0971-4693/94 US $ 5.00
Allelopathy Journal 31 (1): 105-116 (2013) *International Allelopathy Foundation* 2013
Tables: 5, Figs : 2

Effects of *Calotropis procera* and *Citrullus colosynthis* on germination and seedling growth of maize

RABIA NAZ and ASGHARI BANO*

Department of Plant Sciences, Quaid-i-Azam University, Islamabad Pakistan
E. Mail: banoasghari@gmail.com

(Received in revised form: December 16, 2012)

ABSTRACT

The allelopathic potential of leaf extracts (0.8, 0.1 and 1.2%) of *Calotropis procera* and *Citrullus colosynthis,* were studied in laboratory and pot in culture on germination and seedlings growth of maize. The aqueous leaf extracts of *C. procera* enhanced the germination, but all concentrations of *C. colosynthis* extracts were inhibitory. However all concentrations of *C. procera* and *C. colosynthis* extracts stimulated the seedling growth (root length, shoot length, fresh and dry weight of root and shoot) of maize. The highest concentration (1.2%) of both species *C. procera* and *C. colosynthis* increased the seedling growth of maize by stimulating their root length, shoot length, fresh and dry weight in both laboratory and pot study. Thus *Zea mays* can be planted in the vicinity of these naturally growing plant species due to their stimulatory effect on growth.

Keywords: Aqueous leaf extract, *Calotropis procera, Citrullus colosynthis,* germination, maize, phytochemicals, nutrients, seedling growth, Vigour index*, Zea mays*

INTRODUCTION

The term Allelopathy refers to both beneficial and detrimental biochemical interactions among all plants (23). Allelopathy is an ecological phenomenon, in which different types of chemicals produced and released from plants affects the germination and growth of another neighbouring plant (24). The allelopathy play a crucial role in plant-plant interactions due to allelochemicals (32,33,34), released from these plants might be favourable or harmful to the growth of other receptor plants (32). The production of secondary metabolites (Allelochemicals) from the plants influences the growth and development of different agricultural crops and biological systems (13). Many plant species produce and release the secondary metabolites/bioactive compounds (tannins, phenolic acids, lignins, alkaloids, flavonoids, coumarins and terpenoids) into the environment and these suppress the growth of other plants. These are present in all plant tissues (leaves, stems, roots, rhizomes, flowers, fruits and seeds and even in pollen grains (2). However, the growth stimulation of plants by different other plants (17) have been ignored because these are not very strong like the inhibitory effects (37). Allelopathy performs an important function under the natural and managed ecosystems by affecting the seed germination and growth of seedlings (23). The allelopathic effects of some plants

*Correspondence author

have been studied on germination inhibition (24), plumule and radicle length, seedling growth retardation and poor seedling survival (36).

Calotropis procera Decne. (Family Asclepidaceae) is an evergreen, spreading and perennial shrub (18). It grows wild in agricultural areas in sandy warm areas (4). *Citrullus colocynthis* (Family Cucurbitaceae) is dioecious herb, which may be prostate or climber. The widespread occurrence of *C. procera* and *C. colosynthis* near barley, oat, rice, sorghum, wheat, cotton, sugarcane and maize fields, makes it suspicious to cause some adverse effects on these crops through allelopathic interaction. Moreover in the near future, they may invade our crop fields and may become major weeds of our cropping system. Hence, this study aimed to evaluate the allelopathic effects of *C. procera* and *C. colosynthis* on the germination and seedling growth of maize (*Zea mays* L.) in laboratory and pot culture.

MATERIALS AND METHODS

Fresh leaves of *C. procera* and *C. colosynthis* were collected from the arid region, District Mardan, Punjab, Pakistan. The dried leaves of *C. procera* and *C. colosynthis* were finely grinded using mechanical grinder and stored in air tight containers for further use. The experimental treatments consisted of 2 factors, (i). Plant species: 2 (*C. procera*, *C. colosynthis*) and (ii). Leaf extracts concentrations: 3 (0.8%, 0.1%, and 1.2%). The treatments were replicated 3-times in complete randomised design. The experiment was conducted in Laboratory (Petri plates) and pot culture. To prepare the extracts of *C. procera* and *C. colosynthis*, 1.2 g leaves of each were soaked in 100 ml distilled water at room temperature (28-30°C). After 24h the extracts were filtered through four layered cheese cloth. Other concentrations of extracts of both selected species (0.1% and 0.8%) were prepared by diluting the stock solution of 1.2% and stored for further application in Petri plate and pot experiment.

Laboratory Bioassay

The germination test and seedling growth with three replication of each treatment were arranged in completely randomized design and were carried out in sterile Petri dishes (15cm) lined with filter paper Whatman No. 3 and in small pots. Seeds of maize were sterilized with NaOCl 10% for 2-3min, thereafter; seeds were rinsed thrice with sterile water. Aqueous extracts of different concentrations (10 ml) were pipetted to the filter paper placed in Petri dishes and distilled water was used as control. The Petri dishes were kept in the laboratory at room temperature (28-30°C). The laboratory experiment was done for 7 days to allow the last seed germination. A seed was considered as germinated, when the radical emerged. Germination was recorded daily. The number of germinated seeds were counted daily and also measured the lengths of both root and shoot. The vigour index (VI) of the seed was estimated as under: (1)

$$\text{Vigour index} = [\text{Germination (\%)} \times \text{mean (radicle length + plumule length]} / 100$$

After germination test and measuring the root and shoot length, the seedlings were separated into shoot and root parts for measuring fresh and dry weight.

Pot Culture

The germination test and seedling growth with three replication of each treatment were arranged in completely randomized design and were carried out in sterile small pots (11 cm depth, 8 cm dia). Sterilized soil and sand (3:1) was used for pot experiment. Seeds were sterilised with NaOCl (10%) and thoroughly rinsed with sterile water. Aqueous extracts of different concentrations (50 ml) were applied to pots and distilled water was used as control. The pot experiment was done for 15 days under natural conditions. The number of germinated seeds were counted, root and shoot length was measured and recorded the dry weight of root and shoot of maize seedlings.

Data was statistically analyzed by ANOVA on Statistix 8.1. Software and comparison among mean values of all treatments was made by Least Significant Difference (LSD).

II. Qualitative and Quantitative analysis of Phytochemicals

Various chemical tests were done for the presence of phytochemicals analysis in each fraction of both plants by using standard procedures.

(i). Tannins

Presence of tannins in various fractions was determined by Sofowara method (30). Fifty mg of each fraction was boiled in 20 ml distilled water in a test tube and was filtered. A few drops of 0.1% ferric chloride was added in each test tube and observed for colour change, the presence of brownish green or a blue-black coloration shows the presence of tannins.

Tannin content was determined using Van-Burden and Robinson WC (35) method. Absorbance at 120 nm was recorded in spectrophotometer (HITACHI. Model: U-1100 573 × 415) within 10 min and tannins contents were expressed as percentage of dried fraction.

(ii). Saponins

Determination of saponins in various fractions was done as per Harborne (10). Twenty mg of each sample was boiled in 20 ml distilled water in a water bath for 5-minutes and filtered. Ten ml of each filtrate was mixed with 5 ml distilled water and shaken vigorously for froth formation. Three drops of olive oil was mixed in the froth and shaken vigorously, and observed for the emulsion formation.

(iii). Phenolics

Total phenolic contents of *C. procera* and *C. colocynthis* were determined by the Folin-Ciocalteu colorimetric method (14) using gallic acid as standard and the absorbance was measured at 765 nm in spectrophotometer (HITACHI. Model: U-1100 573 × 415). Results were expressed as gallic acid equivalent (GAE) mg/g of dried fraction. Data for each fraction was recorded in triplicate.

(iv). Flavonoids

Two methods were used to determine the presence of flavonoids in the plant extracts (10, 30).

a) Fifty mg of each fraction was heated for 3 min with 10 ml ethyl acetate over a steam. The mixture was filtered and 1 ml of dilute ammonia solution was shaken with 4 ml of filtrate. Appearance of yellow colour indicated the presence of flavonoids.
b) Fifty mg of each extract was suspended in 100 ml distilled water to get the filtrate. Five ml of dilute ammonia solution was added to 10 ml of aqueous filtrate followed by few drops of concentrated H_2SO_4. A yellow colour observed in each fraction indicated the presence of flavonoids.

Total Flavonoids content was determined as per Sakanaka *et al*. (24) method. The absorbance was measured immediately at 510 nm in spectrophotometer (HITACHI. Model: U-1100 573 × 415). Flavonoids were estimated as Rutin equivalent mg/g of dried fraction. All samples were run in triplicate.

(v). Terpenoids (Salkowski test)

Presence of terpenoids in various fractions was determined as per Harbrone (10). Five ml (1 mg/ml) of each extract was mixed in 2 ml chloroform and then 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the inter face showed the presence of terpenoids.

(vi). Alkaloids

Alkaloids in various fractions were detected as per Harbrone (10). 0.4 g of each extract was mixed with 8 ml of 1% HCl, warmed and filtered. Two ml of each filtrate was titrated separately with (a) Mayer's reagent and (b) Dragendroff's reagent. Turbidity of precipitation indicated the presence of alkaloids.

(vii). Phlobatannins

Phlobatannins in various fractions were identified according to Trease and Evans (31). 80 mg of each plant extract was boiled in 1% aqueous hydrochloric acid, the deposition of a red precipitate indicated the presence of phlobataninins.

(viii). Coumarins

In each fraction, presence of coumarins was determined as per Trease and Evans (31). 0.3 g of each plant extract was taken in a small test tube and covered with filter paper moistened with 1 N NaOH. The test tube was placed, for few minutes, in boiling water bath. After removing the filter paper it was examined under UV light, yellow florescence indicated the presence of coumarins.

(ix). Total Proteins

Total protein in both plant extracts was determined using colorimetric method (16). Plant extract (0.4ml) was mixed with 4 ml of copper sulphate solution and incubated at room temperature for 10 min. Then, 4 ml of phenol reagent was added and allowed to react for 30 min. The absorbance was measured at 600 nm against reagent blank.

(x). Total Sugars

Total sugar in the plant extract was determined using calorimetric method (8). Plant extract (1 ml) was mixed with 1 ml of 2% phenol and 5 ml of concentrated sulphuric

acid, allowed to react for 30 min and the absorbance was measured at 430 nm against reagent blank.

(xi). Elemental analysis of Plant extracts

To determine the macro and micronutrients, different stock solutions were made. One hundred ppm stock solution of K, Mg, Ca, Fe, Mn, Cu and Zn were prepared by dissolving required amount of salts in distilled water. The availability of different elements in all plants collected from Mardan district was determined by Perchloric-acid digestion method (3).

Procedure

Oven dried sample (0.25g) were taken in 50 ml flask and added 6.5 ml of mixed acid solution i.e.Nitric acid, Sulfuric acid, Perchloric acid (5:1:0.1) in it and boiled it in fume hood on hot plate till the completion of digestion indicated by white fumes coming out from the flasks. Thereafter, few drops of distilled water were added and allowed to cool. Then these digested samples were transferred in 50 ml volumetric flasks and the volume was made upto 50 ml by adding distilled water in them. Then filter the extract with whatmann No 42 filter paper and filtrate were collected in labeled plastic bottles. Concentration of these elements in the entire sample was determined by Shimadzu AA-670 Atomic Absorption Spectrophotometer.

$$\text{Nutrient cation in plants} = (\text{ppm in extract} - \text{blank}) \times A/W \times \text{dilution factor}$$

Where, A: Total volume of extract (ml), W: Weight of dry plant

RESULTS AND DISCUSSION

Germination

The direct or indirect effect of one plant upon another plant through the release of different chemical compounds into the environment is demonstrated by seed germination test (15) and seedling growth of recipient plants. All leaf extract concentrations of *C. procera* in both the laboratory (5.2%-5.6%) and pot experiment (1.20%-6.8%) increased the germination (%) than control (Table 1).

The germination (%) in laboratory (12.4%-24.8%) and pot experiment (1.2%-6.5%) was non-significantly reduced in all treatments from lowest (8 mg/ ml) to highest concentration (12 mg/ml) of *C. colosynthis* (Table 1). The alterations in the synthesis or activities of gibberellic acid in the seed could be due to the presence of phenolic compounds (21). Therefore, the presence of phenols, tannins and flavonoids in the leaf extract of *C. colosynthis* may be responsible for its inhibitory effect on the germination of maize seeds.

Allelochemicals of plant extracts have both inhibitory and stimulatory effects on growth of other plants. The reduction in germination might due to allelopathic effects of plant extract. The inhibition increased with increase in concentrations of aqueous extracts (26,28). Leaf extracts of *Ougeinia oojeinensis* are toxic to germination of *Brassica*

Table 1. Effects of leaf extracts of *C. procera* and *Citrollus colocynthis* on maize seed germination (G%) and Vigour index in Petri Plate and Pot Experiments

Extract conc. (%)	Petri Plate Experiment		Pot Experiment	
	Germination (%)	Vigour Index	Germination (%)	Vigour Index
		Calotropis procera		
Control	84.3[a]	26.7[a]	83.3[a]	29.3[a]
0.8	88.7[a]	27.9[a]	84.3[a]	29.9[a]
0.1	88.7[a]	21.7[b]	85.7[a]	31.1[a]
1.2	89.0[a]	26.3[ab]	88.7[a]	33.1[a]
Mean	88.8	25.3	86.2	31.4
		Citrollus colocynthis		
Control	88.7[a]	21.6[a]	84.3[a]	29.3[a]
0.8	77.7[a]	22.8[a]	72.3[ab]	25.8[ab]
0.1	66.7[a]	20.5[a]	61.3[b]	22.1[b]
1.2	66.7[a]	20.1[a]	61.3[b]	22.6[ab]
Mean	70.4	21.1	65.0	23.5

Data in Parenthesis indicate % Inhibition/ Stimulation over control

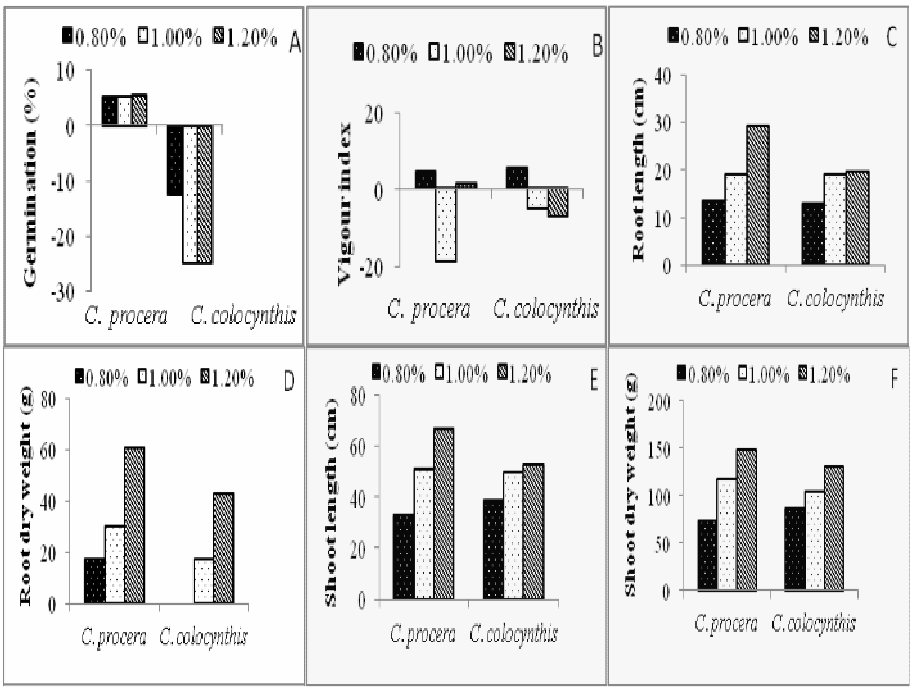

Figure 1. Effects of leaf extracts of *Calotropis procera* and *Citrollus colocynthis* on maize seed germination and Vigour index and seedling growth in Petri Plate Experiment

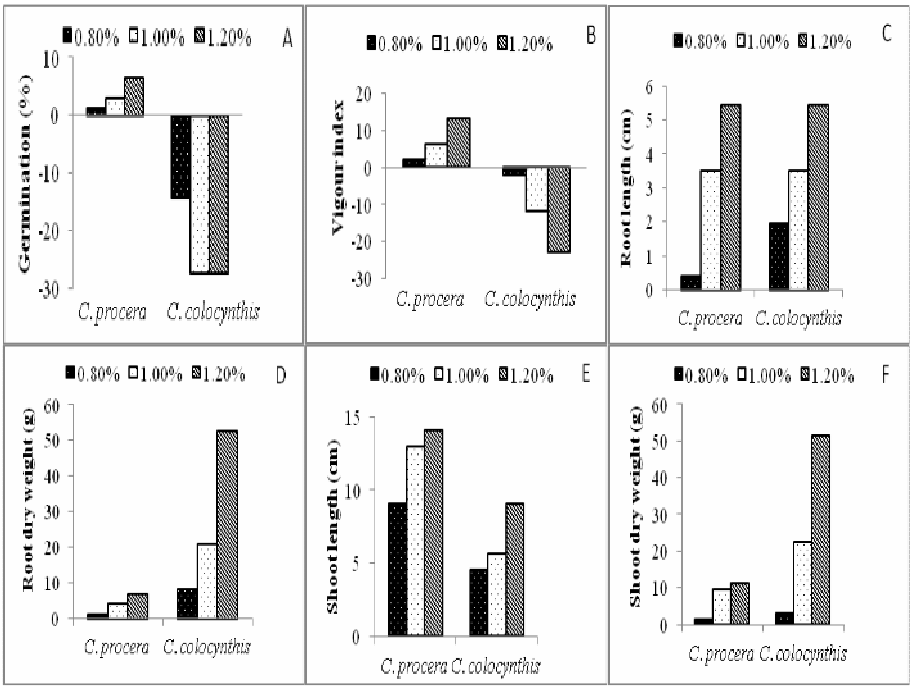

Figure 2. Effects of leaf extracts of *Calotropis procera* and *Citrollus colocynthis* on maize seed germination and Vigour index and seedling growth in Pot Experiment

campestris, *Triticum aestivum* and *Hordeum vulgare* (19). There were non-significant differences in vigour index in both laboratory and pot experiment.

Seedling Growth

Aqueous leaf extracts of both donor plants stimulated the root length, root fresh weight and root dry weight of maize seedlings.

In laboratory experiment all concentrations of aqueous leaf extract from *C. procera* increased the seedling growth and maximum increase was in root length (29%), root fresh weight (93%) and root dry weight (61%) was recorded at highest concentration (12 mg/ml) than control. However in pot experiment, all concentrations of leaf aqueous extracts from *C. procera* increased the root length, root fresh weight and root dry weight of maize seedlings and the increase was concentration dependent (Table 2). Previous studies (20) reported that water extracts of leaf, stem and root of *C. gigantea* increased the root length of rice plant. In both laboratory and pot bioassay, all concentrations of *C. procera* increased the shoot length, shoot fresh weight and shoot dry weight than control. In Petri plate experiment the maximum increase in shoot length was (66%) and in shoot dry weight (48%) at highest concentration (12 mg/ ml). The same pattern of increase in seedling growth was also observed in pot experiment (Table 3).

Table 2. Effects of different concentration of *C. procera* and *C. colocynthis* leaf extracts on Maize seedling growth root length (RL) and root dry weight (DW) in Petri Plate and Pot Experiment

Extract conc. (%)	Petri Plate Experiment		Pot Experiment	
	Root length (cm)	Root dry weight (g)	Root length (cm)	Root dry weight (g)
		Calotropis procera		
Control	19.5[d]	0.023[b]	25.8[a]	0.072[a]
0.8	22.1[c]	0.027[b]	25.9[a]	0.073[a]
0.1	23.2[b]	0.030[ab]	26.7[a]	0.075[a]
1.2	25.2[a]	0.037[a]	27.2[a]	0.077[a]
Mean	23.5	0.031	26.6	0.075
		Citrollus colocynthis		
Control	19.5[c]	0.023[a]	25.8[a]	0.072[b]
0.8	22.0[a]	0.023[a]	26.3[a]	0.078[b]
0.1	23.2[a]	0.027[a]	26.7[a]	0.087[ab]
1.2	23.3[b]	0.033[a]	27.2[a]	0.11[a]
Mean	22.8	0.028	26.7	0.092

Data in Parenthesis indicate % Inhibition/ Stimulation over control

Table 3. Effects of different concentration of *C. procera* and *C. colocynthis* leaf extracts on Maize seedling growth shoot length (SL) and shoot dry weight (DW) in Petri Plate and Pot Experiment

Extract conc. (%)	Petri Plate Experiment		Pot Experiment	
	Shoot length (cm)	Shoot dry weight (g)	Shoot length (cm)	Shoot dry weight (g)
		Calotropis procera		
Control	9.8[d]	0.023[c]	17.7[b]	0.62[a]
0.8	13.0[c]	0.040[b]	19.3[ab]	0.63[a]
0.1	14.8[b]	0.050[ab]	20.0[ab]	0.68[a]
1.2	16.3[a]	0.057[a]	20.2[a]	0. 69[a]
Mean	14.7	0.049	19.8	0.67
		Citrollus colocynthis		
Control	9.83[c]	0.023[b]	17.7[a]	0.062[b]
0.8	13.6[b]	0.043[a]	18.5[a]	0.064[b]
0.1	14.7[a]	0.047[a]	18.7[a]	0.076[b]
1.2	14.97[a]	0.053[a]	19.3[a]	0.094[a]
Mean	14.4	0.048	18.8	0.078

Data in Parenthesis indicate % Inhibition/ Stimulation over control

In Petri plate experiment, all concentrations of *C. colosynthis* significantly increased the root length, root fresh weight but slightly increased the root dry weight of maize seedlings. The maximum increase in root length (19%) and root dry weight (43%) was recorded by the highest concentration (12 mg/ ml) of *C. colosynthis* over control (Table 2). In pot culture, all concentrations of *C. colosynthis* exhibited slight increase in root length, root fresh weight and dry weight. However, the highest concentration (12 mg/

ml) increased the root dry weight (53%) of maize seedlings over the control (Table 2). Seed exudates of *C. colosynthis* did not inhibit the root growth of proso millet seedlings (11). Elongation in root indicates the growth stimulation in plant. In laboratory bioassay all concentrations of leaf extracts of *C. colosynthis* increased the shoot length of maize seedlings over control. The maximum increase in shoot length (52%) was observed at 12 mg/ml concentration of *C. colosynthis*. All concentrations of *C. colosynthis* slightly stimulated the shoot dry weight in Petri plate experiment and the shoot length in pot experiment. In pot experiment, the highest concentration of *C. colosynthis* (12 mg/ ml) significantly increased the shoot dry weight (52%) than control (Table 3).

Highest concentrations of both selected species increased the seedling growth, this might be due to the proteins and sugar content present in the leaf extracts of both plants. The present investigation revealed the allelopathic growth enhancing effects of *C. procera* and *C. colosynthis* and also has the water soluble substances. The higher concentration of *Ficus subincisa*, *Toona hexandra* and *Bauhinia purpurea* stimulates the seedling growth of *Brassica campestris*, *Triticum aestivum* and *Hordeum vulgare* (27). Different types of allelochemicals (alkaloids, phenolic acids and terpenoides) are leached from plants (7,12). The endogenous phenols have only the growth stimulatory properties and also work as analogues of growth hormones and have an effect on the growth and physiology of plants (9). Tea Seed Powder stimulates growth of pot grown oat, beet, barley and mustard and also promotes the growth and yield of strawberries (5).

Phytochemicals analysis

The alkaloids, phenolics, flavonoids, tannin, saponin, terpenoids, phlobetanin and coumarins were found present in *C. procera* (Table 4). In *C. colocynthis* also all these phytochemicals were present except phlobatanins. Total phenolic content (TPC) was determined in comparison to standard Gallic acid and the results were expressed as mgGA/g dry sample of plant (Table 5), while total flavonoid content was determined in comparison to standard Rutin and the results were expressed as mgRU/g of dry sample of plant. There was no significant difference in phenolic content and flavonoid content in extracts of selected plants (*C. procera* and *C. colocynthis*). Both plant species contained tannins, *C. procera* extract (0.503±0.054%) followed by *C. colocynthis* (0.347±0.003%). These phytochemicals possess allelophatic activity on some plant species (25).

Total Protein and Sugar

The maximum protein content in leaf extract was observed in *C. colocynthis* (457.71±0.99µg/g) and *C. procera* leaf extract (428.68±3.7 µg/g) (Table 5). While the maximum sugar content WAS in leaf extract of *C. procera* (1567.52±3.4 µg/g) followed by *C. colocynthis* (1473.1±4.3 µg/g). The protein and sugar content in the plant extracts may also cause stimulatory effects on seedling growth.

Elemental analysis of selected plant species

Macronutrients: The selected plant species differed in their macronutrients content (Ca, K, and Mg) (Table 4). The Ca content was maximum in *C. procera* (3.13±0.007mg/g) followed by *C. colocynthis* leaf extracts (2.93±0.006mg/g). However, the leaf extracts of

C. procera was rich in K (82±0.018mg/g) and Mg (1.113±0.018mg/g) than *C. colocynthis* (1.59±0.009mg/g) and (0.473±0.012mg/g), respectively.

Table 4. Qualitative Phytochemical Analysis of selected plants. The data represents mean of three replicates

Constituents	*Calotropis procera*	*Citrullus colocynthis*
Alkaloids		
Dragondorff's test	+++	+++
Mayer's test	+++	+++
Phenolic	+++	+++
Flavonoids	+++	+++
Tannin	+++	++
Saponin	+	+
Terpenoids	++	++
Phlobetanin	+	-
Coumarin	+	+

+++ : Strong positive , ++: Moderate positive, +: weak positive,-: Negative

Table 5. Quantitative analysis of Chemical constituents and estimation of protein and sugar in dry plant extracts

Constitutents	*C. procera*	*C. colocynthis*
Phenolic (mg/g)	41.775±0.086	41.573±0.034
Flavonoids (mg/g)	52.221±0.119	52.811±0.403
Tannin (%)	0.503±0.054	0.347±0.003
Total Proteins (µg/g)	428.68±3.7	457.71±0.99
Total Sugar (µg/g)	1473.1±4.3	1567.52±3.4

Micronutrients: The selected plant species contains the micronutrients (Fe, Mn, Zn and Cu) (Table 4). The Fe content in *C. colocynthis* was 98.98±0.18µg/g followed by *C. procera* (94.19±0.35 µg/g). The Mn content was higher in *C. procera* (85.20±0.77 µg/g) than *C. colocynthis* (73.44±0.56 µg/g). The Zn content was similar in *C. procera* extract (63.90±0.93 µg/g) and in *C. colocynthis* extract (63.02±0.09 µg/g). The Cu content was higher in *C. procera* (18.47±0.26 µg/g) than *Citrollus colocynthis* (14.92±0.21 µg/g). The macro and micronutrients present in the selected plant extracts can be easily absorbed by host plants, which may promote the growth of host plant by stimulating its specific biological factors.

It was concluded from this study that maize can be grown in vicinity of naturally growing *C. procera* and *C. colosynthis,* as these weed plants have growth promoting substances and less detrimental effects on maize crop.

ACKNOWLEDGEMENT

The authors are thankful to Higher Education Commission, Pakistan for financial support of this research.

REFERENCES

1. Abdul-baki, A. and Anderson, J.D. (1970). Viability and leaching of sugars from germinating barely. *Crop Science* **10**: 31-34
2. Ahmad, S., Arfan, M., Khan, A.L., Ullah, R., Hussain, J., Muhammad, Z., Khan, R., Khan, N. and Watanabe, N. (2011). Allelopathy of *Teucrium royleanum* Wall. Ex Benth. From Pakistan. *Journal of Medicinal Plants Research* **5**: 765-772.
3. Allen, S.E. (1974). *Chemical Analysis of Ecological Materials*. Blackwell Scientific Publications, Oxford.
4. Al-Zahrani, H.S. (2002). Effects of salinity stress on growth of *Calotropis procera* seedlings, *Bulletin of Pure and Applied Sciences* **21**: 109-122
5. Andersen, M. and Cedergreen, N. (2010). Plant growth is stimulated by tea-seed extract: A new natural growth regulator? *Horiculture Science* **45** : 1848-1853.
6. Ashrafi, Z.Y., Mashhadi, H.R. and Sadeghi, S. (2007). Allelopathic effects of barley (*Hordeum vulgare*) on germination and growth of wild barley (*Hordeum spontaneum*). *Pakistan Journal of Weed Science Research* **13**: 99-112.
7. Blum, U., Shafer, S.R. and Lehman, M.E. (1999). Evidence for inhibitory allelopathic interactions involving phenolic acids in field soils: Concepts *vs* an experimental model. *Critical Reviewes in Plant Sciences* **18**: 673-693.
8. Dubois, M., Gills, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**: 350-356.
9. Gantzer, E. (1960). Wirkungen von Cumarin auf Wachstums- und Entwicklungsvorgänge und seine Wanderungs-fähigkeit im Pflanzengewebe. *Planta* **55**: 235-253. (German)
10. Harborne, J.B. (1973). *Phytochemical Methods.* Chapman and Hall, London. pp. 49-188.
11. Howard, F., Harrison, J.R., Levi, A. and Kousik, C.S. (2008). A survey of watermelon germplasm for inhibitory seed exudates. *Horticulture Science* **43**:138-142.
12. Inderijt. (1996). Plant phenolics in allelopathy. *Botanical Review* **62:** 186-202.
13. Khalid, S., Ahmad, T. and Shad, R.A. (2002). Use of allelopathy in agriculture. *Asian Journal of Plant Sciences* **3:** 292-297.
14. Liu, X., Ardo, S., Bunning, M., Parry, J., Zhou, K. and Stushnoff, C., Stoniker, F., Yu, L. and Kendall, P. (2007). Total phenolic content and DPPH radical scavenging activity of lettuce (*Lactuca sativa* L.) grown in Colorado. *Food Science and Technology* **40** : 552-557.
15. Lovett, J. and Ryuntyu, M. (1992). Allelopathy: Broadening the context. In: *Allelopathy, Basic and Applied Aspects*. (Eds., S.J.H. Rizvi and V. Rizvi). Chapman and Hall, London. Pp. 11-19
16. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193:** 265-75.
17. Mallik, M.A.B. and Williams, D.R. (2005). Allelopathic growth stimulation of plants and microorganisms. *Allelopathy Journal* **16**: 175-198.
18. Nasser, R.A., Al-Mefarrej, H.A., Khan, P.R. and Alhafta, K.H. (2012). Technological properties of *Calotropis procera* (AIT) wood and its relation to uses. *American-Eurasian Journal of Agricultural and Environmental Sciences* **12**: 5-16
19. Negi, B.S., Chauhan, D.S. and Todaria, N.P. (2007). Allelopathic effects of *Ougeinia oojeinensis* Roxb. (Fabaceae) on the germination and growth of wheat, barley and mustard. *Allelopathy Journal* **20**: 403.410.
20. Oudhia, P. and Tripthi, R.S. (2001). Allelopathic effects of Ageratum conyzoides and Calotropis gigantea on germination and seedling vigour of rice. *Agricultural Science Digest* **21**(1): 69-70.
21. Olofsdotter, M. (2001). Rice -A step toward use of allelopathy. *Agronomy Journal* **93**: 3-8.
22. Rice, E. L. (1984). *Allelopathy*. 2nd ed. Academic Press, New York.
23. Sadeghi, S., Rahnavard, A. and Azhrafi, Z.Y. (2010). Allelopathic effect of *Helianthus annuus* (sunflower) on *Solanum nigrum* (Black Nightshade) seed germination and growth in laboratory condition. *Journal of Horticulture Sciences and Ornamental Plants* **2**: 32-37.
24. Sakanaka, S. Tachibana, Y. and Okada, Y. (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chemistry* **89**: 569-575.
25. Seigler, D.S. (1996). Chemistry and mechanism of allelopathic interactions. *Agronomy Journal* **88**: 876-885.

26. Seyyednejad, S.M., Koochak, H., Najafabade, F.P. and Kolahi, M. (2010). Allelopathic effect of aquatic hull extract of rice (*Oryza sativa* L.) on growth of *Silybum marianum* and *Echinochloa crus-galli. African Journal of Agricultural Research* **5**: 2222-2226.
27. Singh, B., Jhaldiyal, V. and Kumar, M. (2009). Effects of aqueous leachates of multipurpose trees on test crops. *Estonian Journal of Ecology* **58**: 38-46
28. Sisodia, S. and Siddiqui, M.B. (2008). Allelopathic effect of *Lantana camara* on *Bidens pilosa. Vegtos* **20**: 29-32.
29. Sisodia, S. and Siddiqui, M.B. (2009). Allelopathic potential of rhizosphere soil of *Croton bonplandianum* on growth and establishment of some crop and weed plants. *African Journal of Agricultural Research* **4**: 461-467.
30. Sofowara, A. (1993). *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books, Ibadan, Nigeria. p. 289.
31. Trease, G.E. and Evans, W.C. (1989). *Pharmacognosy*. 11th Edn. Macmillian publishers.
32. Thapaliyal, S., Bali, R.S., Singh, B. and Todaria, N.P. (2007). Allelopathic effects of trees of economic importance on germination and growth of food crops. *Journal of Herbs Spices and Medicinal Plants* **13**: 11.23.
33. Turk, M.A. and Tawaha, A.M. (2003). Allelopathic effect of black mustard (*Brassica nigra* L.) on germination and growth of wild oat (*Avena fatua* L.). *Crop Protection* **22** : 673-677.
34. Turk, M.A., Lee, K.D. and Tawaha, A.M. (2005). Inhibitory effects of aqueous extracts of black mustard on germination and growth of radish. *Research Journal of Agriculture and Biological Sciences* **1**: 227-231.
35. Van-Burden, T.P. and Robinson, W.C. (1981). Formation of complexes between protein and tannin acid. *Journal of Agriculture Food Chemistry* **1**: 77-82.
36. Vankar, P.S. and Srivastava, J. (2008). Comparative study of total phenol, flavonoid contents and antioxidant activity in *Canna indica* and *Hibiscus, Rosa sinensis*, prospective natural food dyes. *International Journal of Food Engineering* **4**: 1-17.
37. Yamada, K. and Hirose, K. Hideyuki Shigemori1, Koji Hasegawa. Plant Growth Promotive Allelochemicals. www.niaes.affrc.go.jp/marco/.../**W3-10**