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RESEARCH ARTICLE

CALOTROPIS PROCERA- A BIOHERBICIDAL AGENT TO CONTROL BARNYARD GRASS AND BERMUDA GRASS IN PADDY FIELDS

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ABSTRACT

Barnyard grass *(Echinochloa crus-galli* L.) and Bermuda grass (*Cynodon dactylon* L.) are the most prominent weeds in rice field which significantly affected the growth and development of rice from germination stage to yielding stage. The allelopathic effect of dry leaves (mature and senesced) aqueous extracts (5%, 10%, 15%, 20% and 25%) of *Calotropis procera* Decne., was investigated to evaluate the herbicidal efficacy on the germination and growth of Barnyard grass (*Echinochloa crus-galli* L.) and Bermuda grass (*Cynodon dactylon* L.). The results showed that the germination delayed when concentration increased from lower to the higher concentrations. The most affected seeds of the tested plants were *Echinochloa crus-galli*, i.e. up to 89%. Generally, the radical length was more sensitive than plumule length against aqueous concentration. *C.dactylon* showed minimum retardation effect when treated with *C. procera* aqueous extract. Aqueous mature leaves extract of *C. procera* showed maximum inhibited (upto 89%) at the higher concentrations (25%). *C.dactylon* shown stimulatory effect on its all physio-biochemical parameters, when treated with low concentrated (up to 5%) aqueous concentration of *C.procera.* The phytotoxic effect of matured leaves was more pronounced than senesced leaves.

INTRODUCTION

Rice is a staple food in numerous countries around the globe serving daily as a source of carbohydrate, protein, lipid, vitamins, and minerals (Walter *et al.*, 2008). Therefore, rice production should maintain its trend in order to support the constantly growing consumption demand (Bennett, 2008). Weed infestation in rice fields results in huge economic losses and low quality crop yields (Taiwo and Makinde 2005, Khan *et al*. (2008). This may be due to phytotoxic allelochemicals which released by weeds in crop fields (Naqvi and Mullar1975; Hussain and Abidi, 1991). Worldwide, a large amount of money is spent every year to control them. While control of weeds can be achieved through several means such as mechanical, chemical and biological, in which use of synthetic herbicides is common and provides an effective method. Unfortunately, the use of synthetic herbicides may affect the environment and human health, and also leads to increasing herbicidal resistance among many weed species (Om *et al*., 2002 and Heap, 2006). Therefore, efforts to develop alternative means of weed control, which are not only eco- friendly, but also cost effective and bioefficaceous are needed (Duke *et al*., 2002). Allelopathy is a natural and environment friendly technique which may prove to be a tool for weed management and thereby increase crop yields. The term allelopathy is commonly denotes the interaction in which one plant could cause suffering to another plant (Rice, 1984). As mentioned it may be environmental friendly since it could be extracted from flower, leaves, stem and roots. These allelopathic extracts could be used to control the growth of weeds (Chon *et al*., 2003 and Singh *et al*., 2003). The main principle in allelopathy arises from the fact that compounds which is known as allelochemical which can alter the growth and physiological activities of plants. Allelopathic plants produce thousands of chemicals; some of those compounds are released to the environment, from leaching, litter decomposition, root exudation, or direct volatilization and could affect (either positively or negatively) germination and growth of neighbor species. The most commonly found allelochemicals are cinnamic acid, benzoic acids, flavonoids, and various terpenes (Singh *et al*., 2003); these compounds are known to be phytotoxic. Phytotoxicity of allelochemicals are assumed to be associated with the presence of strong electrophilic or nucleophilic system. Action by such as systems on specific positions of protein or enzyme would alter their configuration and affect their activity (Macias *et al*., 1992). Rice cropping which is characterized by the heavy use of fertilizers, herbicides and pesticides may cause environmental problems in the future. Therefore, low- input sustainable agriculture may be an alternative way to minimize environmental costs. *E.crus-galli* is one of the greatest yield- limiting weeds in the irrigated rice systems of India. Barnyard grass is better adapted for growth under dry rather than wet conditions and is expected to

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become a greater problem in dry, direct- seeded rice. Ecological control through the application of naturally occurring allelopathic substances to agriculture practice has been an important and useful method to control weed (Macias, 1995). *Calotropis procera* is one of the poisonous plant belongs to family Asclepiadaceae and abundantly used to grow in barren land. It grows commonly around farms, agricultural areas, and in the sandy warm parts especially in the western coastal plain (Narwal 1994). It is world widely distributed but mostly abundant in tropical and sub-tropical countries . Intensive scientific research on the effect of weeds on crops, crops on weeds, crops on crops has only occurred over past few decades. Several researchers have studied the impact of allelochemicals on different plants in crop and agro forestry systems, such as Narwal *et al*, (1994) and Oudhia *et al*; (1999), However, reason behind crop yield losses depend on some factors like weeds depend on the cultivars used, duration of crop weed competition, the major weed species involved, weather variation between years and the agronomic practices used to modify the conditions at the specific sites. Hence the present investigation has been made to determine the bioherbicidal efficacy of a weed *Calotropis procera* on two different weed verities viz. Barnyard grass and Bermuda grass.

MATERIALS AND METHODS

Experimental sites

Experiments were carried out during the month of January to May 2013 in Department of Botany and Botany wing- DDE, Annamalai University, Annamalai Nagar, Tamilnadu, which is situated at 11.4° North latitude, 79.73° East longitude and about 5.248 meters altitude from the sea level. Physio-chemical properties of experimental soil has been described in Table no 1.

Weed sample collection

Aerial part (mature and senesced leaves) of *Calotropis procera* and seeds of Barnyard grass and Bermuda grass were collected from the nearby locality areas of Annamalai Nagar, Tamilnadu, and stored in polyethene bags in dry and dark place until the further use.

Weed extract formation

C.procera leaves (fresh and senesced) were thoroughly rinsed under the tap water by 2 to 3 times. Leaves were further washed by 0.1% Mercuric Chloride ($HgCl_2$) for maximum sterilization. Thereafter, leaves were cutted into small pieces. These pieces were dried in to the hot air oven at 80°c for about 24 hours .After this these dried pieces were crushed with the help of ordinary grinder until a powder form is formed. For preparing the different desirable concentration of plant sample, 500 gm of fine power of *C.procera* leaves were was soaked in 5 liter of water for 48 hrs under aseptic condition. Finely extract was filtered by muslin cloth and filtrate was considered as 100% concentrated extract. From that filtrate, different desirable extracts (5%, 10%, 15%, 20% and 25%) were prepared by the water dilution.

5%= 50ml.L-^{1}
10%=100ml.L-^{1}
15%=150ml.L-^{1}
20%=200ml.L-^{1}
25ml=250ml.L-^{1}

Bioassay for germination percentage

Earthen pots (20cm×30cm×10cm) were used for the germination of Barnyard grass and Bermuda grass seeds. Three kilogram of normal garden soil (description of soil is mentioned in table no- 1) used as a medium for the bioassay experiments. The 50 seeds of each Barnyard grass and Bermuda grass were taken and steeped in water to determine their viability; those seed that floated were discarded. The viable seeds were sterilized for two minutes in 0.2% mercuric chloride ($HgCl_2$) solution. The seeds were then thoroughly washed with tap water and the seeds were sown to the normal garden soil in earthen pot. Each pot was irrigated uniformly by different concentrations of weed extracts and the distilled water was used as control. Each experiment was carried out with five replicates. The extracts/water was irrigated to the pots in alternative day's upto 29th day from the day of seed sown. Germination percentage was recorded on 7th day of sowing of seeds and calculated by following formula.

Germination percentage= No of seed germinated/No seed sown ×100

Bioassay for seedling growth (physical and chemical parameter)

After 15 days and 30 days of sowing, both the varieties (Barnyard grass and Bermuda grass) of weed seedlings were uprooted, washed thoroughly and used as material for analysis of shoot and root length, fresh weight, dry weight, chlorophyll content, sugar content and protein content. All parameters were calculated by reference to the control plants.

Preliminay phytochemcial analysis of *C. procera*. (Suganya *et al.* 2012)

Results is given in Table no :1

Data analysis

All the experiments were performed in a completely randomized block design (RBD) and repeated thrice. For each treatment five replicates were maintained. The data of barnyard grass and Bermuda grass were subject to the analysis of variance (ANOVA) by using IBM SPSS Statistics version 20 and thereafter significance was tested for all the values by the variance ration (i.e. F-value) at the 5% level. Tukey's Multiple Range Test (TMRT) is used for understanding the significance among treatments at significance ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of mature and senesced leaves of *C.procera* on germination percentage of Barnyard grass and Bermuda grass after 15 DAS

Germination of Barnyard grass and Bermuda grass was adversely affected by aqueous extract of mature and senesced

Table 1. Preliminary Phytochemical analysis of *Calotropis procera*

Phytochemical	Organic solvent					
	Pet. Ether	n-Hexane	Chloroform	Ethyl acetate	Ethanol	Methanol
Alkaloid	-	+	+	+	+	-
Cardic glycosides	+	-	+	+	+	+
Flavonoid	-	+	+	+	+	+
Glycosides	+	+	+	+	-	-
Saponins		+	+	+	-	-
Saponins glycosides	-	+	+	+	+	+
Steroids	-	-	+	+	-	+
Tannins	-	-	+	+	+	+
Volatile oil	-	+	+	+	+	+

(+) symbol represent availability of Phytochemical and (-) symbol represents lacking of Phytochemical.

Table 2. Physiological properties of soil of Experimental pots (pre- treatment and post – treatment)

	Mature leave extract treatment on 30 DAS		Senesced leave extract treatment on 30 DAS	
	Bernyard grass		Bermuda grass	
Parameters	Pre-treatment	Post- treatment	Pre- treatment	Post- treatment
Texture	Light clay	Light clay	Light clay	Light clay
Sand (%)	57.5	57.2	57.5	54.2
Silt (%)	22.4	21.8	22.4	20.8
Clay (%)	13.2	12.5	13.2	9.5
pH	8.1	8.9	8.1	7.2
EC(ds/m)	0.59	0.83	0.59	0.83
Organic carbon (%)	1.4	2.3	1.4	2.5
Total nitrogen (%)	0.13	0..9	0.13	0.6
Available P (ppm)	5.3	4.6	5.3	3.4

Table 3. Effect of mature and senesced leaves of *C.procera* on germination percentage of Barnyard grass and Bermuda grass on 15 DAS

	Aqueous Mature leaves extract treatment on 15 DAS		Aqueous Senesced leaves extract treatment on 15 DAS	
Treatment	Barnyard grass	Bermuda grass	Barnyard grass	Bermuda grass
C	98a	92a	98a	94a
5	73.5b	96.8b	78.4b	99b
	(-24)	(+7)	(-1)	(+6)
10	61.5c	63.88c	66.4c	65.8c
	(-36)	(-31)	(-31)	(-29)
15	30.72d	34.78d	36.26d	38.54d
	(-68)	(-62)	(-62)	(-58)
20	22.54e	28.52e	27.44e	38.02e
	(-76)	(-69)	(-71)	(-66)
25	9.8f	19.98f	16.66f	17.86f
	(-89)	(-82)	(-82)	(-80)

- Mean values followed by the same letters within each column are not significantly different according to LSD (P =0.05) n = 4, C, control; 5, 10, 15, 20 and 25 denote the aqueous concentration of *C.procera*.
- (-) and (+) symbols represent inhibitory and stimulatory percentage over control respectively.
- Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) (P < 0.05).

Table 4. Effect of Mature and senesced leaves of *C.procera* on root length and shoot length of Barnyard grass and Bermuda grass after 30 DAS

Treatment	Aqueous Mature leaves extract treatment on 30 DAS				Aqueous Senesced leaves extract treatment on 30 DAS			
	Barnyard grass		Bermuda grass		Barnyard grass		Bermuda grass	
	root length	shoot length	root length	shoot length	root length	shoot length	root length	shoot length
C	6.9a	15.8a	4.7a	12.4	6.9a	15.8a	4.7a	12.4a
5	5.25b	12.64b	5.40b	14.01b	5.46b	12.96b	5.21b	13.76b
	(-23)	(-19)	(+14)	(+12)	(-20)	(-17)	(+10)	(+7)
10	4.01c	9.48c	2.87c	7.82c	4.21c	10.27c	3.06c	8.19c
	(-41)	(-39)	(-38)	(-36)	(-38)	(-34)	(-34)	(-33)
15	2.28d	6.04d	1.70d	4.96d	2.56d	6.64d	1.88d	5.58d
	(-66)	(-61)	(-63)	(-59)	(-67)	(-57)	(-59)	(-54)
20	1.45e	4.3e	1.18e	3.84e	1.73e	4.43e	1.37e	4.47e
	(-78)	(-72)	(-74)	(-68)	(-74)	(-71)	(-70)	(-63)
25	0.81f	2.37f	0.81f	2.01f	1.32f	3.32f	0.94f	2.86f
	(-86)	(-84)	(-82)	(-81)	(-80)	(-80)	(-79)	(-76)

- Mean values followed by the same letters within each column are not significantly different according to LSD (P =0.05) n = 8. C, control; 5, 10, 15, 20 and 25 denote the aqueous concentration of *C.procera*.
- (-) and (+) symbols represent inhibitory and stimulatory percentage over control respectively.
- Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) (P < 0.05).

leaves extract of *Calotropis procera*. However, germination responses varied with the test grass species. In our experiments, we found that phytotoxicity of extract is directly proportional to extract concentration. Our findings were supported by (Travlos *et al*., 2007 and Travlos and Paspatis, 2008) who mentioned that Magnitude of Phytotoxicity activity is dependent upon the concentration and chemical stability of the active compound. Table no 2 is showing that aqueous extract of *C.procera* were highly effective in reducing the germination of Barnyard grass and Bermuda grass. except 5%, in which mature leaves and senesced leaves shown stimulatory effect on Bermuda grass. Similar stimulatory effects were also observed by Batish *et al.* (1997) who stated that, a slight promotion in root length at low Parthenin concentration was previously found in *Triticum aestivum* L. In case of Barnyard grass, significant negative impact (upto -89%) on germination was shown at 25%. Similar results were found by Anjum *et al*. (2005) and Javed *et al*., (2006) who mentioned that aqueous extracts of allelopathic grassed *Imperata cylindrica* and *Desmostachya bipinnata* not only suppress the germination and growth of *P.hysterophorus,* but also reduce the spread of this noxious weeds in the field. Least negative impact was at 5%, which shown only (-24%) phytotoxicity effect on seedling germination of Barnyard grass. Result shown that Bermuda grass is more recessive than barnyard grass. In the case of senesced leave extract treatment, Phytotoxicity impact was least significant than mature leave extract treatment. Barnyard grass shown maximum negative Phytotoxicity impact (-82%) at 25% followed by 20% extract concentration which shown (-71%). Minimum Phytotoxicity impact was shown at 5% which was (-19%). In the case of Bermuda grass maximum Phytotoxicity was shown (-80% and -29%) at 25% concentration. At 5% concentration Bermuda grass showed stimulatory impact (+6%). These findings are agreed with Batish *et al*. (2007) who observed that at low concentration (0.5%) of *Anisomeles indica* shown stimulatory effect over the plumule length of *Phalaris minor.*

Effect of aqueous extracts of *C.procera* on seedling growth and weed biomass of barnyard grass and Bermuda grass

Table no 3 and 4 are showing similar results in the case of seedling growth and weed biomass of barnyard grass and Bermuda grass when treated with aqueous extract of *C.procera*. 25% weed extract shown maximum inhibitory effect on both the weed species. Phytotoxicity in root length, shoot length and weed biomass (i.e. -86%, -84% and -85%) respectively was observed in barnyard grass. This may be due to that the phenolic compunds (ferulic acid, p-hydroxybenzoic acid, p- coumaric acid, and m- coumaric acid) possessed various allelopathic activities and most has inhibitory effects on seed germination and early seedlings growth of barnyard grass, irrespective of concentration. (Chung *et al*. 2002). Here root is showing more inhibitory effect when treated by weed extract, since it is a first receptor site for allelochemicals. Our findings are supported by Javaid and Anjum (2005) who mentioned the same thing in case of *Parthenium* seedlings. In case of Bermuda grass the percentage of Phytotoxicity in root length and shoot length and weed biomass at 25% was (-82%, -81% and -79%) respectively. These reductions in tested plants may be due to the reduced rate of cell division and cell elongation due to the presence of allelopathic in the aqueous extracts (Backolova, 1971). In contrast of 25% aqueous extract of *C.procera*, 5% extract shown stimulatory impact (+16%, +12% and +8%) on root length, shoot length and weed biomass respectively in case of Bermuda grass. Our findings are supported by Javaid *et al* (2006) who reported that at lower concentration, plant extract may be stimulatory to the test plant growth. Senesced leaves extract shown less phytotoxicity and stimulatory effect than mature leaves. 25% extract of senesced leaves shown (-80%, -76% and -74%) significant negative phytotoxicity effect on root length, shoot length and weed biomass respectively in barnyard grass. These findings were favored by (Kil and Yun, 1992; Noor and Khan, 1994) who stated that, aerial parts have more phytotoxic effect than the sub aerial parts. Least phytotoxicity was shown in the 5% of aqueous extract by (-20%), (-17%) and (-27%) on root length, shoot length and weed biomass of barnyard grass respectively. These findings are supported by Chung *et al*. (2001) who stated that inhibition of barnyard grass germination and seedling length and weight and of agronomic characteristics were related to allelopathic compounds released or produced by rice cultivars. In case of Bermuda grass, 5% extract treatment shown stimulatory effect by (+10% and +7% and +18%) on root length, shoot length and weed biomass respectively. Similar observation is reported by (Shaukat *et al*., 1983; Reigosa *et al*., 1999) who mentioned that Lower aqueous extracts of *Inula grantioides Boiss,* and *Capsicum annum* L., stimulated the seedling growth of test species. At 25% concentration Bermuda grass shown (-79%, -76% and -77%) phytotoxicity effect on root length, shoot length and weed biomass respectively. Similar findings were observed by (Travelos and Paspatis, 2008) who reported that Duckweed species are highly sensitive to chemicals that inhibit the function of photosystem II and their responses by chlorosis is readily measurable through the drastic decrease in their fresh weight.

Effect of aqueous extracts of *C.procera* on biochemical properties of barnyard grass and Bermuda grass

Table no 5 showed chlorophyll content in which lowest concentration (at 5%) of C.procera mature leave extract shown minimum negative effect (-34%) on barnyard grass. But in the case of Bermuda grass at the same 5% concentration, it shown stimulatory effect (+22%). As soon as the extract concentration increased (up to 25%), Phytotoxicity intensity was also increased. In the case of Barnyard grass maximum negative Phytotoxicity (-83%) was shown at 25%, followed by 20% (Phytotoxicity effect -76%). Such type of reduction may be due to the reduction in the amount of chlorophyll might be due to inhibition of synthesis of enzymes, protein and cofactors required for synthesis of chlorophyll or it may be due to excessive breakdown of chlorophyll under the influence of allelochemicals, (Kohli 1997). In Bermuda grass maximum and minimum Phytotoxicity effect (-81% and -45%) were shown in 25% and 10% extract concentration respectively. Similar findings were observed by Batish *et al*., (2004) who stated tha amount of chlorophyll content and the respiratory activity in treated seedlings of *C.occidentalis* and *E.crus-galli* were drastically reduced in response to eucalypt oil. Senesced leaves shown less negative and stimulatory impact than the mature

Table 5. Effect of mature and senesced leaves of *C.procera* on fresh weight (mg/g) and dry weight on 30 DAS

Treatment	Aqueous Mature leaves extract treatment on 30 DAS				Senesced leaves treatment after 15 DAS			
	Barnyard grass		Bermuda grass		Barnyard grass		Bermuda grass	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
C	3.71a	3.53a	3.92a	3.74a	3.71a	3.53a	3.92a	3.74a
5	2.87b	2.64b	4.19b	3.30b	2.98b	2.98b	4.28b	3.96b
	(-30)	(-33)	(+24)	(+8)	(-24)	(-27)	(+21)	(+18)
10	2.36c	2.27c	2.69c	2.58c	2.56c	2.5c	2.69c	2.54c
	(-49)	(-51)	(-41)	(-45)	(-40)	(-43)	(-41)	(-43)
15	1.95d	1.85d	2.20d	2.11d	2.11d	2.03d	2.26d	2.17d
	(-64)	(-67)	(-58)	(-61)	(-57)	(-61)	(-56)	(-59)
20	1.76e	1.53e	1.99e	1.94e	1.93e	1.82e	2.11e	2.03e
	(-71)	(-79)	(-65)	(-67)	(-64)	(-69)	(-61)	(-64)
25	1.47f	1.27f	1.67f	1.49f	1.66f	1.48f	1.70f	1.54f
	(-82)	(-85)	(-76)	(-79)	(-76)	(-80)	(-75)	(-77)

- Mean values followed by the same letters within each column are not significantly different according to LSD (P =0.05) n = 8. C, control; 5, 10, 15, 20 and 25 denote the aqueous concentration of *C.procera*.
- (-) and (+) symbols represent inhibitory and stimulatory percentage over control respectively.
- Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) (P < 0.05).

Table 6. Effect of mature and senesced leaves of *C.procera* on chlorophyll content (mg/g) of Barnyard grass and Bermuda grass after 30 DAS

Treatment	Aqueous Mature leaves extract treatment on 30 DAS		Aqueous Senesced leaves extract treatment on 30 DAS	
	Barnyard grass	Bermuda grass	Barnyard grass	Bermuda grass
	Total chl.	Total chl.	Total chl.	Total chl.
C	0.912a	0.966a	0.912a	0.966a
5	0.602b	1.186b	0.634b	1.156b
	(-34)	(+22)	(-30)	(+19)
10	0.407c	0.528c	0.432c	0.576c
	(-55)	(-45)	(-52)	(-40)
15	0.301d	0.413d	0.337d	0.458d
	(-67)	(-56)	(-63)	(-52)
20	0.212e	0.246e	0.251e	0.267e
	(-76)	(-74)	(-72)	(-72)
25	0.157f	0.178f	0.196f	0.204f
	(-83)	(-81)	(-79)	(-78)

- Mean values followed by the same letters within each column are not significantly different according to LSD (P =0.05) n = 8. C, control; 5, 10, 15, 20 and 25 denote the aqueous concentration of *C.procera*.
- (-) and (+) symbols represent inhibitory and stimulatory percentage over control respectively.
- Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) (P < 0.05).

Table 7. Effect of Mature and Senesced leaves of *C.procera* on sugar content (mg/g) of Barnyard grass and Bermuda grass after 30 DAS

Treatment	Aqueous Mature leaves extract treatment on 30 DAS		Aqueous Senesced leaves extract treatment on 30 DAS	
	Barnyard grass	Bermuda grass	Barnyard grass	Bermuda grass
	Total sugar	Total sugar	Total sugar	Total sugar
C	8.95a	9.36a	8.95a	9.38a
5	6.45b	11.51b	6.99b	11.23b
	(-27)	(+22)	(-21)	(+11)
10	4.89c	5.62c	6.18c	6.01c
	(-42)	(-39)	(-30)	(-35)
15	4.12d	4.59d	4.09d	4.97d
	(-53)	(-50)	(-50)	(-46)
20	2.15e	2.32e	2.24e	2.53e
	(-75)	(-74)	(-74)	(-72)
25	1.35f	1.60f	1.62f	1.88f
	(-84)	(-82)	(-81)	(-79)

- Mean values followed by the same letters within each column are not significantly different according to LSD (P =0.05) n = 8. C control; 5, 10, 15, 20 and 25 denote the aqueous concentration of *C.procera*.
- (-) and (+) symbols represent inhibitory and stimulatory percentage over control respectively.
- Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) (P < 0.05).

Table 8. Effect of mature leaves and Senesced leaves of *C.procera* on protein content (mg/g) of Barnyard grass and Bermuda grass after 30 DAS.

	Aqueous Mature leaves extract treatment on 30 DAS		Aqueous Senesced leaves extract treatment on 30 DAS	
Treatment	Barnyard grass	Bermuda grass	Barnyard grass	Bermuda grass
C	2.91(±0.34)a	3.06a	2.91a	2.83a
5	2.04b	3.82b	2.13b	3.67b
	(-11)	(+23)	(-26)	(+19)
10	1.78c	1.96c	1.90c	2.09c
	(-38)	(-35)	(-14)	(-31)
15	1.17d	1.35d	1.34d	1.53d
	(-59)	(-55)	(-53)	(-32)
20	0.67e	0.80e	0.76e	0.89e
	(-76)	(-73)	(-73)	(-70)
25	0.44f	0.53f	0.53f	0.61f
	(-84)	(-80)	(-81)	(-76)

- Mean values followed by the same letters within each column are not significantly different according to LSD (P =0.05) n = 8. C, control; 5, 10, 15, 20 and 25 denote the aqueous concentration of *C.procera*.
- (-) and (+) symbols represent inhibitory and stimulatory percentage over control respectively.
- Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) (P < 0.05).

leaves treatment of *C.procera*. At 5% negative impact (-30%) and stimulatory impact (+19%) was observed in barnyard grass and Bermuda grass respectively. At higher concentration (i.e. 25%) barnyard grass and Bermuda grass had significant inhibitory effect (-79% and -78%) respectively. Similar results were found by Riti (2011) who observed that dry leaf residue of *Hyptis suaveolens* can caused significant inhibition of total chlorophyll and sugar content of potted *Perthenium*.

Table no 6 showed sugar content of both the test weed varieties. At lower concentration (5%) of *C.procera* extract, barnyard grass shown least negative effect (-27%) and Bermuda grass shown maximum stimulatory effect (+22%) over the control. As soon as the concentration increased, Phytotoxicity influence also increased. At 25% mature leaves concentration, barnyard grass shown (-84%) negative impact and Bermuda grass shown (-82%) negative phytotoxic effect. Such type of sugar reduction may be due to the allelochemicals which may alter the leaf diffusibility, transpiration rate and stomatal aperture in test plants and these might also affect photosynthesis and directly carbohydrates amount of test plants, (Polova and Vicherkova (1986) and Vicherkova and Polova (1986). When both the test weed verities treated with senesced leave of *C.procera*, both the negative and stimulatory effects was observed but it was lesser than mature leave extract. At 5% senesced leave extract shown (-21%) inhibitory impact on barnyard grass and (+11%) stimulatory impact on Bermuda grass. Similar findings were observed by Sodaeizadeh *et al*. (2010) who mentioned that at lower dose of Peganum haumala L. showed stimulatory effect on chlorophyll content of *Avena fatua* L. At 25% senesced leave extract shown maximum inhibitory impacts were shown in both the test weed varieties. At 25% extract concentration inhibitory impacts were (-81% and -79%) in barnyard grass and Bermuda grass respectively. Table no 7 shown Phytotoxicity effect of *C.procera* mature and senesced leaves on protein content of barnyard grass and Bermuda grass. Similar results were found in both the test weed varieties. At 5% mature leave aqueous extract concentration, barnyard grass shown minimum inhibitory effect (-11%) and Bermuda grass shown maximum stimulatory effect (+23%) over the control treatment respectively. At higher concentration (25%) both the test weeds (Bermuda grass and Barnyard grass) showed maximum inhibitory effect (-84% and -80%) over the control treatment. It may be due to allelochemicals which are disturb the hormonal balance, protein synthesis, respiration, photosynthesis, chlorophyll formation, permeability and plant water relation (Yamne *et al* 1992). In the case of senesced leaves treatment at 5% concentration barnyard grass shown negative effect (-26%) and stimulatory effect (+19%) than the mature leaves treatment. At 25% senesced leaves treatment both the weed verities (i.e. barnyard grass and Bermuda grass) shown inhibitory effect (-81% and -76%) respectively over the control treatment.

Conclusion

From the present study, it could therefore be concluded that aqueous extract of *Calotropis procera* possess strong weed – suppressing ability. The data revealed that Bermuda grass was more recessive than Barnyard grass. At 5% concentration Bermuda grass showed stimulatory effect over the control treatment. At 5% mature leaves concentration, inhibitory and stimulatory effect was more pronounced than senesced leaves treatment. Mature leaves of *C.procera* shown maximum phytotoxic effect than the senesced leaves. Hence, *Calotropis procera* could be one of plant for developing bioherbicides to controlling such type of noxious weeds.

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