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Bioherbicidal potential of some allelopathic agroforestry and fruit plant species against *Lepidium sativum*

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Abstract

Herbicide resistance and environmental pollution are the risks associated with chemical control of weeds. Allelopathic plant extracts may be exploited for weed management as an alternative to commercial herbicides. Before development of allelochemical-based eco-friendly herbicides, bioherbicidal potential of plants need to be evaluated. Present study was conducted to evaluate phytotoxic allelopathic impact of leaf extract from eight agroforestry and fruit plant species against Lepidium sativum. The results showed that all plant species delayed germination and inhibited root length, shoot length and seedling dry weight of Lepidium sativum. Four plant species such as Moringa oleifera, Mangifera indica, Albizia procera and Delonix regia were most phytotoxic with Lepidium sativum root growth inhibition of ≥85% as compared with control and seedling persistence index <30% of control. Phenolic contents were maximum in Mangifera indica (137 mg g^{-1} leaf dry weight) followed by Delonix regia (130 mg g^{-1} leaf dry weight). The results suggest that phytotoxic action of leaf extract of plant species may be due to presence of phenolic allelochemicals that may be exploited further either directly for weed management or development of bioherbicides.

Keywords: Allelopathy, allelochemicals, growth inhibition, phytotoxicity, weed management

Introduction

Weeds have been a major threat to crop productivity in agroecosystems since start of the agriculture on earth and lack of weed control is a pressing concern expressed by the farmers (Stokstad, 2013). Herbicide use have been the most effective way to control weeds especially in the last few decades. The share of herbicides in total pesticide use in the world is about 47.5% with an annual consumption of 0.95 million tonnes (De *et al.*, 2014). Nonetheless, there is growing public, scientific and farmers' concern regarding harmful impact of herbicides on environment and development of herbicide resistant weeds (Edwards, 2013, Dayan and Duke, 2014). Presently, 255 weed species (148 dicots and 107 monocots) have developed resistance to 163 herbicides in 92 crops in 70 countries (Heap, 2018). Furthermore, in the last 20 years, no new mode of action of the herbicides has been reported (Dayan and Duke, 2014; Romano-Armada *et al*., 2017). Keeping in view all above-mentioned problems associated with use of commercial herbicides, there is growing need for alternative options for weed control in agroecosystems.

Higher plants produce various secondary metabolites (natural products) which may be released into the environment as allelochemicals to inhibit growth of the other plant species including weeds (Cheng and Cheng, 2015). Phenolic compounds are a major group of allelochemicals ranging from phenols, hydroxy acids, aldehydes, benzoic acids cinnamic acids, coumarins, tannins and flavonoids. They are produced by various plant species and their inhibitory effect on crops and weeds have been well reported (John, 2012, Mushtaq *et al.*, 2013; Tariq *et al*., 2018; Perveen *et al.*, 2019). At times, the secondary metabolites are not released by plants into the environment; however, they still act as natural phytotoxins. Either exploited as allelochemicals or phytotoxins, the natural products offer a viable option for weed control in agroecosystems as an alternative to commercial herbicides.

Many agroforestry and fruit plant species have been reported as allelopathic thus showing bioherbicidal potential to be used for management of weeds. For example, *Dalbergia sissoo* leaf extract inhibited germination and growth of maize, pearl millet and rice (Akhtar *et al.*, 2010). Mulberry leaf extract suppressed the growth of bermuda grass (Haq *et al.*, 2010). Growth of understory plant species was reduced by allelochemicals from *Delonix regia* (Chou and Leu, 1992).

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Though few reports are available regarding phytotoxic potential of common agroforestry and fruit plant species (*Moringa oleifera, Morus alba, Mangifera indica, Albizia lebbeck, Albizia procera, Delonix regia, Ziziphus jujube, Ziziphus mauritiana*) of Asia including Pakistan; nevertheless, these plants have been tested against different target crops and weed species with extreme variation in the extract concentrations from species to species under variable test conditions, which makes it difficult to decide the most potent allelopathic plant species to exploit further for bioherbicidal efficacy (Chou and Leu, 1992, Uddin *et al.*, 2007; Alshahrani *et al.*, 2009; Haq *et al.*, 2010; Phiri and Mbewe, 2010; Piyatida and Kato-Noguchi, 2010; Saleem *et al.*, 2013). The present study was therefore conducted to explore and compare bioherbicidal potential of plant species using an established model test plant species (*Lepidium sativum*) for allelopathic studies under identical controlled environment for germination, early seedling growth and total phenolic contents of all donor plant species. *Lepidium sativum* is a leafy edible plant which has been used as a test species for initial screening in laboratory bioassays to evaluate the phytotoxic effect of allelochemicals and/or natural phytotoxins (Vaughn and Berhow, 1999; Macias *et al.*, 2000; Perveen *et al.*, 2015). Furthermore, we used modified equation to determine seedling persistence index, which has been seldom employed to evaluate persistence of target plant seedlings, for comparison of phytotoxic potential of multiple plant species and concentrations.

Materials and Methods

The extract was prepared as previously described (Perveen *et al.*, 2015). Briefly, the leaves of tree species were collected from University of Agriculture, Faisalabad. The oven-dried leaves were ground to powder. The possible phytotoxins from leaf powder of each plant were extracted by adding 10 g powder to 100 mL distilled water on an ultrasonic device for 30 min. The extract was sieved through cheesecloth to obtain 10% extract. Further dilutions (2.5, 5 and 7.5%) were made using distilled water.

The seeds of *Lepidium sativum* were surface sterilized with 2% NaClO for 2 minutes and were rinsed with distilled water to remove traces of NaClO. Ten seeds of *Lepidium sativum* were placed on a filter paper soaked with 4 mL of respective concertation of each species in Petri dishes (9.5 cm diameter). The control (0% extract) received only distilled water. The petri dishes were sealed and placed in a growth cabinet at 24±2ºC and 14/10 (light/dark) for 6 days. The germinated seeds were counted daily and time to start germination was noted. Mean germination time (MGT) was calculated by following formula (Ellis and Roberts, 1981):

$$MGT=\frac{\Sigma nD}{\Sigma n}$$

where n= number of newly germinated seeds at time D; D= Number of days from the sowing, ∑n= Final germination

Germination index (GI) was calculated by following formula as reviewed and described earlier (Ranal and Santana, 2006):

$$GI=\frac{\text{Number of germinated seeds}}{\text{Days of first count}}+\text{---}+\frac{\text{Number of germinated seeds}}{\text{Days of final count}}$$

Root and shoot length and dry weight of seedlings were measured 6 days after sowing. Seedling persistence index (SPI) was calculated using formula modified from the previously reported (Mishra and Misra, 1997) and expressed as % of control:

$$SPI\,(\%\text{ of control})=\frac{\text{root length }(\%\text{ of control})+\text{shoot length }(\%\text{ of control})+\text{seedling dry weight }(\%\text{ of control})}{3}$$

Total phenolic compounds from leaf extract of eight allelopathic plant species were measured using Folin-Ciocalteu reagent (Saleem *et al.*, 2013). Chlorogenic acid was used as a standard and its concentrations (0-1000 ppm) were measured in the same way as leaf extract samples to draw a calibration curve and calculate phenolic contents. To 200 µL of the samples or respective chlorogenic acid concentration or blank, 4 mL of 4% Na_2CO_3 and 200 µL of Folin-Ciocalteau reagent was added while vortex-mixing. Thereafter, the mixture was incubated at room temperature for 30 min and absorbance was measured at 750 nm on a spectrophotometer (Hitachi U-2800, Japan).

The results are reported as mean ± SEM of five replications. The treatment means were statistically compared by ANOVA followed by post-hoc Duncan's Multiple Range Test (DMRT) at $p<5\%$.

Results

Effect of allelopathic plant leaf extract on germination of *Lepidium sativum*

Leaf extract of all plant species significantly delayed time to start germination and mean germination time and reduced germination index in a concentration-dependent manner (Table 1). The time to start germination and mean germination time was delayed by ≥1 day as compared with control after application of 10% leaf extract of all donor plant species except *Morus alba.* The same concentration (10%) of *Moringa oleifera, Mangifera indica, Albizia procera* and *Delonix regia* decreased germination index by ≥50. The final germination was significantly inhibited by plant species at any concentration except *Moringa oleifera* that reduced final germination by 26% and *Mangifera indica* where no seed was germinated at 10% leaf extract (Table 1).

Table 1: Inhibitory effect of allelopathic aqueous leaf extract of agroforestry and fruit tree species on germination of *Lepidium sativum*

Name of plant species	Extract concentration (%)	Time to start germination (days)	Mean germination time (days)	Germination index	Final germination (%)
Control	0.0	1.0 ± 0.0g	1.0 ± 0.0q	10 ± 0.0bc	100 ± 0.0a
Moringa oleifera	2.5	2.0 ± 0.0f (100)	2.0 ± 0.0lmno (100)	5.10 ± 0.09efghijk (-49)	100 ± 0.0a (0)
	5.0	2.0 ± 0.0f (100)	2.46 ± 0.21ghi (146)	6.22 ± 0.45defg (-37.8)	98 ± 1.88a (-2)
	7.5	2.0 ± 0.0f (100)	2.24 ± 0.14ijkl (124)	5.53 ± 0.45fghij (-44.7)	100 ± 0.0a (0)
	10	3.0 ± 0.0bc (200)	3.0 ± 0.0d (200)	2.42 ± 0.07no (-75.8)	74 ± 2.31c (-26)
Morus alba	2.5	1.0 ± 0.0g (0)	1.13 ± 0.12q (13)	10.0 ± 0.0bc (0)	100 ± 0.0a (0)
	5.0	1.0 ± 0.0g (0)	1.70 ± 0.11op (70)	12.40 ± 0.98a (24)	100 ± 0.0a (0)
	7.5	1.2 ± 0.19g (20)	1.97 ± 0.13lmno (97)	9.53 ± 1.17bc (-4.7)	100 ± 0.0a (0)
	10	1.8 ± 0.19f (80)	1.94 ± 0.05lmno (94)	5.80 ± 0.80defgh (-42)	100 ± 0.0a (0)
Mangifera indica	2.5	1.0 ± 0.0g (0)	4.0 ± 017c (300)	10.5 ± 0.98bc (5)	96 ± 3.76a (-4)
	5.0	3.2 ± 0.35b (220)	5.58 ± 0.14a (458)	5.58 ± 0.21defghi (-44.2)	98 ± 1.88a (-2)
	7.5	3.80 ± 0.20a (280)	5.22 ± 0.06b (422)	3.21 ± 0.31lmn (-67.9)	74 ± 5.64c (-26)
	10	-	-	-	-
Albizia lebbeck	2.5	2.0 ± 0.0f (100)	1.90 ± 0.09mnop (90)	10.8 ± 0.80b (-67.9)	100 ± 0.0a (0)
	5.0	1.0 ± 0.0g (0)	1.87 ± 0.02nop (87)	6.40 ± 0.24de (-36)	100 ± 0.0a (0)
	7.5	2.0 ± 0.0f (100)	2.0 ± 0.0lmno (100)	5.0 ± 0.0ghijk (-50)	100 ± 0.0a (0)
	10	2.0 ± 0.0f (100)	2.20 ± 0.12ijklm (-220)	6.13 ± 0.69defg (-38.7)	100 ± 0.0a (0)
Albizia procera	2.5	1.0 ± 0.0g (0)	2.0 ± 0.0lmno (100)	5.0 ± 0.0ghijk (-50)	100 ± 0.0a (0)
	5.0	2.4 ± 0.20e (140)	2.40 ± 0.23hij (-240)	4.33 ± 0.4ijkl (-56.7)	100 ± 0.0a (0)
	7.5	2.60 ± 0.24de (160)	2.60 ± 0.23fgh (-240)	3.0 ± 0.41jkl (-70)	100 ± 0.0a (0)
	10	2.0 ± 0.0f (100)	2.0 ± 0.0lmno (100)	5.0 ± 0.0ghijk (-50)	100 ± 0.0a (0)
Delonix regia	2.5	1.0 ± 0.0g (0)	1.0 ± 0.0q (0)	10.0 ± 0.0bc (0)	100 ± 0.0a (0)
	5.0	2.0 ± 0.0f (100)	2.0 ± 0.0lmno (100)	5.20 ± 0.12efghij (-48)	100 ± 0.0a (0)
	7.5	2.0 ± 0.0f (100)	2.0 ± 0.0lmnop (100)	5.0 ± 0.0ghijk (-50)	100 ± 0.0a (0)
	10	2.0 ± 0.0f (100)	2.0 ± 0.0klmno (100)	5.0 ± 0.0fghijk (-50)	100 ± 0.0a (0)

Ziziphus jujube	2.5	1.0 ± 0.0g (0)	1.69 ± 0.01op (-240)	9.4 ± 0.0c (-6)	100 ± 0.0a (0)
	5.0	2.0 ± 0.0f (100)	2.0 ± 0.0mno (100)	5.0 ± 0.12ghijk (-50)	100 ± 0.0a (0)
	7.5	2.0 ± 0.0f (100)	2.32 ± 0.12hijk (-240)	6.60 ± 0.0d (-34)	100 ± 0.0a (0)
	10	2.0 ± 0.0f (100)	2.32 ± 0.20ijk (-240)	6.39 ± 0.0def (-36.1)	100 ± 0.0a (0)
Ziziphus mauritiana	2.5	2.0 ± 0.0f (100)	1.64 ± 0.02p (-240)	10.6 ± 0.24bc (6)	100 ± 0.0a (0)
	5.0	2.0 ± 0.0f (100)	2.10 ± 0.09jklmn (-240)	6.0 ± 1.0defgh (-40)	100 ± 0.0a (0)
	7.5	2.0 ± 0.0f (100)	2.0 ± 0.0klmno (100)	5.0 ± 0.0fghijk (-50)	34 ± 0.0e (-66)
	10	2.0 ± 0.0f (100)	2.74 ± 0.13defg (-240)	9.64 ± 0.22bc (-3.6)	100 ± 0.0a (0)

Data are presented as mean ± SEM; mean values having different letters in a column are statistically different at $p<0.05$; Values in parenthesis show % (+) increase or (-) decrease as compared with control.

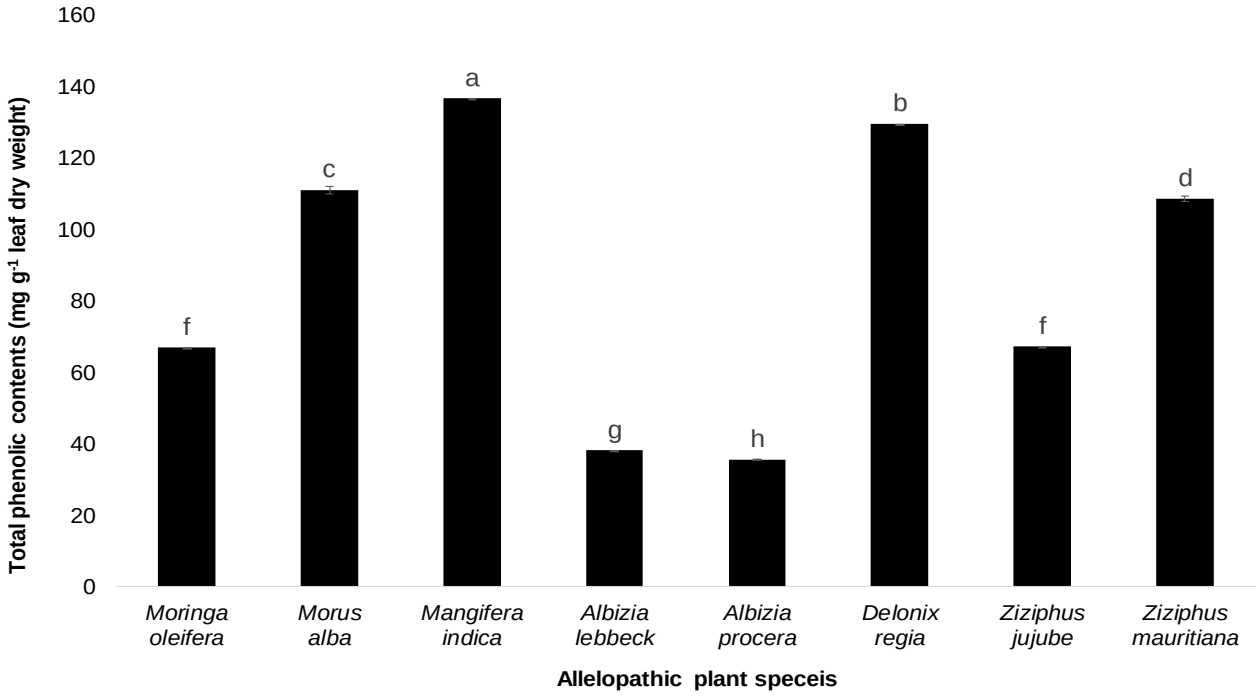

Figure 1: Total phenolic contents in leaf extract of eight allelopathic plant species.
Data are presented as mean ± SEM (n=5); Bars having different letters at top are statistically different at $p<0.05$.

Total phenolic contents in leaf extract and allelopathic effect on seedling growth of *Lepidium sativum*

All plant species significantly suppressed shoot length, root length and seedling dry weight of *Lepidium sativum* at ≥5% leaf extract concertation (Table 2). *Moringa oleifera*, *Mangifera indica*, *Albizia procera*, *Delonix regia* and *Ziziphus mauritiana* inhibited root length by ≥85% and seedling dry weight by >55% at 10% leaf extract as compared with control (Table 2). Regarding seedling persistence index, four plant species such as *Moringa oleifera*, *Mangifera indica*, *Albizia procera* and *Delonix regia* were most phytotoxic with *Lepidium sativum* seedling persistence index <30% of control (Table 2). Among test species, *Mangifera indica* had maximum (137 mg g^{-1} leaf dry weight) phenolic contents followed by *Delonix regia* with 130 mg g^{-1} leaf dry weight (Figure 1).

Table 2: Phytotoxic allelopathic effect of aqueous leaf extract of agroforestry and fruit tree species on seedling growth of *Lepidium sativum*

Name of plant species	Extract concentration (%)	Shoot length (cm)	Root length (cm)	Seedling dry weight (mg)	Seedling persistence index (% of control)
Control	0.0	4.16 ± 0.04d	6.53 ± 0.11b	10.60 ± 0.38a	100
Moringa oleifera	2.5	5.57 ± 0.13a (33.89)	5.54 ± 0.24f (-15.16)	8.20 ± 0.19cd (-22.64)	99
	5.0	4.56 ± 0.07b (9.6)	4.12 ± 0.03kl (-36.90)	7.40 ± 0.23defg (-30.18)	81
	7.5	4.40 ± 0.09c (5.7)	2.12 ± 0.02s (-67.53)	4.60 ± 0.23op (-56.60)	61
	10	2.17 ± 0.01kl (-47.3)	0.91 ± 0.05vw (-86.06)	2.80 ± 0.19s (-73.58)	31
Morus alba	2.5	4.10 ± 0.03d (-1.44)	5.09 ± 0.0h (-22.05)	7.6 ± 0.23def (-28.30)	83
	5.0	3.20 ± 0.02g (-23)	4.06 ± 0.02i (-37.82)	7.0 ± 0.0efgh (-33.96)	68
	7.5	2.20 ± 0.03jk (-47)	4.30 ± 0.02jk (-34)	5.80 ± 0.0jkl (-45.28)	58
	10	2.06 ± 0.02kl (-50.5)	3.75 ± 0.05m (-42.57)	4.80 ± 0.19no (-54.71)	51
Mangifera indica	2.5	1.99 ± 0.07l (-52)	4.19 ± 0.03kl (-35.83)	7.40 ± 0.23defg (-30.18)	61
	5.0	1.43 ± 0.02no (-85.7)	1.44 ± 0.02u (-77.94)	6.40 ± 0.23hij (-39.62)	39
	7.5	0.52 ± 0.04r (-65.6)	0.36 ± 0.03xy (-94.48)	3.20 ± 0.19rs (-69.81)	16
	10	-	-	-	0
Albizia lebbeck	2.5	4.58 ± 0.04b (10.09)	6.34 ± 0.02bc (-2.90)	9.0 ± 0.0b (-15.09)	97
	5.0	3.77 ± 0.05e (-9.3)	4.13 ± 0.02kl (-36.75)	7.80 ± 0.19de (-26.41)	76
	7.5	3.42 ± 0.04f (-17.8)	4.18 ± 0.02kl (-35.98)	7.20 ± 0.19efgh (-32.07)	71
	10	2.16 ± 0.02jk (-48)	2.08 ± 0.02s (-68.14)	5.80 ± 0.19jkl (-45.28)	46
Albizia procera	2.5	2.52 ± 0.05i (-39.42)	2.36 ± 0.01r (-63.85)	7.0 ± 0.0efgh (-33.96)	54
	5.0	1.84 ± 0.03m (-55.7)	1.11 ± 0.01v (-83.00)	5.80 ± 0.19jkl (-45.28)	39
	7.5	1.48 ± 0.04no (-64)	1.12 ± 0.02v (-82.84)	5.0 ± 0.0mno (-52.83)	33
	10	1.16 ± 0.01pq (-72)	0.92 ± 0.03vw (-85.91)	3.80 ± 0.19qr (-64.15)	26
Delonix regia	2.5	4.46 ± 0.07bc (7.2)	5.32 ± 0.03g (-18.52)	8.20 ± 0.19cd (-22.64)	89
	5.0	3.75 ± 0.04e (-9.8)	4.10 ± 0.01kl (-37.21)	7.80 ± 0.19cde (-26.41)	76
	7.5	2.54 ± 0.04i (-39)	2.15 ± 0.02s (-67.07)	6.0 ± 0.0ijk (-43.39)	50
	10	1.17 ± 0.02pq (-72)	0.97 ± 0.05v (-85.14)	4.60 ± 0.23op (-56.60)	29

Ziziphus jujube	2.5	4.37 ± 0.06c (5)	5.10 ± 0.01d (-21.89)	8.60 ± 0.23bc (-18.86)	88
	5.0	4.42 ± 0.04c (6.2)	6.09 ± 0.01d (-6.73)	7.80 ± 0.23de (-26.41)	91
	7.5	3.19 ± 0.04g (-23)	5.78 ± 0.05e (-11.48)	7.0 ± 0.0fgh (-33.96)	77
	10	3.17 ± 0.01g (-23)	4.83 ± 0.06i (-26.03)	5.80 ± 0.23jkl (-45.28)	68
Ziziphus mauritiana	2.5	3.19 ± 0.03g (-23)	7.16 ± 0.02a (-9.64)	7.80 ± 0.19de (-26.41)	87
	5.0	3.35 ± 0.03f (-19.5)	6.21 ± 0.0cd (-4.90)	6.80 ± 0.19fgh (-35.84)	80
	7.5	2.77 ± 0.05h (-33.4)	3.43 ± 0.07n (-32.15)	5.60 ± 0.23klm (-47.16)	57
	10	1.38 ± 0.09o (-67)	1.84 ± 0.06t (-71.82)	5.20 ± 0.19lmno (-50.94)	37

Data are presented as mean ± SEM; mean values having different letters in a column are statistically different at $p<0.05$; Values in parenthesis show % (+) increase or (-) decrease as compared with control.

Discussion

Allelopathic extract of donor plant species on seedling growth (root and shoot length and dry biomass) was variable depending upon the concentration and species (Table 2). Root length, shoot length and seedling dry weight are three important growth indictors to assess phytotoxic effect of allelochemicals and herbicides (Kamble, 2006, Mushtaq *et al.*, 2010a). Sometimes it is difficult to compare large data and decide most phytotoxic allelopathic plants when multiple plant species and extract concentrations are used as the impact on the three growth parameters may be different (Akhtar *et al.*, 2010, Piyatida and Kato-Noguchi, 2010). In the present study; therefore, seedling persistence index was calculated, which accounts for root and shoot length as well as seedling dry weight to set a criterion for easy and simple comparison of many species and concentrations. Based on seedling persistence index, four plant species *Moringa oleifera*, *Mangifera indica*, *Albizia procera* and *Delonix regia* resulted in highest bioherbicidal potential (seedling persistence index <30% of control) at 10% leaf extract against target plant species, *Lepidium sativum*. The phytotoxic effect was less at lower leaf extract concentrations. Few plant species showed a growth promontory effect at lowest leaf extract concentration (2.5%). This is the characteristic of allelochemicals from plant extract that they inhibit growth of other plants at higher concentrations and promote at lower concentrations (Akhtar *et al.*, 2010, Haq *et al.*, 2010).

The bioherbicidal effect of plant species might be due to presence of allelochemicals in the leaf extract, which is also supported by higher total phenolic contents in most phytotoxic plant species except *Albizia procera* (Figure 1). Phenolic compounds are the largest class of allelochemicals inhibiting growth of various crop and weed species (John, 2012). Previously, allelochemicals have been isolated and identified from leaves of *Moringa oleifera*, *Mangifera indica* and *Delonix regia* (Chou and Leu, 1992; Bennett *et al.*, 2003; Saleem *et al.*, 2013). The allelochemicals from these plant species have shown bioherbicidal potential against weed species. *Albizia procera* was among the most phytotoxic four plant species; however, total phenolic contents were very low in it as compared with other species. It is likely that *Albizia procera* might have allelochemicals other than phenolic compounds (Koodkaew *et al.*, 2012).

Though allelopathic leaf extract of the plant species in the present study significantly delayed germination of test species as indicated by enhanced time to start germination and mean germination time, the final germination was only inhibited by two species (Table 1). It has been reported that allelochemicals did not cause significant reduction in germination of target species, rather they inhibit early seedling growth (Mushtaq *et al.*, 2010a). The reduction of early seedling growth is very critical regarding control of weeds because critical period for weed-crop competition starts with germination of crop from very 1st week and lasts until 4-6 weeks after sowing (Kasasian and Seeyave, 1969). Furthermore, most herbicides are applied as early postemergence or postemergence to control weeds in field crops (Carey and Kells, 1995; Mushtaq *et al.*, 2010b).

There is a dire need to explore new phytotoxins with diverse mode of actions for managing herbicide resistant weeds in agroecosystems worldwide (Dayan and Duke, 2014). The leaf extract of tested plant species may be exploited as bioherbicide or to develop eco-friendly future herbicides. However, future studies might be conducted to investigate allelopathic impact of these plant species against

weeds under field conditions before general recommendation. In conclusion, among eight species investigated, four (*Moringa oleifera, Mangifera indica, Albizia procera* and *Delonix regia*) were most phytotoxic and have potential to be exploited as eco-friendly bioherbicides.

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