 105-110.
- Kato-Noguchi, H and Y. Tanaka. 2006. Abscisic acid-glucose ester as an allelopathy agent from citrus fruit. *Acta Physiologica Plantarum* 28: 635-639.
- Kayode, J. 2004. Allelopathic effect of aqueous extracts of *Calotropis procera* on germination and seedling growth of maize. *Pak. J. Sci. Ind. Res.* 47: 69-72.
- MacDonald, I. and C.A. Omonhinmin. 1998. Allelopathic effect of *Calotropis procera* on the germination of *Helianthus annuus* seeds. *Comp. Newslet.* 32: 31.

- Macias, F.A., J. M. G. Molinillo, R. M. Varela and J. C. G. Galindo. 2007. Allelopathy: A natural alternative for weed control. *Pest Manag. Sci.* 63: 327-348.
- Moradi, D.P., F. Sharif-zadeh and M. Janmohammadi. 2008. Influence of priming techniques on seed germination behavior of maize inbred lines (*Zea mays* L.). *ARPN J. Agri. Biol. Sci.* 3(3): 22-25.
- Nenaah, E.G and M.E. Ahmed. 2011. Antimicrobial activity of extracts of *Calotropis procera* (Ait.) and synergistic effect with reference antimicrobials. *Res. J. Med. Pl.* 5(6): 706-716.
- Norton, A.P., A.C. Blair, J. Hardin, S.J. Nissen and G.R. Brunk. 2008. Herbivory and novel weapons: No evidence for enhanced competitive ability or allelopathy induction of *Centurea diffusa* by biological control. *Biol. Invasions* 10: 79-88.
- Orchard, T. 1977. Estimating the parameters of plant seedling emergence. *Seed Sci. Technol.* 5: 61-69.
- Oudhia, P. 2001. Allelopathic effects of root leachates of some obnoxious weeds on germination and seedling vigour of wheat. *Ecol. Env. Cons.* 7(1): 111-113.
- Parihar, G., A. Sharma, S. Ghule, P. Sharma, P. Deshmukh and D. N. Srivastava. 2011. Anti-inflammatory effect of *Calotropis procera* root bark extract. *Asian J. Pharm. Life Sci.* 1(1): 29-44.
- Parsons, W. T and E. G. Cuthbertson. 1992. Weeds of Natural Ecosystems. In: *Noxious Weeds of Australia*. Inkata Press, Melbourne, Victoria, Australia, pp. 173-175.
- Qureshi, R., W. A. Khan and B. Khan. 2009. Study of vegetation and smooth coated otter in chotiari wetlands complex, sanghar, sindh, pakistan. *Pak. J. Bot.* 41(5): 2507-2516.
- Razzaq, A., Z.A. Cheema, K. Jabran, M. Farooq, A. Khaliq, G. Haider and S.M.A. Basra. 2010. Weed management in wheat through combination of allelopathic water extracts with reduced doses of herbicides. *Pak. J. Weed Sci. Res.* 16: 247-256.
- Razzaq, A., Z.A. Cheema, K. Jabran, M. Hussain, M. Farooq and M. Zafar. 2012. Reduced herbicide doses used together with allelopathic sorghum and sunflower water extracts for weed control in wheat. *J. Plant Prot. Res.* 52: 281-285.
- Samreen, U., F. Hussain and Z. Sher. 2009. Allelopathic potential of *Calotropis procera* (AIT.) AIT. *Pak. J. Pl. Sci.* 15 (1): 7-14.
- Sastry, C.S.T. and K.Y. Kavathekar. 1990. Plants for reclamation of wasteland. Publication and Information Directorate, CSIR, New Delhi, pp. 175-179.
- Shahia, M., A.A. Hanafi-Bojdd, M. Iranshahic, H. Vatandoostb and M.Y. Hanafi-Bojdd. 2010. Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepiadaceae) against *Culex*

- quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae). Vect. Borne Dis. 47: 185–188.
- Singh, A.N., A. K. Shukla, M. V. Jagannadham and V. K. Dubey. 2010. Purification of a novel cysteine protease, procerain B, from *Calotropis procera* with distinct characteristics compared to procerain. Process Biochem. 45: 399-406.
- Singh, H.P., D. R. Batish, K. Shalinder, R. K. Kohli and K. S. Dogra. 2005. Allelopathic interference of *Ageratum conyzoides* L. against some crop plants. Weeds management: balancing people, planet, profit. Proc. 14th Aus. Weeds Conf., Wagga Wagga, New South Wales, Australia, 6-9 September-2004, pp. 558-561.
- Stavrianakou, S., V. Liakoura, E. Levizou, P. Karageorgou, C. Delis, G. Liakopoulos, G. Karabourniotis, G. Manetas and Y. Manetas. 2004. Allelopathic effect of water-soluble leaf epicuticular material from *Dittrichia viscosa* on seed germination of crops and weed. Allelopath. J. 14: 35-41.
- Steel, R.G.D., J.H. Tori and D. Dicky. 1997. Principles and Procedures of Statistics. Multiple comparisons. 3rd Ed., MC Graw Hill Book Co, NY USA, pp. 178-198.
- Tanveer, A., A. Rehman, M.M. Javaid, R.N. Abbas, M. Sibtan, A.U.H. Ahmad, M.S. Ibin-i-zamir, K.M. Chaudhary and A. Aziz. 2010. Allelopathic potential of *Euphorbia helioscopia* L. against wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medic.). Turk. J. Agric. For. 34: 75-81.