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ALLELOPATHIC ASSESSMENT OF SELECTED INVASIVE SPECIES OF PAKISTAN

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Abstract

Invader species are a great threat to local flora. Eight invader species of Pakistan were screened for their allelopathic activity through sandwich method. Toxic (inhibitory) and non-toxic (stimulatory) effects were assessed by recording their effect on germination and growth of lettuce. Radicle and plumule growth of lettuce were recorded at 5, 10 and 50 mg leaves concentrations of each species. Among all species the growth activity was found to be concentration dependent. Except *Eutcalyptus glabra* all species resulted in inhibitory effects at 5, 10 and 50 mg leaves concentrations. *Xanthium strumarium* and *Cannabis sativa* showed strong inhibitory effects on radicle and plumule growth of lettuce. Maximum inhibition was recorded at highest concentration; even growth of lettuce was stopped with 50 mg leaves concentration of *C. sativa*.

Key words: Allelopathic, Invasive species *Xanthium strumarium* and *Cannabis sativa*, Pakistan

Introduction

Allelopathy is recognized as an interesting aspect of biological science. The concept of Allelopathy is popular worldwide because of its wide scope and unlimited opportunities that it provides. Many studies confirmed the presence of allelochemicals in different plant parts including leaves, stems, flowers, roots and buds. The allelochemicals have great potential to be used as pesticides because they are free from the harmful effects as compared to the pesticides in use (Khan *et al.,* 2011). An invader is a species that has colonized, successfully persisted and spread into an area in which it its existence was not possible before (Reichard & Hamilton, 1997). Invasive organisms are a main interest in ecology because of their tremendous destruction, but their mechanism of invasiveness needs to be explored (Callaway & Maron, 2006). Invasive species have potential to change the structure of the local plant communities (Woitke & Dietz, 2002; Mack *et al.,* 2000; Vitousek, 1990). Invasive species are introduced accidently or intentionally by human beings (Qureshi *et al*., 2014). Recent studies presents strong evidence that some of the most strong exotic species are successful because of allelopathic interactions between foreign and native plant species (Baise *et al.,* 2003; Callaway & Reidenour, 2004; Prati & Bossodorf, 2004; Stinson *et al.,* 2006; Qureshi *et al*., 2014). Invader plants may spread and replace native species through competition, changes in ecosystem process or allelopathy (Shabbir & Bajwa, 2006; Orr *et al.*, 2005; Hierro & Callaway, 2003; Malik & Prescott, 2001).

Pakistan has a long history of introduction of exotic plant species. Out of 700 alien species, few species are strong invaders (Altaf & Zarif, 2003). Some invader species of Pakistan are *Broussonetia papyrifera, Eucalyptus camaldulensis, Cannabis sativa, Prosopis juliflora, Lantana camara, , Parthenium hysterophorus* and *Xanthium strumarium* (Marwat & Hashmi, 2002; Khan *et al.,* 2010; Khan *et al.,* 2011; Qureshi *et al.,* 2014). Among these *E. camaldulensis* was introduced purposely. In Pakistan research on allelopathy was initiated in early seventies (Khan *et al.,* 2011).

B. papyrifera and *P. hysterophorus* has been recognized as one of the worst invader plants of Pakistan (Qureshi *et al.,* 2014; Riaz & Javaid, 2011; Malik & Hussain, 2006). During February to April *B. papyrifera* contributes to severe pollen allergy (Birsel, 2007). Pollen counts reach approximately 40000 per m^3 causing severe asthma related problems (Ali & Malik, 2010). *P. hysterophorus* has the ability to support or replace both native and non-native flora (Timsina *et al.,* 2011).

Bioassays are necessary in all stages of the identification and isolation of active allelopathic compounds. There are a number of simple and inexpensive bioassays that are in use since many years to detect allelopathic activity. Lettuce was considered to be best for bioassays because of its fast germination and high sensitivity. Because of these reasons, it is used extensively in allelopathic studies (Macias *et al.,* 2000; Shinwari *et al*., 2013). Keeping in view the importance of allelopathic activity of various plant species, the experiment was conducted under laboratory conditions with the objectives to find out allelopathic activity of some invader species on the germination and seedling growth of lettuce.

Materials and Methods

Eight invader species (*Broussonetia papyrifera, Cannabis sativa, Eucalyptus glabra, Lantana camara, Leucaena leucocephala, Parthenium hysterophorus, Prosopis juliflora* and *Xanthium strumarium*) were screened for their allelopathic activity by sandwich method. Fresh leaves of the invader species were collected from Rawalpindi and Islamabad, Pakistan. The fresh leaves were washed with running water to remove dust and other undesired materials. Fresh leaves were then oven dried at 60˚C for gradual drying. Properly oven dried leaves were stored in plastic bags at 4˚C for further use. Three different concentrations (5, 10 and 50 mg each) of dry leaves were weighed and were used for the experiment.

Media preparation: Agar powder (Nacalai Tesque, Kyoto, Japan) with gelling temperature 30-31˚C was used as a media. Agar solution (0.75%w/v) was prepared and autoclaved at 121˚C for 15 minutes. Three different concentrations of leaves (5, 10 and 50mg) were placed in multi-dishes (10 cm^2 area per each dish) (Nalge Nunc Intl., Roskilde, Denmark). The experiment was performed in the triplicate (Fujii *et al.*, 2003; Anjum *et al.*, 2010).

Inoculation procedure: Using a pipette first layer of agar (5 ml) was added to each well of the multi-dish, as a result dried plant material rise up. The agar medium was allowed to solidify in each Multidish at room temperature. Again 5 ml (second layer) of agar medium was added to each well of Multidish and allowed to solidify. In each dish, five *Lettuce* seeds were placed above agar at equal distances. All multidishes were sealed and were placed in plastic box having moistened filter paper at the bottom. Multidishes were incubated in complete darkness at 25˚C. After 72 h lengths of radical and hypocotyls were noted for each plant. Percentage germination and percentage lengths were calculated according to method of Iqbal *et al*., (2004). For each plant, mean and standard error were calculated to determine the growth pattern of 5, 10 and 50 mg of leaves (Fujii *et al.*, 2003).

Statistical analysis: The data was analyzed by Microsoft Excel. Percentage germination was recorded and presented in the table. Percent growth of radical and plumule of test plant under the influence of various invader species is represented by line graphs.

Results and Discussion

Table 1 represent the percentage germination of 8 invader plant species from different families that have been used to check their allelopathic action. In the given species allelopathic action was concentration dependent. The 50 mg leaves concentration inhibited the growth of radicle in *lettuce* up to 80-90% with *B. papyrifera, L. leucocephala, P. hysterophorus, P juliflora* and *X. strumarium* (Figs. 1-8). *L. camara* resulted in 50% growth inhibition at 50 mg concentration, whereas *E. glabra* resulted in 45% growth inhibition. *L. camara* significantly reduced the germination of tef with 75% aqueous leaf extract, however at lower concentrations, no significant effects were shown (Tadele, 2014). Strong allelopathic activity was recorded by *C. sativa*. This species completely inhibited the germination of lettuce at 50 mg leaves concentration (Table 1).

Table 1. Percentage germination of *Lettuce* seedlings under the effect of different invader species.

Species	Family	Treatments			
		Control	5mg leaf extract	10mg leaf extract	50mg leaf extract
B. papyrifera	Moraceae	100	100	100	100
C. sativa	Cannabaceae	100	100	100	0
E. galbra	Myrtaceae	100	100	100	100
L. camara	Verbenaceae	100	100	100	100
L. leucocephala	Fabaceae	100	100	100	100
P. hysterophorus	Asteraceae	100	100	100	100
P. juliflora	Fabaceae	100	100	100	100
X. strumarium	Asteraceae	100	100	100	80

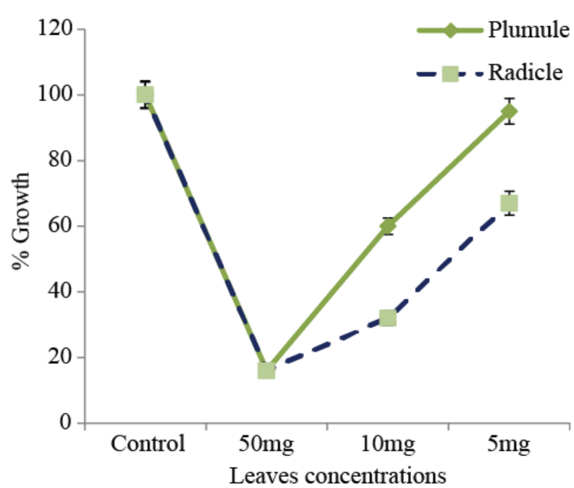

Fig. 1. Effect of *B. papyrifera* on growth of plumule and radical of lettuce.

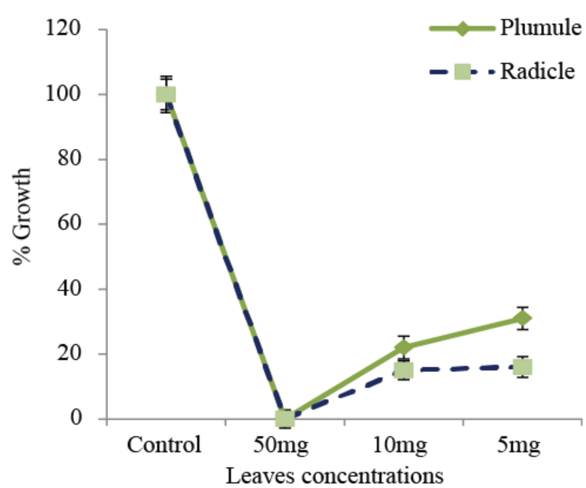

Fig. 2. Effect of *C. sativa* on growth of plumule and radical of lettuce.

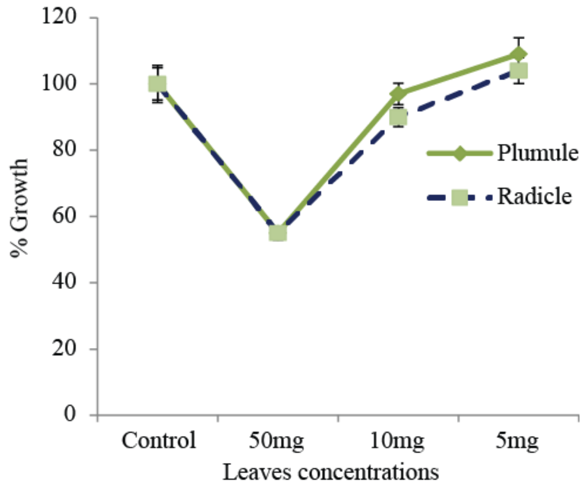

Fig. 3. Effect of *E. glabra*on growth of plumule and radical of lettuce.

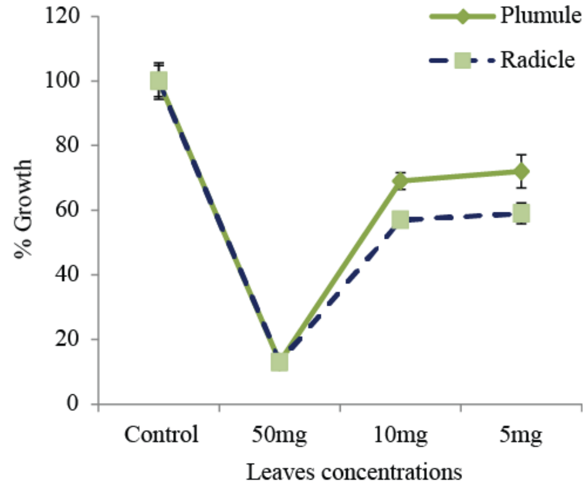

Fig. 5. Effect of *L. leucocephala* on growth of plumule and radical of lettuce.

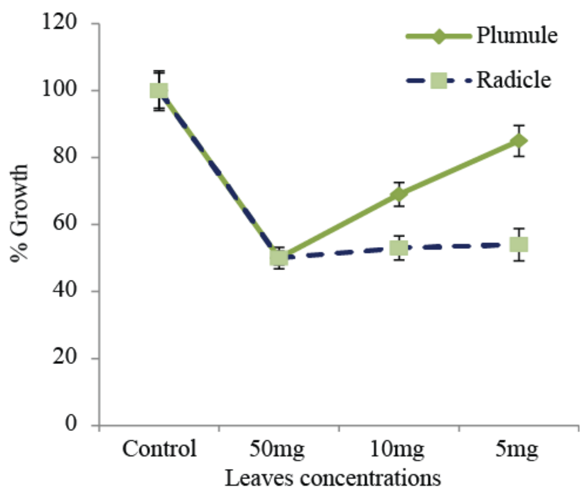

Fig. 4. Effect of *L. camara* on growth of plumule and radical of lettuce.

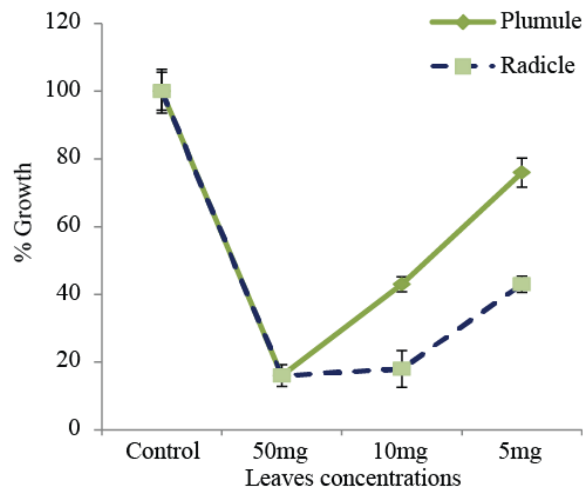

Fig. 6. Effect of *P. hysterophorus* on growth of plumule and radical of lettuce.

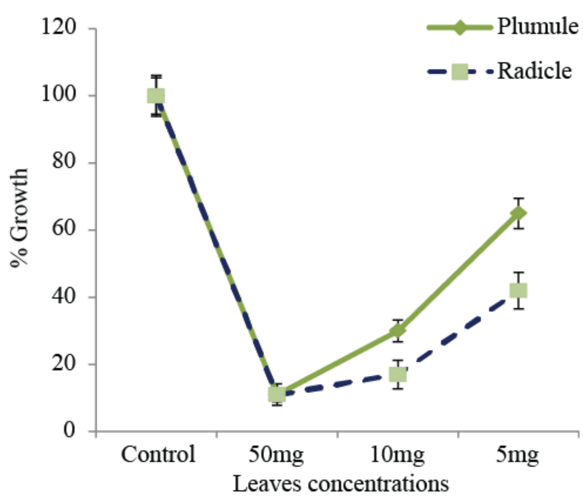

Fig. 7. Effect of *P. juliflora* on growth of plumule and radical of lettuce.

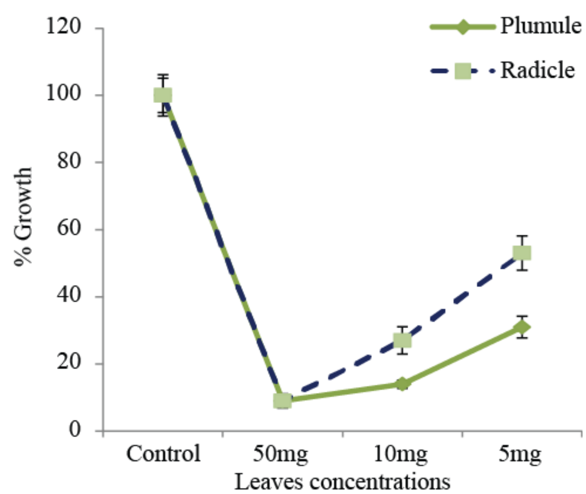

Fig. 8: Effect of *X. strumarium* on growth of plumule and radical of lettuce.

Growth activity of tested species was less than 71% at 5 and 10 mg leaves concentration (Fig. 2). *E. glabra* resulted in stimulatory effects up to 9% in plumule and 4% radicle length at 5 mg leaves concentrations (Fig. 3). However, there was a reduction in growth activity at 10 and 50 mg leaves concentration. Less than 10% growth inhibition was recorded at 10 mg leaves concentration of *E. glabra*, where 95% growth was recorded. At 50 mg leaves concentration (*E. glabra*) more than 40% growth inhibition was recorded (Fig. 3). Radicle length of lettuce under the effect of 5mg concentration of *L. camara, L. leucocephala, P. hysterophorus, P. juliflora* and *X. strumarium* were reduced up to 40-60%. At 10 mg concentration these species shows 50-85% radicle inhibition of lettuce (Figs.4-8). *B. papyrifera* resulted in 35-70% inhibition of radicle growth of test plant at 5 and 10 mg leaves concentration. Aqueous extracts of *Eucalyptus citroides* and *Azadiracta indica* were highly effective in reducing germination and growth of wheat. Growth was significantly reduced when 100% aqueous extract concentration was used (Rao & Mamta, 2013).

Among all invader species *B. papyrifera, L. leucocephala, P. hysterophorus, P. juliflora* and *X. strumarium*, plumule inhibitory activity was more than 80% at 50mg leave concentration (Figs. 2-8). Leaf leachates of different concentrations of *L. leucocephala* resulted in strong inhibitory effects towards the germination percentage, plumule and radicle length of Oats and this inhibition was concentration dependent (Rishi & Dhillon, 1997). Leaf extracts and leaf leachates of *P. juliflora* resulted in similar inhibitory effects (Geol *et al*., 1989). Similarly, no plumule growth was recorded with 50mg concentration of leaves of *C. sativa*. The results are in contrast to Anjum *et al.* (2010), where stimulatory growth effects were recorded with *C. sativa*, *L. camara* and *P. hysterophorus* on plumule growth, 50% plumule inhibition was recorded with 50 mg leaves concentration of *E. glabra* and *L. camara*. However, at 10 mg leaves concentrations higher growth activity was recorded on same species in comparison to 50mg leaves concentration. At 50mg leaves concentration, maximum plumule and radicle inhibition was recorded by *X. strumarium*, where growth inhibition was up to 85% (Fig. 8). The plant was active in its allelopathic potential at 10mg leaves concentration, where less than 40% growth activity was recorded. Among other species 3-60% plumule inhibition was recorded at 50 mg leaves concentrations (Figs. 1-8).

C. sativa and *X. strumarium* also resulted in 69% plumule inhibition at 5mg leaves concentration (Figs. 2&8). *E. glabra* resulted in 9% stimulatory effects at 5mg leaves concentration (Fig. 3). Among other species 5-35% plumule inhibition was recorded (Figs. 1-8). Leaf leaches from 71 ground cover plant species on lettuce were tested. Eight species of Oxalis showed strong inhibition to lettuce where, 4-27% radicle growth inhibition was recorded (Shiraishi *et al*., 2002). The leachates from Oxalis species resulted in more than 84% inhibition of the radicle length of lettuce seedlings, seed germination of lettuce remained unaffected (Shiraishi *et al.*, 2005).

X. strumarium resulted in strong allelopathic activity at all concentrations. Maximum radicle and plumule growth inhibitions were recorded at all concentrations (Fig. 8). *E. glabra* resulted in a stimulatory effect at 5 mg leaves concentration, however slight inhibitory effects were recorded at 10 and 50 mg leaves concentrations (Fig. 3). Anjum *et al.* (2010) performed laboratory experiments to examine the allelopathic potential of some medicinal plants on the germination and growth of lettuce. Using sandwich method, *Albezzia lebbeck* and *B. papyrifera* have strong inhibitory effects on the radicle and hypocotyls growth of lettuce. However, stimulatory effects were recorded with *C. sativa* and *P. hysterophorus* at 5mg leaves concentrations (Anjum *et al.,* 2010). Different plant parts of *P. hysterophorus* resulted in reduced rate of germination and growth rate, leaves were more allelopathic against the germination and growth of *Avena fatua* and *Phalaris minor* (Aslam *et al.,* 2014). Germination of different test species was significantly affected at higher concentrations of *Digeria muricata* (Aziz & Shaukat, 2014).

Conclusion

Allelochemicals produced by less desirable plants including invasive species have the potential to be used as allelopathic plants and can be used to control weeds/or pests when applied as bio herbicide, in the form of aqueous extracts, mulch, plant residues and companion plant in the field. This is a preliminary research to screen plants with allelopathic potential. Once the bioactivity of the plant is established, it could be viable economic opportunities for the farmers to apply it as bioherbicide.

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