PHYTOTOXIC EFFECTS OF *Calotropis procera* (Ait.) R. Br. EXTRACT ON THREE WEED PLANTS

Aasifa GULZAR*, Mohammad Badruzamman SIDDIQUI*, Usman ARERATH*

* *Department of botany, Aligarh Muslim University, Aligarh, 202002, India,*
Corresponding author: Aasifa Gulzar, Research Scholar, Department of Botany, A.M.U., Aligarh, 202002, India, phone: 09760931189, e-mail: aasifa4gulzar@gmail.com, aasifa.gulzar@rediffmail.com

Abstract. A study was conducted to determine the potential and nature of allelopathic interference of *Calotropis procera* on seed germination and seedling growth of three weed species (*Ageratum conyzoides* L., *Cannabis sativa* L. and *Trifolium repens* L). Aqueous extracts of *Calotropis procera* at 0.5, 1.0, 2.0 and 4.0% concentrations were applied to determine their effect on seed germination and seedling growth of test plants under laboratory conditions. The aqueous extracts had retardary effect on seed germination, root length and shoot length. Germination percentage, root length and shoot length of weed species decreased progressively when treated with increasing extract concentration (0.5, 1, 2 and 4%). The pH values did not increase at all extract concentrations. Therefore, the change in pH values in this experiment is not responsible for the inhibition of test species growth.The phenolic content analysed show more pronounced increase in its contents at 4% concentrations. The study concludes that *C. procera* releases phenolics into the extract and these are probably involved in the growth inhibitory effect, which causes allelopathy operative in the community dominated by *C. procera* and provide an advantage to the weed.

Keywords: Allelopathy, aqueous extract; *Calotropis procera*, Phenolics; pH.

INTRODUCTION

Association and disassociation patterns between c ertain plants may be governed by direct competition for necessary growth factors or through addition of allelopathic chemicals into the soil environment [21]. It has been documented that allelopathy may play an important role in plant-plant interference by allelochemicals [21]. These chemicals are released to the environment, from leaching, litter decomposition, root exudation, or direct volatilization, they could affect (either positively or negatively) germination and growth of other species. The allelopathic effects studied included germination inhibition [28], plumule and radicle length [27, 28], seedling growth retardation [4, 14] and poor seedling survival [7, 17]. Allelochemicals produced by plants may be released into the surrounding environment in sufficient amounts with enough persistence to effect neighbouring and succession species [19]. Due to the action of allelochemicals, a large number of physiological functions and biochemical reactions are affected, such as seed germination, cell division, cell elongation, membrane permeability and ion uptake [2, 24]. The effect of various allelochemicals of different plants on physiological and biochemical processes was reported in recent decades by [9]. The subject of allelopathy currently receives much attention from scientists, the increasing interest in allelopathy in recent years has been stimulated by the recognition that agro-ecological applications of allelopathy may provide alternatives to synthetic herbicides for weed management [26] and with the evidence allelopathy has the potential for weed control [20].

Calotropis procera represents a highly invasive species that has reached the status of weed in many regions (Fig. 1). The high potential of *C. procera* to invade pristine or economic important areas is of much concern, as it is also very difficult to eradicate [15]. It is a member of family Asclepiadaceae [18] 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 [23]. 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 [11]. Phytotoxic impact of these anxious compounds on other plants is usually dominant at early growth stages, causing inhibition of seed germination and seedling growth [10]. Its widespread and persistent occurrence near barley, oat, rice, sorghum, maize, cotton, sugarcane fields and especially around wheat crop fields makes it suspicious

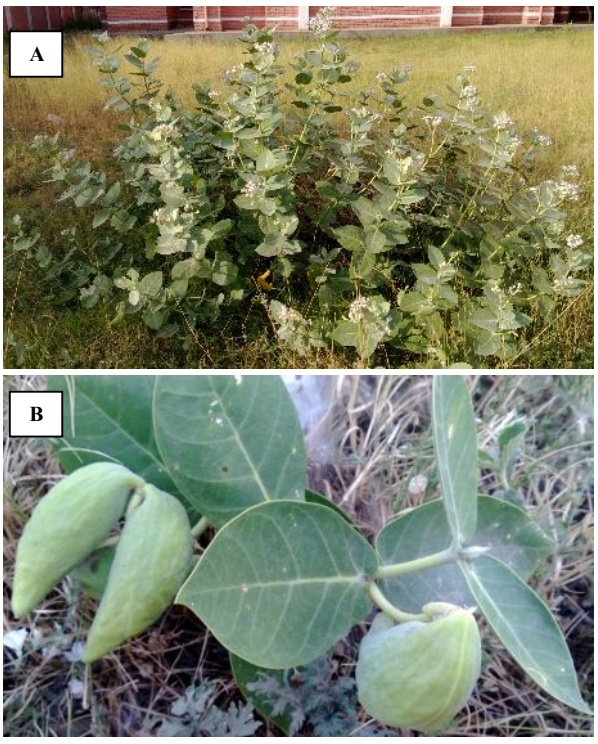

Fig. 1. *Calotropis procera* (**A**) and *C. procera* with attached fruit (**B**)

to cause some adverse effect on these crops through allelopathic interaction [29].

The objective of this study is to evaluate the effect of *C. procera* leaves extract on the seed germination and seedling growth of some weed plants (*Ageratum conyzoides*, *Cannabis sativa* and *Trifolium repens*) and the effect of pH and Phenolic variation in different parts of *Calotropis procera*.

MATERIAL AND METHODS

C. procera was collected from a selected area where no other vegetation was present from the campus of the Aligarh Muslim University, Aligarh, Uttar-pradesh. The plants were uprooted at maturity. A number of fresh leaves from the aerial shoots were separated from the plants, chopped into small pieces with scissors, air-dried and ground into fine powder with morter and pestle. Stock aqueous extract was obtained by soaking 4 g air-dried plant material in 100 mL of cold distilled water (4% w/v) at room temperature (20^0C) for 24 h with occasional shaking. The mixture was filtered through two layers of cheesecloth and centrifuged for 20 m at 10.00 rpm in to remove particulate material and the purified extract was adjusted to pH 6.8 with 1M HCl. Different concentrations (0.5%, 1% and 2%) were prepared from the stock solution in addition to the control (distilled water). The aqueous extracts were individually bottled and tagged. Ten seeds of test plants (*Ageratium conyzoides, Trifolium repens, Cannabis sativa*) were arranged in 9 cm diameter Petri-dishes lined with two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from 26-32^0C and night temperature from 19-23^0C. The seeds were surface sterilized with 2% sodium hypochlorite for 2 min before sowing, then rinsed four times with distilled water. The sterilized seeds were soaked in aerated distilled water for 24 h. The germination percentage (GP), shoot length (SL) and root length (RL) were recorded after twenty days at the end of the experiment. The study was carried out in the Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, Uttar-pradesh, during 2010. A completely randomized design (CRD) with four replications was used to conduct four experiments. Germinated seeds were counted daily according to the seedling evaluation procedure in the Handbook of Association of Official Seed Analysts [2].The seeds were considered as germinated when the radical size was 2 mm. Ten days after sowing, germination percentage was calculated using the formula Germination percentage= (Germinated seed/Total seed × 100) for each replication of the treatment. The length of root and shoot were measured by using a meter scale. The amount of total phenolics in leachates, extracts and soil was determined using Folincio-calteu reagent as per the method of Swain and Hillis [25]. The pH of each extract prepared from different parts of *C. procera* was determined by immersing the electrode of a digital PH meter (EcoScan). The mean of four replicates were taken and presented.

Statistical analysis

The data were subjected to ANOVA followed by Duncan's Multiple Range Test (DMRT) [8] and 2 sample t-test, wherever applicable. The statistical analysis was done using SPSS/PC version 10 software.

RESULTS

Calotropis procera leaf extract were found to be phytotoxic and decreased the germination traits and growth of test species compared with control (Fig. 1). Germination percentage was significantly reduced with *C. procera* extract when compared with distilled water (Fig. 1). Significantly lowest germination percentage was recorded at 4% concentration (40%), followed by 84.3% at 1% and 66.6% at 2% concentration in *Ageratium conyzoides*. Generally, GP of *C. sativa* seeds is supported statistically ($p \leq 0.01$). However, the reduction goes to a markedly lower level at 2 and 4% concentrations (50 and 37%, respectively). The percentage was reduced at 2 and 4% extract concentration levels in *T. repens*. This may be due to presence of phytotoxins leachates present in the water extract of *C. procera*. Data related to root and shoot length presented in Fig. 2 indicates that *Caltropis procera* extract significantly reduced both the root length and shoot length. Both root and shoot lengths, were severely affected and the effect was more on root length than on shoot length respectively when compared with distilled water. In aqueous extract, shoot length reduced by nearly 41%, 21% and 32% respectively at 4% concentration in *Trifolium repens*, *Cannabis sativa* and *Ageratum conyzoides*. Maximum root length (7.1cm) was attained by seedlings supplied with distilled water, which shows a significant decrease, by treatment with aqueous extract of different concentrations in three test species. Similarly, the root length was reduced at 4% concentration in *Trifolium repens*, *Cannabis sativa* and *Ageratium conzyzoides* (Fig. 2). In the present study, pH of extracts (that is root, stem and leaf) ranged from 6.00 to 6.79 (Table 1). A significant higher amount of water-soluble phenolics (µg/ml) was found in leaf at 4% (928.06), followed by root (600.27), and stem extract (98.57) (Table 1).

DISCUSSIONS

Allelopathic effect of 0.5, 1, 2 and 4% aqueous extract besides the control from leaves of *C. procera* (donor species) was clearly demonstrated on germination percentage, shoot and root length of three weeds (*A. conyzoides, C. sativa* and *T. repens*). This inhibition was more marked in *T. repens* than *A. conyzoides* indicating that *T. repens* is more sensitive to the tested donors, while the *A. conyzoides* is more adapted. After making these observations, it could be concluded that extract might posses some growth inhibitors. Some recent studies indicating the phytotoxic/allelopathic effect of aqueous extracts of

weeds include *Brassica nigra* [26], *Raphanus raphanistrum* [16], *Andrographis panicula* [1], *Baccharis dracunculifolia* [13], *Chenopodium murale* [3], *Terminalia arjuna* [13]. The phenolic allelochemicals of *Calotropis procera* thus cause a major biological implication for the growth of target plants as observed in the present study. Phenolics are the most common water-soluble allelochemicals known to play a significant role in plant-plant interactions including allelopathy [5]. These being water-soluble easily leach out from green foliage as well as decaying plant debris through rainfall and accumulate in soil, where they bring about substantial changes in soil dynamics and growth retardatory effects on the other plants [6]. Germination percentage (GP) of the three investigated recipient species demonstrated a gradual decrease related to the application of higher concentrations of the donor species as follows: *T. repens>C. sativa>A. conyzoides.* Other workers have indicated that effect of a given compound or plant metabolite may be inhibitory or stimulatory depending on their concentration in the surrounding medium. The study concluded that *C. procera* leaf extract exerted allelopathic effects by releasing water-soluble phenolic acids as putative allelochemicals and pH change at different concentrations.

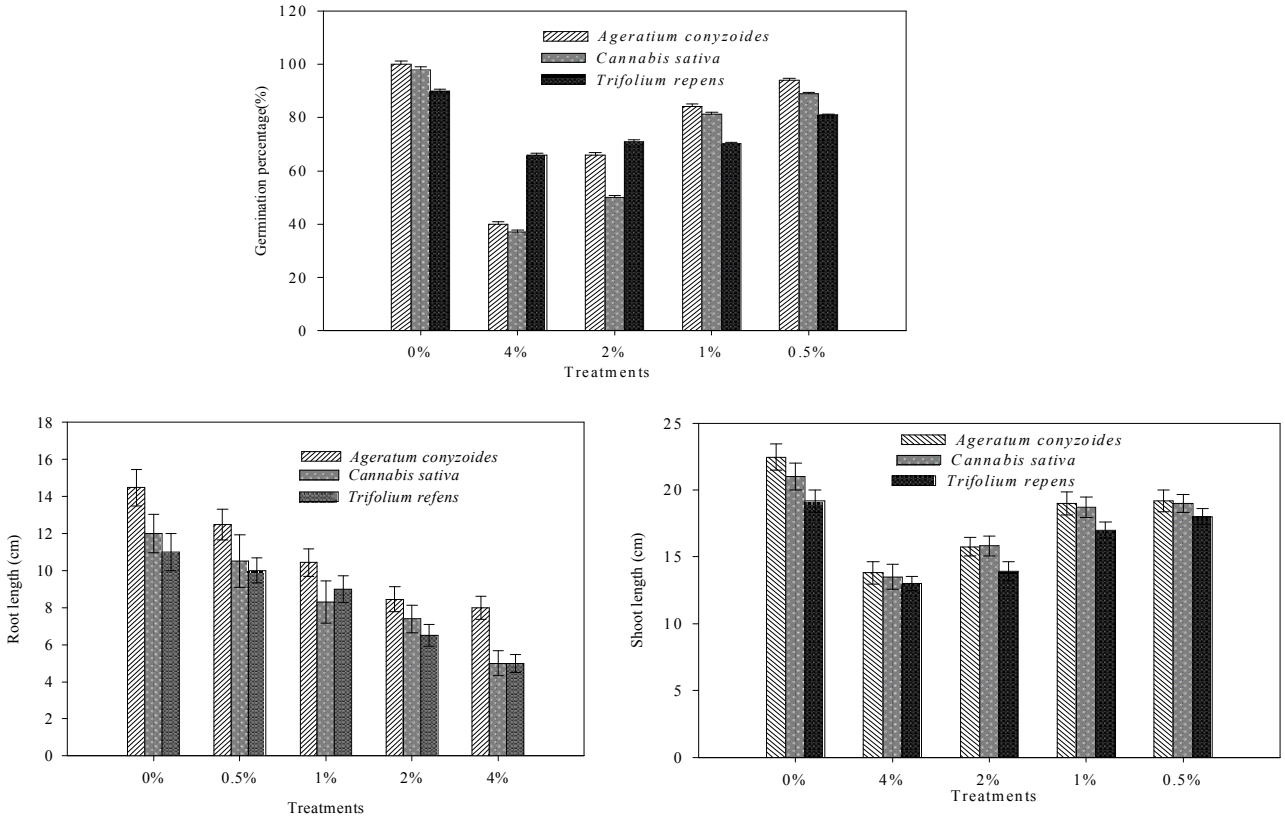

Figure 1. Allelopathic effect of *C. procera* infested soil on Germination percentage, root length and shoot length of test species. Data are the means of four replicates with standard error

Table 1. Values of pH and content of phenolic in different concentration of extracts of leaves, stem and roots of *C. procera*

Parameters	Extract Concentration	Root	Stem	Leaves
pH	0.5	6.62^{a}	6.72^{a}	6.79^{a}
	1.0	6.51^{b}	6.58^{a}	6.68^{b}
	2.0	6.30^{c}	6.49^{b}	6.58^{c}
	4.0	6.00^{d}	6.38^{a}	6.52^{d}
Total phenolic content (µg/ml)	0.5	137.2^{d}	69.13^{d}	501.13^{d}
	1.0	279.56^{c}	72.73^{c}	572.30^{c}
	2.0	389.11^{b}	87.23^{b}	599.46^{b}
	4.0	600.27^{a}	98.57^{a}	928.06^{a}

Different alphabets within a column represent significant difference at $P<0.05$

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