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Effects of allelochemical extracts from medicinal plants on physiological and biochemical mechanisms of maize (*Zea mays* L.) seedlings

Rabia Naz[1, 2*], Asghari Bano[2]

[1]*Dept. of Biosciences, COMSATS Institute of Information Technology, Islamabad, Pakistan*

[2]*Department of Plant Sciences, Quaid-i-Azam University, Islamabad Pakistan*

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Abstract

Aqueous extract of *Ricinus communis* and *Lantana camara* contains substances that have allelopathic potential on maize (*Zea mays L.)*. The present study showed that different concentrations of the water soluble leaf extracts of *R. communis* and *L. camara* inhibit the germination and growth of maize. These extracts also affect activity of some enzymes. Leaf aqueous extracts of both the plants were made to determine their effects on germination, seedling growth, fresh and dry weight of root and shoot and their effects on activity of superoxide dismutase (SOD), peroxidase (POD), Catalase (CAT), polyphenoloxidase (PPO) of root and shoot and lipidperoxidatio (MDA) of root of 8 d old maize seedlings. Results revealed that higher concentration of *Lantana camara* leaf extract exhibited significant inhibitory effect on germination and seedling growth. The biochemical assay indicate that the *Ricinus communis* and *Lantana camara* leaf extracts at higher concentration of 1.2% have increased the SOD, POD and CAT activity of root as compared to control. The enzyme activity of shoot was not significantly increased by the *Ricinus communis* and *Lantana camara* leaf extract except the catalase activity. The allelopathic effect of these extracts was more pronounced on root than the shoot parts. The higher concentration of extracts had stronger inhibitory effects, whereas the lower concentrations showed stimulatory effect in some cases.

* **Corresponding Author:** Rabia Naz ✉ rabia.naz@comsats.edu.pk

Introduction

Allelopathic effects are mediated through release of allelochemicals. Allelochemicals are usually called secondary plant products of the main metabolic pathway in plants (Haddadchi and Gerivani, 2009). Several phytotoxic substances causing germination and growth inhibitions or stimulations have been isolated from plant tissues and soils (Turk and Tawaha, 2003). These substances, collectively known as allelochemicals, are usually secondary plant products or waste products of main metabolic pathways and most of them originate from the shikimic acid and acetate pathway (Rice, 1984; Turk and Tawaha, 2003). Allelochemicals are present in almost all plants and in many tissues, like leaves, stems, flowers, fruits, seeds and roots (Putnam, 1988). They are often water-soluble substances that are released into the environment through root exudation, leaching and decomposition of plant residues (Aminidehaghi *et al.,* 2006). Allelochemicals that inhibit the growth of some species at certain concentrations might in fact stimulate the growth of the same or different species at different concentrations (Narwal, 1996).

It has been documented that allelopathy play an important role in plant-plant interference by those chemical compounds (Turk and Tawaha., 2003; Turk and Tawaha., 2005; Ashrafi *et al*., 2007). If some of those compounds 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 of some plants were studied including germination inhibition (Sadeghi *et al.,* 2010), plumule and radicle length (Oudhia *et al*., 1998), seedling growth retardation (Oudhia, 2000a; 2000b) and poor seedling survival (Vankar and Srivastava, 2008).

Multiple physiological effects have also commonly been observed from treatments with many allelochemicals. These effects include decreases in plant growth, absorption of water and mineral nutrients, ion uptake, leaf water potential, shoot turger pressure, and osmotic potential caused by phenolic compounds (Barkosky and Einhellig, 2003). In agriculture, the inhibitory effect of weed species on germination and growth of crops has been attributed to phytotoxic chemicals released from the leaf litter and roots. *Lantana camara*, one of the world's 10 worst weeds was introduced in the Indian subcontinent during the early part of the nineteenth century (Bansal, 1998). The weed is aggressively growing in forest, agriculture, tea garden and wastelands of all over the country (Ahmed, 1997). This obnoxious weed poses a serious problem to flora and fauna because of its toxic substance and it contains certain allelopathic compounds (Jain *et al.,* 1989). Although several researches have so far worked on the invasion and allelopathic effects of *Lantana* on various agricultural crops throughout the world (Bansal, 1998) however such scientific activities are scarce in the context of Pakistan.

Ricinus communis is a plant commonly found in both the tropical and temperate climates of the world (Lakshmamma, and Prayaga, 2006; Raoof, and Yasmeen, 2006). The study of allelopathic effect of Ricinus leaf extract on agricultural crops is scarce throughout the world.

Such information should be beneficial when planning for sowing maize in close vicinity of Lantana weed or Ricinus trees. Hence this study was conducted to investigate the effect of different concentrations of aqueous leaves extract of *L. camara* and *R. communis* on the seed germination and physiological responses of the crop plant maize (*Zea mays* L.).

Materials and methods

Plant sampling and preparation of phenolic extracts

Fresh leaves of *Lantana camara* were collected from Quaid-i-Azam University campus *and Ricinuc communis* were collected from arid region of Punjab, Pakistan.

Plant leaves were washed properly with distilled water. The leaves were shade dried at room temperature. The dried leaves of *L. camara* and *R.*

communis were uniformly grinded using mechanical grinder and stored in air tight containers for further use.

Aqueous extracts of *L. camara* and *R. communis* were prepared as 1.2 g of leaves were soaked in 100 mL distilled water and kept at room temperature of 28–30°C. After 24 h the aqueous extracts were filtered through four layered cheese cloth. Other concentrations of aqueous extract (1.0% and 0.8%) were also prepared and stored for seed treatment experiment.

Treatments

Seven treatments C, T1, T2, T3, T4, T5, and T6 were used during experiment. C: Seeds of receptor plant soaked in distilled water (control) whereas T1, T2 and T3 were seeds of receptor plant soaked in *R. communis* extracts at concentration of 0.8%, 1.0%, 1.2% and T4, T5 and T6 seeds of receptor plant soaked in *L. camara* extracts at concentration of 0.8%, 1.0%, 1.2% concentrations, respectively.

Germination and Growth records

The germination test was carried out in the sterile Petri dishes (15 cm) lined with filter paper Whatman No. 3. Seeds of maize were sterilized with NaOCl 10% for 2–3 min, thereafter; the seeds were thoroughly rinsed three times with sterile water. Aqueous extract of different concentrations (10 mL) were pipetted to the filter paper placed in petri dishes and distilled water was used as control treatment. The Petri dishes were set in the laboratory at room temperature ranging from 28–30 °C. The experiment was extends over a period of 7 d to allow the last seed germination. A seed was considered as germinated, when radical emerged. The germination was recorded on daily basis. The results were determined by counting the number of germinated seeds and measuring the lengths of both root and shoot. Germination rate was calculated by using R=G n/G (Dn) formula. Where, n is the number of germinated seeds, D is the number of spent days from beginning and R is germination rate mean. The vigour index (VI) of the seed was estimated as follow: (Abdul-Baki and Anderson, 1970)

Vigour index = [germination percentage × mean (radicle length + plumule lenght] / 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 and assay of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), polyphenoloxidase (PPO) activity and Lipid peroxidation (LP).

Enzyme extraction

Freezing shoot and root (0.2 g) were extracted with 4 mL of 50 mM phosphate buffer (pH 7.0) (NG *et al.*, 2003). The extract was centrifuged at 15000 *g* for 20 min at 4 °C and supernatant was used to determine the activity of SOD, POD, CAT and PPO.

SOD activity

One unit of SOD activity was defined as the amount of SOD which produced one half of the maximum competition against NBT in the specified system; absorbance was measured at 560 nm (Beauchamp and Fridovich, 1971).

POD activity

The POD activity was measured by the method of Vetter *et al.* (1958) and further modified by Gorin and Heidema (1976). The assay mixture contained 0.1 mL enzyme extract, 1.35 mL 100 mM MES buffer (pH 5.5), 0.05% H_2O_2 and 0.1% phenylenediamine. Changes in absorbance were recorded at 485 nm for 3 min with the spectrophotometer. The activity of POD was presented as change in OD485 nmol /min /mg protein.

CAT activity

Catalase activity was determined with some modifications (Goel *et al.*, (2003). The 3 mL reaction mixture contained 50 mM phosphate buffer (pH 7), 15 mM H_2O_2, 0.1 mL enzyme extract. The decrease in H_2O_2 was followed as the decline in absorbance at 240 nm, and activity was calculated using the extinction coefficient for H_2O_2

PPO activity

The activity of polyphenoloxidase was determined with some modification (Kar and Mishra, 1976). The 3 mL reaction mixture contained 25 mM phosphate buffer (pH 6.8), 0.1 mM pyrogallol, and 0.1 mL enzyme extract and blank without pyrogallol. The absorbance of the purpurogallin formed was recorded at 420 nm, and activity was calculated using the extinction coefficient for purpurogallin.

Lipid peroxidation (LP)

The level of lipid peroxidation was measured in terms of TBARS content (Prochazkova *et al.*, 2001). Radicle sample (0.1 g) was homogenized in 2 mL 0.1 % trichloroacetic acid (TCA). The homogenate was centrifuged at 15000×g for 15 min. To 1 mL aliquot of the supernatant, 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95 °C for 30 min and than cooled in an ice bath. After centrifugation at 10000×g for 10 min the absorbance of the supernatant were recorded at 440, 532 and 600 nm. Malondialdehyde equivalents were calculated in the following manner (nmol·ml-1) (Du and Bramlage, 1992):

[[(A532 - A600) - [(A440 - A600) (MA of sucrose at 532nm/MA of sucrose at 440nm)]]/157000]×106

The MA (molar absorbance) of 1–10 mM sucrose at 532 nm and 440 nm was calculated to be 8.4 and 147, respectively, giving a ratio of 0.0571.

Statistical analysis

Germination and seedling growth bioassay were conducted in a complete randomized design (CRD) with three replications. Means were compared by LSD tests at P≤0.05 using Statistix 8.1.

Results and discussion

Germination (%) assay

The percentage germination was non-significantly affected by all the treatments with extract from *R. communis* and *L. camara* except the highest concentration i.e. treatment with 1.2% extract which resulted in significant decrease (50% and 55.3%) respectively as compared to control. The allelopathic effects of *L. camara* on germination and growth behavior of some agricultural crops have been reported (Ahmed *et al.,* 2007). The allelopathic effect of aqueous extract of *Bambusa arundinacea* was observed on *Arachis hypogaea* (Eyini *et al.*, 1989). Germination rate was non-significantly reduced by all the treatments with extract from *R. communis* and *L. camara* (Figure 1).

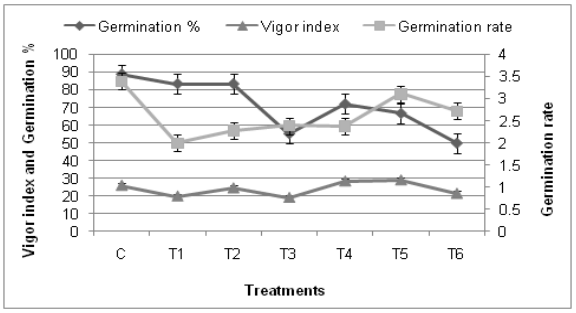

Fig. 1. Effect of different concentrations of aqueous extracts from *R. communis* and *L. camara* on Germination %, Germination rate and Vigor index of maize C: Maize seeds soaked in distilled water (control) whereas T1, T2 and T3 were seeds soaked in *R. communis* extracts at concentration of 0.8%, 1.0%, 1.2% and T4, T5 and T6 seeds soaked in *L. camara* extracts at concentration of 0.8%, 1.0%, 1.2% concentrations, respectively.

Seedling growth

The *L. camara* extracts caused reduction in root length, while exhibited stimulatory effect on shoot. The *R. communis* extracts exhibited stimulation in both root and shoot length at all concentrations. Maximum elongation (13%) of root was observed in T1 (*R. communis* 0.8%). The root length of maize seedlings was found to be significantly inhibited with the increase of the *L. camara* extract concentration (Figure 2). Some researchers found that aqueous extracts of black mustard (*Brassica nigra*) caused the reduction in germination, hypocotyl and radicle length of *Avena fatua* (Turk and Tawaha, 2003). The leaf extracts having more pronounced inhibitory effects on radicle growth than on hypocotyl growth. Scientists stated that aqueous extracts of *Eucalyptus* leaves significantly reduced seed germination, root and shoot length, fresh and dry weight of maize compared to control treatment (Khan *et al,*. 2004).

The lowest concentration of *R. communis* and *L. camara* extracts at 0.8% exhibited significant stimulation in root fresh weight, while *L. camara* at

highest concentration of 1.2% exhibited significant inhibition in root fresh weight as compared to control.

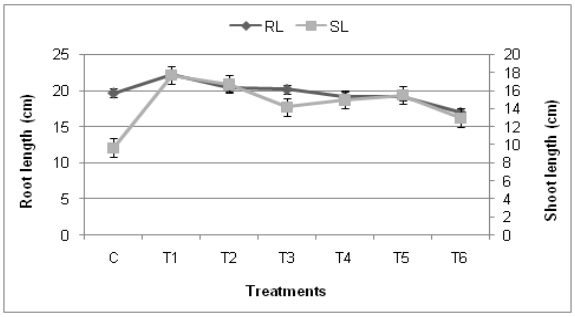

Fig. 2. Effect of different concentrations of aqueous extracts from *R. communis* and *L. camara* on Root length (RL), Shoot length (SL) of maize The labeling of treatments is same as described in Figure 1.

All the concentration of the extracts from *R. communis* and *L. camara* exhibited significant increase in shoot fresh weight. The maximum increase (93.4%) was observed in *R. communis* extract at concentration of 0.8% (Figure 3).

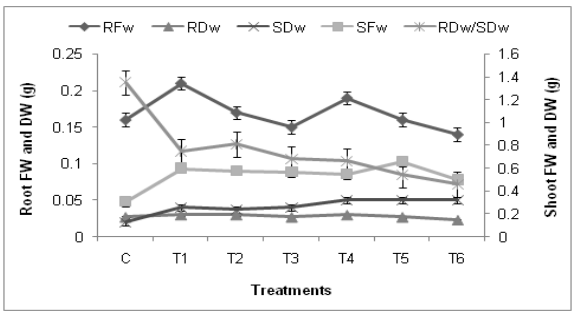

Fig. 3. Effect of different concentrations of aqueous extracts from *R. communis* and *L. camara* on Root Fresh weight (RFw), Shoot fresh weight (SFw), Root Dry weight (RDw), Shoot Root Dry (SDw) of maize The labeling of treatments is same as described in Figure 1.

Maximum reduction in root dry weight was observed in T6 treatment (*Lantana* 1.2%). Some researchers reported the concentration dependent decrease of *Lantana* extract (Daniel, 1999). There was no significant effect of *L. camara* and *R. communis* leaf extracts on root dry weight but significant increase on shoot dry weigh was recorded (Figure 3).

Biochemical assay

Many studies employing mutants and antisense lines for catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase have revealed a strong link between ROS and process such as growth, development and biotic and abiotic stress responses (Alexandra *et al.*, 2010). To control of ROS and to protect cells under stress conditions, plants contain several enzymes scavenging ROS (SOD, CAT and POD). Enhanced formation of ROS under stress conditions induces both protective responses and cellular damage.

The *R. communis* leaf extract at 0.8% concentration decreased the SOD activity of root as compared to control (Figure 4). Increase in the concentration of the extract linearly increased the SOD activity with that of control. Similarly *L. camara* extract was inhibitory at low concentration (0.8%) whereas higher concentrations were stimulatory, the magnitude of stimulation being higher with the increase in concentration.

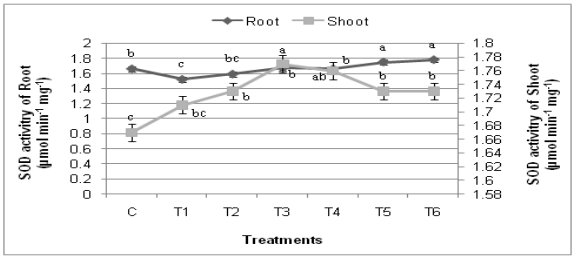

Fig. 4. Effect of different concentrations of aqueous extracts from *R. communis* and *L. camara* on SOD activity (units min^{-1} mg^{-1} protein) of root and shoot of maize The labeling of treatments is same as described in Figure 1.

The SOD activity of maize shoot showed linear increase with the increase in concentration of both *R. communis* and *L. camara* extract as compared to the controls (Figure 4). Highest (5.4%) SOD activity was observed in T3 treatment with *R. communis* extract at 0.8%.

The POD activity of root (Figure 4) was non-significantly decreased following the treatments T1–T3 with the leaf extracts of *R. communis*, while the *L. camara* extracts showed linear increase in POD activity. The maximum increase (74%) in POD activity was observed in the treatment T6 as

compared to control. All the concentration of *R. communis* and *L. camara* leaf extracts non-significantly decreased the POD activity of shoot (Figure 5). These results are in agreement with Ullah *et al.*, (2013) who reported that the allelochemical extracts of *Phytolacca latbenia* significantly reduced the POD activity in *Brassica napus* and *Triticum aestivum*. The peroxidases are associated with biochemical and physiological processes such as growth, cell formation, fruit development, ethylene biosynthesis, as well as the response to various stresses (Matamoros *et al.*, 2003).

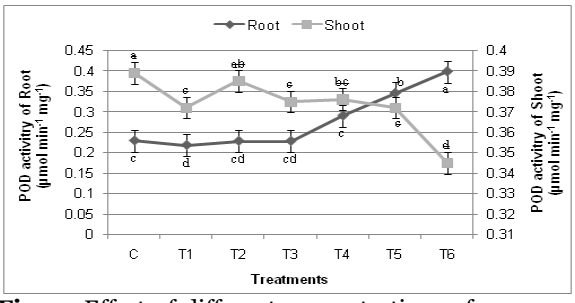

Fig. 5. Effect of different concentrations of aqueous extracts from *R. communis* and *L. camara* on POD activity (nmol min^{-1} mg^{-1} protein)) of root and shoot of maize The labeling of treatments is same as described in Figure 1.

The results revealed that all the concentration of *R. communis* extracts showed non-significant increase in CAT activity of root but the *L. camara* extracts exhibited significant increase in CAT activity of root and shoot as compared to control (Figure 6).

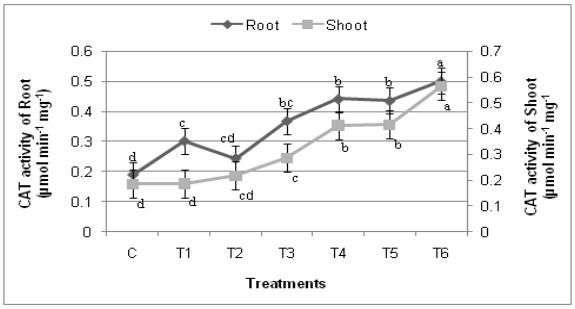

Fig. 6. Effect of different concentrations of aqueous extracts from *R. communis* and *L. camara* on Catalase activity (µmol min^{-1} mg^{-1} protein) of root and shoot of maize The labeling of treatments is same as described in Figure 1.

All the *R. communis* concentrations (0.8%, 1.0%, 1.2%) non-significantly decreased the polyphenol oxidase activity, while the *L. camara* concentrations significantly increased the PPO activity of root as compared to untreated control. The PPO activity of shoot was decreased by all the treatments except *L. camara* 1.2% concentration which non-significantly increased the PPO activity as compared to untreated control. Increasing of PPO activity was associated by decreasing of radical length (Figure 7).

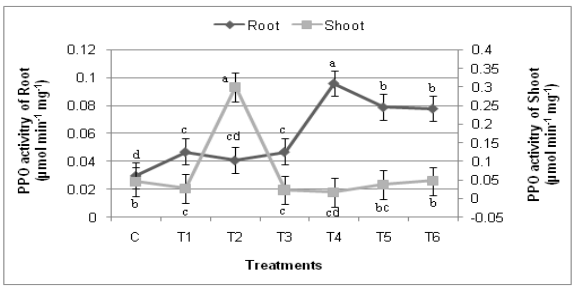

Fig. 7. Effect of different concentrations of aqueous extracts from *R. communis* and *L. camara* on PPO activity (µmol min^{-1} mg^{-1} protein) of root and shoot of maize The labeling of treatments is same as described in Figure 1.

The lipid peroxidation (MDA) of radical increased with extracts concentrations compared to the controls (Figure 8). The *R. communis* extract at concentration of 0.8% and 1.0% decreased the lipid peroxidation while all the *L. camara* extract concentrations (0.8%, 1.0%, 1.2%) non-significantly increased the activity. In this study increasing and/or decreasing of lipid peroxidation associated by POD activity. Malondialdehyde (MDA) is formed through auto oxidation and enzymatic degradation of polyunsaturated fatty acids in cells (Hodges *et al.*, 1998). Thus development of seedlings and enzyme activity, both affected on lipid peroxidation. An increase in CAT activity and MDA content have been observed in *Brassica napus* and *Triticum aestivum* with the increasing concentrations of *Phytolacca latbenia* extracts (Ullah *et al.*, 2013).

These free radicals are extremely dangerous to cells because they cause enzyme inactivation, membrane lipid peroxidation and decrease in the absorption by the roots (NG *et al.*, 2003. Furthermore, the increase of POD and PPO activities accompanied by the

reduction of root growth strengthens the hypothesis of phenolic acids synthesis by the phenylpropanoid pathway incorporation in lignin, increase in the cell wall rigidity and growth reduction (NG *et al*., 2003). There was a close association between POD and PPO (Nkang, 2001).

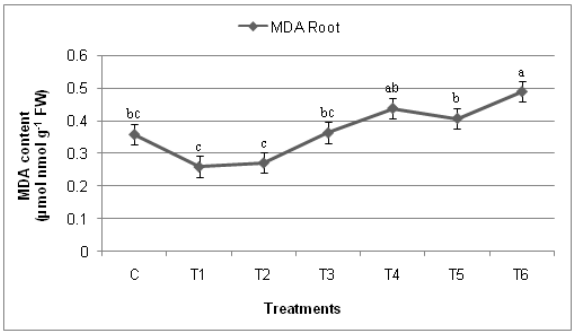

Fig. 8. Effect of different concentrations of aqueous extracts from *R. communis* and *L. camara* on MDA content (nmol g^{-1} FW) of root and shoot of maize The labeling of treatments is same as described in Figure 1.

To counteract the toxicity of reactive oxygen species, plants have developed a highly efficient antioxidant enzymes defense system, mainly including SOD, APX, CAT and Prx, increasing tolerance to different stress factors (Jiang and Zhang 2002).

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