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FULL LENGTH ARTICLE

Allelopathic effect of *Calotropis procera (Ait.) R. Br.* on growth and antioxidant activity of Brassica *oleracea* var. botrytis

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Peroxidase (POD) and catalase (CAT) activity

Abstract The present study intended to investigate the effect of aqueous extract from *Calotropis procera* on the growth of *Brassica oleracea var botrytis*. Seeds of brassica were soaked in solutions containing 20%, 40%, 60% and 80% concentrations of leaf, fruit and flower extract of *C. procera*. For control, distilled water was used. The effects of extracts on germination percentage, seedling growth, dry biomass, and relative water content were investigated. Higher concentrations of extract (60% and 80%) significantly reduced germination percentage, radicle length, plumule length, dry matter accumulation, and relative water content of the brassica seedlings as compared to control. The retardatory effect increases with the increase in the concentration of three types of extract used, with more pronounced effect noticed by leaf extract followed by fruit and flower extract. There were significant interactions among the different concentrations of extracts used, etype of extract with respect to gemination percentage, seedling length, dry biomass, and relative water content. The effect of pot based assay in relation to chlorophyll content was significantly reduced and antioxidant enzymes [superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities] show both significant and non-significant effect on antioxidant enzymes based on concentrations of extract and extract type used. The antioxidant enzymes show the significant decrease in its activity at low concentrations (20% and 40%) and non-significant increase at higher concentration (60% and 80%) of extracts in contrast to control. Based on the investigation, it could be speculated that the delayed germination and low germination rate of the test species after treatment by extracts could be due to the fact that extracts damaged the membrane system of the seeds and *C. procera* might release phenolics into the soil and these are probably involved in the growth inhibitory effect of test species.

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1. Introduction

Allelopathy is fascinating and perplexing subject that concern with the interaction of plants as influenced by the chemical substances that they release into the environment (Bais et al., 2003; Machado, 2007; Willis, 2004). Allelochemicals from plants are released into the environment by exudation from roots, leaching from stems and leaves or decomposition of plant material (Rice, 1984; Inderjit et al., 2006). The multiple effects resulting from allelochemicals include effect on cell division, production of plant hormones, membrane permeability, germination of pollen grains, mineral uptake, movement of stomata, pigment synthesis, photosynthesis, respiration, protein synthesis, nitrogen fixation, and specific enzyme activities (El-Khatib et al., 2004; Rafael et al., 2005; Jamali et al., 2006; Hegazy et al., 2007; Farrag, 2007; Zeng et al., 2008; Inderjit et al., 2008; Pisula and Meiners, 2010; Kim and Lee, 2011; Djurdjevic et al., 2012; Mansour, 2013). 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., 2010; Gulzar and Siddiqui, 2014). 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; Norton et al., 2008; Jabran et al., 2008; Razzaq et al., 2012; Macias et al., 2007).

To defend with stress conditions, Plants are equipped with several ROS scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (POX) which are activated to buttress plant strength against abiotic and biotic stresses. Reactive oxygen species are produced in huge amounts in plants upon exposure to stressful conditions such as sub-optimal temperature, high light, salt, and pathogen infection (Yamamoto et al., 2003; Halliwell, 2006). Enhanced activity of ROS-scavenging enzymes along with increased degree of membrane lipid peroxidation by allelochemical stress has been studied by several authors (Baziramakenga et al., 1995; Yu et al., 2003; Lara-Nunez et al., 2006; Ye et al., 2004, 2006).

Calotropis procera, known as apple of Sodom or mudar, is a member of family Asclepiadaceae (Parihar et al., 2011). The milky sap of this plant is known to contain three toxic glycosides: (i) calotropin, (ii) uscharin, and (iii) calotoxin as well as steroidal heart poisons, known as cardiac aglycones (Zeng et al., 2008). The plant also received much attention from researchers due to its allelopathic behavior and has extensively been used for the control of many plants. A number of secondary metabolites have been isolated from this plant that include many flavonoids (Heneidak et al., 2006; Srivastava et al., 2012), cardiac glycosides (Hanna et al., 2002), triterpenes (Bhutani et al., 1992) and sterols (Chundattu et al., in press) that might contribute its allelopathic potential. However, previous studies investigated regarding its phytotoxic and allelopathic effects of this plant in various crops have been carried out by (Kayode, 2004; Samreen et al., 2009; Yasin et al., 2012; Gulzar et al., 2014a; 2015). 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 (Yasin et al., 2012). 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, seedling growth, dry biomass, total chlorophyll content and antioxidant enzymes (SOD, POD, CAT activity) of *B. oleracea*.

2. Materials and methods

2.1. Preparation of aqueous extract

Insect-free, disease-free plants of *C. procera* were collected from the campus of Aligarh Muslim University, Aligarh (27°, 29–28°, 100 N.L and 77°, 29–78°, 38° E.L) where it was growing abundantly. They were washed thoroughly with distilled water and air-dried at room temperature for 96 h. The leaf, fruit and flower portions were separated, chopped into 1-cm long pieces, and were kept in the oven at 28°C for 72 h. The dried sample was then crushed in a mortar and pestle to make powder. Powdered materials of each part (8g) were added into 100 ml distilled water (1:8 w/v) and kept in shaker for 1hour. After shaking for an hour, extracts were placed at room temperature for 48 hours following the method of Wardle et al. (1992). The extracts were then filtered with muslin cloth followed by Whatman filter paper No. 1. This served as the stock solution from which other concentrations (20%, 40%, and 60%) were prepared by way of dilution.

2.2. Determination of germination percentage, root length, shoot length, dry biomass and relative water content (RWC)

The seed of *B. oleracea* was procured from IARI, New Delhi. These were surface sterilized with 95% ethanol and 10% chlorax for 5 min and thoroughly washed with sterile water several times. Next, five sets of autoclaved petri dishes were prepared, each containing a single layer of Whatman No. 1 filter paper and 5 ml of test extract for each concentration (20%, 40%, 60%, and 80%) of leaf, fruit, and flower. The petri dishes treated with distilled water were taken as a control. In each prepared petri dish, ten surface sterilised brassica seeds were placed. A total of four replications of the sets with the previously described concentrations were kept undisturbed at room temperature (24 ± 2°C) in the laboratory for seven days. The number of germinating seeds was recorded on the sixth day, whereas root length, shoot lengths, dry biomass and RWC of the brassica seedlings were determined after fifteen days. The emergence of a radical approximately 1 mm in diameter was taken as the index of germination. The dry biomass was determined after oven drying at 80 °C for 24 hours. Using the equation of Deef and Abd El-Fattah (2008), the relative water content (RWC) was evaluated as: $RWC\% = (FW - DW)/FW \times 100$.

2.3. Determination of chlorophyll and antioxidant enzymes

To observe the direct effect of allelochemicals on crop in the field, 20-cm pots were filled with 300g of soil collected from brassica-growing fields. Ten surface-sterilised brassica seeds

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