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EVALUATION OF DOMINANT ALLELOPATHIC WEED THROUGH EXAMINING THE ALLELOPATHIC EFFECTS OF FOUR WEEDS ON GERMINATION AND SEEDLING GROWTH OF SIX CROPS

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Abstract

Leaf aqueous extracts were used to examine the allelopathic effects of *Melilotus indica, Medicago polymorpha, Elusine indica* and *Fumaria indica* at four concentrations (10, 20, 30 and 40% W/V) on germination percentage, radicle and hypocotyl growth of six crops, namely *Triticum aestivum, Hordeum vulgare, Medicago sativa, Trifolium* spp., *Raphanus sativus* and *Trigonella foenum-graecum.* All weed extracts showed pronounced inhibitory effect on germination and seedling growth of tested crops, however inhibition was not consistent over studied parameters. Weeds exerted different allelopathic effects on test crops. *Fumaria indica* exhibited a significant negative effect on germination of all tested species at 20, 30 and 40% aqueous extracts, followed by *Elusine indica* and *Medicago polymorpha*, and *Melilotus indica at* 10% concentration level. *Melilotus indica* halted the hypocotyl growth of tested crops at 10 and 20% aqueous extracts, followed by *F. indica* at 30 and 40% dosage. Radicle growth of all recipient species was also stunted under the aqueous extract of *M. indica* at 20, 30 and 40%, followed by *Medicago polymorpha* except 10% concentration. *Elusine indica* showed negative effects on hypocotyl growth at higher concentrations as compared to *Medicago polymorpha*, whereas radicle growth was not affected under different aqueous extracts of *E. indica*. Leaf debris method was used in greenhouse to further authenticate the allelopathic effects of dominant allelopathic weed viz., *Melilotus indica* at four concentrations (0, 25, 50 and 75 g per 300 g of soil) on germination percentage, shoot and root length of four crops, namely *Medicago sativa, Trifolium* spp., *Hordeum vulgare* and *Triticum aestivum.* Powdered leaf debris of *M. indica* mixed with clay loam soil appeared to have strong allelopathic inhibition under higher concentrations on germination and shoot growth of *Medicago sativa* and *Trifolium* spp. as compared to *Hordeum vulgare* and *Triticum aestivum*, whereas inhibitory effects were more pronounced on *Trifolium* spp. and *Triticum aestivum*, in terms of their root length. Hence, *M. indica* proved a strong allelopathic weed that should be removed from field to avoid harmful effects during early growth stages of tested crops. Moreover, it is recommended that all these species be phytochemically examined for their allelopathic potential and possible development of environmental safe bio-herbicides to control weeds.

Key words: Allelopathy, *Melilotus indica, Medicago polymorpha, Elusine indica, Fumaria indica,* Leaf aqueous extract, Seedling growth, *Triticum aestivum* L.

Introduction

Weeds cause a serious threats in crop production. Due to lack of knowledge and financial resources, farming communities cannot remove them from their fields. Weeds compete with crop plants for resources like water, air, space, sun light, nutrients, lowering the grain quality and cash value of crops resulting in higher production cost (Alam, 1991; Nasira & Ahmed, 2009). There is a need to better understand weed nature and economical weed control methods to avoid their harmful effects on field crops. Presently, herbicidal weed control method is popular and considered best among other weed control methods (Santos, 2009), whereas manual and mechanical weed control methods are time consuming, laborious and not feasible over large areas. Usage of herbicides leads to threats in environmental pollution and health hazards. Therefore, allelopathy could be considered the basis for biological weed management and safe alternative to chemical weed control method. Allelopathy is a universal mechanism between crops and between crop and weed, in which some chemicals are released from one plant species changing the growing environment in their vicinity and potentially affecting in positive or negative ways the associated crops (Colton & Einhellig, 1980; Tajuddin *et al.,* 2002; Khan *et al.,* 2011; Bagheri *et al.,* 2014). Allelo-chemicals occur almost in all plant tissues like leaves, seeds and fruits, flowers, etc., and can be released together in the form of secondary metabolites (Singh *et al.,* 2005; Batish *et al.,* 2007) that may exert the effects in an additive or synergistic manner (Putnam & Tang, 1986). Allelochemicals are released into atmosphere through exudation as water soluble toxins, leaching of organic matter through decomposition and microbial by-products through non-living entities present in soil (Tukey, 1966; Fay & Duke, 1977; Chou, 1990; Mulatu *et al.,* 2009). Allelopathic phenomena is not easy to understand because plant growth maybe influenced by immobilization of nutrients through microbes involved in decomposition or allelochemicals, or maybe through interaction effects of both phenomena (Batish *et al.,* 2007). Several weed species are reported to have allelochemicals that affect the growth of crop plants (Mulatu *et al.,* 2009; Fischer & Quijano, 1985). Aqueous extract of *Melilotus indica* reduces the germination, root and shoot length of maize at 10% concentration (McCalla & Duley, 1948; Nasrine *et al.,* 2011). Chaghtai *et al.,* (1986) reported that allelo-chemicals of *Fumaria indica* halted the germination and seedling growth of *Triticum aestivum*. Reinhardt *et al.,* (1994) reported the negative effects of *Chenopodium album* on

onion germination and growth stages. Leaf aqueous extracts of *Parthenium* spp. at 10% concentration retarded the seed germination of teff (*Eragrostis teff*) (Tefera, 2002). Iqbal *et al.,* (2006) presented the inhibitory effect of *Lycoris radiate* on alfalfa crop. Nasira *et al.,* (2013) concluded that pot culture, containing the mixture of dried powdered of *Fumaria indica* and soil, enhanced the inhibition of seedling growth of wheat under higher concentrations. Dried powdered of *Melilotus indica* showed pronounced inhibition on maize germination and growth (Nasira & Ahmed, 2009). The main objective of this study was to evaluate the allelopathic effects of *Melilotus indica, Medicago polymorpha, Elusine indica and Fumaria indica* on germination and seedling growth of economically important crops through leaf aqueous extract method, and then to further validate the allelopathic effect of dominant allelopathic weed through leaf debris method, present research work could be interested in better understanding the weed nature and to control their harmful effects on initial growth of crop plants.

Materials and Methods

Aqueous extract method

Collection of donor weed species: Leaves of four weed species (*Melilotus indica, Medicago polymorpha, Elusine indica* and *Fumaria indica*) were collected at flowering stage during May-July 2013 from experimental fields of Arid Agriculture University, Rawalpindi, and National Agricultural Research Centre (NARC), Islamabad, Pakistan.

Collection of test crops: Six test crops namely *Triticum aestivum, Hordeum vulgare, Medicago sativa, Trifolium* spp*., Raphanus sativus, Trigonella foenum-graecum* were used to examine the allelopathic effects of leaf aqueous extracts of *Melilotus indica, Medicago polymorpha, Elusine indica* and *Fumaria indica.* The seeds of test crops were purchased from NARC, Islamabad, Pakistan.

Preparation of leaf aqueous extracts of weeds: Collected leaves were washed several times, oven dried, ground, and then stored in dark place at room temperature (25 ± 3°C) in glass jars until used. To obtain extracts, each 25 grams of shaded-dry leaves of weed species were soaked in 100 ml distilled water in a conical flask and mixed in blender to make it homogeneous. Aqueous extracts were centrifuged for few minutes at orbital shaker (2000 rpm), then filtered material was diluted with distilled water to make 10, 20, 30 and 40% aqueous extracts, based on weight by volume (W/V), of four weeds considered as stock solution. Five sterilized seeds of all crops were placed in each petri dish lined with Whatman No.1 filter paper soaked with 5 ml volume of aqueous extract, concentration 10, 20, 30 and 40% of each weed species. Experiment was arranged in completely randomized design (CRD) with three replications. Finally, petri dishes were placed at room temperature (25 ±3°C) for incubation. Afterward, each petri dish was irrigated on alternate days with respective dosages, whereas distilled water was used as control treatment. Seeds with 3.0 mm radicle were considered as germinated. Germination percentage was calculated on daily basis up to maximum height i.e. 14 days after planting. Seedlings were removed from petri dishes for the assessment of radicle and hypocotyl growth at 21th day after planting.
Following parameters were examined as:

Data collection: Germination test was conducted after 72 hours of incubation period. Germination percent was calculated through counting of germinated seeds on total number of seeds sown over 3 weeks period. Germination percentage was calculated by the following formula:

$$\text{Germinaton percentage} = \frac{\text{Germinated seed}}{\text{Total number of seed sown}} \times 100$$

Radicle length was measured manually by using scale in millimeter (mm) and data was shown in percentage of control. After 72 hours of incubation, length of radicle of three germinated seeds, in each dish, was recorded and then means were calculated. These lengths were expressed as percent growth in terms of control by the formula:

$$\text{Radicle length (\%)} = \frac{\text{Average length of radicle at particular treatment}}{\text{Average length of radicle at control}} \times 100$$

Hypocotyl length was calculated manually using scale in mm and data was expressed in percentage of control. After 72 hours of incubation, length of hypocotyl of three germinated seeds, in each dish, was recorded and means were determined. These lengths were expressed as percent growth in terms of control by the formula:

$$\text{Hypocotyl length (\%)} = \frac{\text{Average length of hypocotyl at particular treatment}}{\text{Average length of hypocotyl at control}} \times 100$$

Leaf debris method: Greenhouse experiment was conducted to further validate the effects of dominant allelopathic weed viz *Melilotus indica* on germination percentage, shoot and root length of four test crops, namely *Medicago sativa, Trifolium* spp*., Hordeum vulgare* and *Triticum aestivum.*

Methodology: Fresh and mature leaves of *Melilotus indica* were dried at 60°C for 48 hours and kept in polythene bags at room temperature until used**.** Each 300 g clay loam soil, additionally excavated from controlled conditions, was mixed with 0, 25, 50 and 75 g ground leaves of *Melilotus indica*. Ten seeds of each test crop were sown in each pot, having 9 cm diameter, properly irrigated with distilled water. All pots were placed in greenhouse under adjusted temperature at 30°C day and 20°C night to secure field-like conditions. Treatments were arranged in randomized complete block design (RCBD) with 3 replications. Pots were irrigated everyday with the consideration of moisture level of soil. Thinning was done after one week of planting when seedlings were established: only five plants were kept in each pot. Plants were harvested after 4 weeks of planting to examine shoot and root lengths.

Data collection: Germination percentage was calculated by same procedure as in aqueous extract method. Root and shoot length of receptor crop species were measured as explained by Zahid *et al.,* (2004).

Inhibition (%) was calculated by using the following formula:

$$\text{Inhibition (\%)} = 100 - \text{growth (\%)}$$

Statistical analysis

Average data of each replication was subjected to statistical analysis at 5% probability level using Statistics 8.1 software.

Results

Aqueous extract method: Allelopathic potential of the selected weed species on the growth of various crop plants under the concentration of 10 % aqueous extract.

Germination percentage: Germination percentage of the test crops was reduced significantly by leaf aqueous extracts of donor weeds at 10% concentration (Table 1). Among test crops, *H. vulgare* exhibited resistance in relation to germination percentage (79.01%), while *T. aestivum* was highly sensitive with germination percentage (66.22%). Among donor plants, maximum germination percentage (77.86%) was observed under aqueous extract of *F. indica*, which was therefore proved to be the lowest allelopathic weed species. Compared to this, there was a slight decrease in germination percentage (76.32, 74.05 and 63.20%) over aqueous extract of *E. indica, M. polymorpha* and *M. indica,* respectively. Interaction study revealed that there was a significant difference in germination percentage of the crop plants against the aqueous extracts of donor weeds. *Medicago sativa* was proved sensitive under the aqueous extract of *M. indica*, while *T. foenum-graecum* was proved a resistant species under aqueous extract of *E. indica*.

Radicle length: Radicle length showed significant differences under aqueous extracts of 4 weeds under 10% rate (Table 2). Among crop plants, *R. sativus* showed the maximum radicle growth (72.91%) indicating tolerance against donor plants, while *H. vulgare* showed a reduced radicle length (61.64%) indicating sensitivivity under aqueous extracts of weeds. *M. polymorpha* showed highest allelopathic effects on radicle growth (53.54%) of crops, while *E. indica* observed minimum allelopathic potential (82.22%) on radicle length. Comparative analysis expressed significant differences in the radicle growth of the plants when subjected to aqueous extracts of weeds. *F. indica* had no allelopathic effects on *Trifolium* spp. and *R. sativus*, as maximum radicle length (99.07, 94.79%) was observed. However, *E. indica* also showed minimum inhibitory effects on radicle growth (94.10 %) of *M. sativa*. *M. polymorpha* exerted highest allelopathic effect on radicle growth (42.27%) of *M. sativa*, followed by *M. indica* in case of *Trifolium* spp.

Hypocotyl length: *Hordeum vulgare* was proved to be a tolerant crop, whereas *T. aestivum* was sensitive in case of hypocotyl length under 10 % aqueous extract of four weed species (Table 3). *M. indica* was found to be a strong allelopathic weed species, while *F. indica* showed a minimum inhibition effect on hypocotyl growth of test crops. A significant interaction was found in comparative analysis of hypocotyl growth of test plants when subjected to aqueous extracts of donor species at 10% concentration, as *M. indica* showed strongest allelopathic effects on hypocotyl growth (53.04%) of *T. foenum-graecum*, whereas lowest effect was observed under *F. indica* on *T. foenum-aestivum* (87.64%).

Allelopathic potential of the selected weed species on the growth of various crop plants under the concentration of 20 % aqueous extract.

Germination percentage: Significant difference was observed regarding germination percentage of test crops as shown in Table 4. *Hordeum vulgare* proved highest resistant crop with maximum germination (83.74%) followed by *R. sativus* (80.27%) under aqueous extract of 20 % of four weeds, whereas *T. foenum-graecum* was found sensitive with lowest germination rate (50.29%). Among donor plants, *M. indica* showed minimum allelopathic effects on test crops exhibiting highest germination percentage (74.53%), while *F. indica* showed significant effects on germination percentage (62.06%) of six crops. Significant interactions were found between the germination percentage of crop species and aqueous extracts of the donor plants at 20%. *M. indica* showed minimum inhibitory effects on germination percentage (89.73, 89.70%) of *H. vulgare* and *R. sativus*, respectively. *F. indica* had strong allelopathic effects on *T. foenum-graecum* with minimum germination (44.93%). *F. indica* showed negative effects on germination percentage of all test crops under 20 %leaf aqueous extract.

Radicle length: Data regarding radicle length of test crops against aqueous extracts at 20% concentration of four weeds showed significant differences (Table 5). Among the crop plants, highest radicle length (65.49%) was recorded in *R. sativus*, which was statistically at par with *Trifolium* spp. *(*64.13%), whereas minimum length was observed in *M. sativa* (48.55%), which proved to be a sensitive crop against aqueous extract of four weeds. Among the donor plants, *M. indica* exhibited maximum inhibitory effects on radicle growth of test species, while minimum suppression of the radicle growth of crops was observed in *E. indica.* Comparative analysis showed significant fluctuations in the radicle length of crops under 20% concentration. *F. indica* showed a strong allelopathic effect on radicle growth of *M. sativa*, followed by *M. indica* in case of *Trifolium* spp. and *T. foenum-graecum. F. indica* showed least allelopathic effects on radicle growth of *Trifolium* spp. (95.23%) and *R. sativus* (92.38%), followed by *E. indica* in case of *Trifolium* spp. *M. indica* had strong allelopathic effects on radicle growth of all crop plants.

Table 1. Effect of aqueous extract of four weed species (10%) on the germination percentage (% of control) of six crop plants.

Test plants	Donor plants				
	Melilotus indica	***Medicago polymorpha***	***Elusin indica***	***Fumaria indica***	**Means**
Triticum aestivum	50.62 fg	66.05 cdefg	73.13 abcde	75.10 abcd	66.22* B
Hordeum vulgare	76.30 abcd	81.54 abc	82.52 abc	75. 68 abcd	79.01 A
Medicago sativa	49.10 g	84.06 abc	85.68 abc	81.93 abc	75.19 AB
Trifolium spp.	69.74 bcdefg	73.21 abcde	71.56 abcdef	75.23 abcd	72.43 AB
Raphanus sativus	68.65 bcdefg	58.54 defg	52.42 efg	89.82 ab	67.35 B
Trigonella foenum-graecum	64.79* cdefg	80.88 abc	92.58 a	69.44 bcdefg	76.92 AB
Means	**63.20 B**	**74.05 A**	**76.32 A**	**77. 86 A**	**-**

$LSD_{(0.05)}$ for Donor plants = 8.88, $LSD_{(0.05)}$ for Crop plants = 10.87, $LSD_{(0.05)}$ for Donor plants × Crops plants = 21.75
*Any two means carrying the same letter(s) in a column or row are non-significantly different at $p \leq 0.05$.

Table 2. Effect of aqueous extract of four weed species (10%) on the radicle growth (% of control) of six crop plants.

Test plants	Donor plants				
	Melilotus indica	***Medicago polymorpha***	***Elusin indica***	***Fumaria indica***	**Means**
Triticum aestivum	54.70 ef	57.57 def	77.41 bcd	62.84 def	63.13* AB
Hordeum vulgare	62.13 def	54.31 ef	86.55 abc	43.58 f	61.64 B
Medicago sativa	59.10 def	42.27 f	94.10 ab	72.39 cde	66.96 AB
Trifolium spp.	43.66 f	55.48 ef	84.44 abc	99.07 a	70.66 AB
Raphanus sativus	60.33 def	48.67 f	87.87 abc	94.79 ab	72.91 A
Trigonella foenum-graecum	46.86* f	62.95 def	62.95 def	85.18 abc	64.48 AB
Means	**54.46 B**	**53.54 B**	**82.22 A**	**76.31 A**	**-**

$LSD_{(0.05)}$ for Donor plants = 1.20, $LSD_{(0.05)}$ for Crop plants = 2.02, $LSD_{(0.05)}$ for Donor plants × Crops plants = 4.99
*Any two means carrying the same letter(s) in a column or row are non-significantly different at $p \leq 0.05$

Table 3. Effect of aqueous extract of four weed species (10%) on the hypocotyl growth (% of control) of six crop plants.

Test plants	Donor plants				
	Melilotus indica	***Medicago polymorpha***	***Elusin indica***	***Fumaria indica***	**Means**
Triticum aestivum	64.78 abcdef	80.31 abcd	63.37 abcdef	87.64 a	58.40* C
Hordeum vulgare	59.37 cdef	86.56 ab	68.76 abcde	78.01 abcde	81.90 A
Medicago sativa	65.70 abcdef	80.36 abcd	72.49 abc	63.16 abcdef	74.6 B
Trifolium spp.	68.69 abcde	82.51 abcd	70.03 abcde	87.29 ab	79.20 B
Raphanus sativus	62.21 bcdef	72.59 abcde	77.99 abcde	74.77 abcde	78.43 BC
Trigonella foenum-graecum	53.04* ef	68.56 abcde	59.16 def	77.37 ab	62.90 C
Means	**51.34 B**	**59.05 AB**	**60.57 AB**	**66.16 A**	**-**

$LSD_{(0.05)}$ for Donor plants = 2.88, $LSD_{(0.05)}$ for Crop plants = 2.99, $LSD_{(0.05)}$ for Donor plants × Crops plants = 5.88
*Any two means carrying the same letter(s) in a column or row are non-significantly different at $p \leq 0.05$

Table 4. Effect of aqueous extract of four weed species (20%) on the germination (% of control) of six crop plants.

Test plants	Donor plants				
	Melilotus indica	***Medicago polymorpha***	***Elusin indica***	***Fumaria indica***	**Means**
Triticum aestivum	77.160e	75.18 f	73.05 g	70.05 h	73.86* C
Hordeum vulgare	89.73 a	83.59 b	81.79 c	79.84 d	83.74 A
Medicago sativa	56.59 k	51.93 l	50.43 l	46.96 m	51.48 E
Trifolium spp.	76.97 e	69.41 h	64.64 i	60.37 j	67.85 D
Raphanus sativus	89.70 a	83.22 bc	77.93 e	70.24 h	80.27 B
Trigonella foenum-graecum	57.02* k	51.43 l	47.77 m	44.93 n	50.29 F
Means	**74.53 A**	**69.12 B**	**65.93 C**	**62.06 D**	**-**

$LSD_{(0.05)}$ for Donor plants = 1.00, $LSD_{(0.05)}$ for Crop plants = 0.85, $LSD_{(0.05)}$ for Donor plants × Crops plants = 1.68
*Any two means carrying the same letter(s) in a column or row are non-significantly different at $p \leq 0.05$

Hypocotyl length: Significant differences in hypocotyl growth of crop plants were observed (Table 6). *H. vulgare* was proved to be a resistant crop with maximum hypocotyl growth (81.90%), while *T. aestivum* proved highly sensitive with minimum growth (58.40%) against the aqueous extract of donor plants at 20% concentration. Among the donor plants, minimum hypocotyl growth (51.34%) was observed under aqueous extract of *M. indica*, whereas *F. indica* showed least inhibitory effects on hypocotyl growth (66.16%) of test plants. Significant interactions were found in hypocotyl length of crops against the aqueous extract of donor plants at 20% concentration. *M. indica* proved highest allelopathic weed in case of hypocotyl length (53.04%) of *T. foenum-graecum*. On the other hand, *F. indica* exhibited lowest allelopathic activity on hypocotyl growth (87.64%) of *T. aestivum*.

Allelopathic potential of the selected weed species on the growth of various crop plants under the concentration of 30 % aqueous extract.

Germination percentage: There was a significant difference in the germination percentage of crop plants against the aqueous extract of four weeds at 30% concentration (Table 7). *H. vulgare* proved a resistant crop species and exhibited maximum germination percentage (79.79%) followed by *R. sativus* (77.38%), whereas *T. foenum-graecum* was shown a sensitive species with minimum germination percentage (47.73%) against leaf aqueous extracts. *F. indica* showed strong inhibitory effects on germination percentage (58.88%), followed by *E. indica*. *M. indica* indicated minimum inhibition on germination percentage of the test plants. Comparative analysis revealed that *R. sativus* had a resistant potential against aqueous extract of *M. indica* with germination (86.81%), whereas lowest germination (42.37%) was recorded in *T. foenum-graecum* under 30% aqueous extract of *F. indica*.

Radicle length: Among test crops, *Raphanus sativus* was seen a resistant crop with maximum radicle growth (63.70%), whereas lowest radicle growth (45.34%) was recorded in *M. sativa*, which was statistically at par with *T. foenum-graecum* (46.69%) against 30% leaf aqueous extract of donor plants as shown in Table 8. *M. indica* showed a strong inhibitory effects on radicle growth whereas minimum inhibition was observed in *E. indica*. Comparative analysis stated that *M. polymorpha* strongly inhibited the radicle growth of *M. sativa*, whereas *F. indica* showed the minimum suppression of the radicle length of *Trifolium* spp. at 30% aqueous leaf extract.

Hypocotyl length: Data regarding hypocotyl growth of test crops exhibited significant variation when subjected to 30% aqueous extract of the donor plants (Table 9). Among crop plants, *T. foenum-graecum* was shown resistant in case of hypocotyl length, which was statistically at par with *M. sativa* while *Trifolium* spp. proved to be highly sensitive against allelopathic effects of weeds. *F. indica* had strong allelopathic activity against hypocotyl growth of test plants at 30% aqueous extract as compared to other donor plants. A significant interaction was observed under allelopathic potential of donor species on hypocotyl growth of the test species. Maximum inhibition was exhibited through *F. indica* on hypocotyl growth of *Trifolium* spp., whereas minimum inhibition was shown by *M. indica* on *T. foenum-graecum*.

Allelopathic potential of the selected weed species on the growth of various crop plants under the concentration of 40 % aqueous extract.

Germination percentage: Among the test plants, germination of *H. vulgare* was not affected under 40% leaf aqueous extract of four weeds, whereas minimum germination (45.42%) was recorded in *T. foenum-graecum* (Table 10). On the other hand, *F. indica* had a negative effect on germination percentage (56.42%) of all crops, and *M. indica* showed minimum allelopathic effects on germination percentage (68.88%) of test crops at 40% leaf aqueous extract. Interaction analysis demonstrated that aqueous extract of *M. indica* showed minimum allelopathic effects on germination percentage (83.44%) of *H. vulgare*, whereas *F. indica* was recorded as highest allelopathic weed species in case of *T. foenum-graecum* germination (40.06%).

Radicle length: Data regarding radicle length of six test crops against the aqueous extract of the donor weeds at 40% concentration showed a significant difference (Table 11). Among the test species, maximum radicle length (61.14%) was observed in *R. sativus* whereas minimum length (42.69%) was recorded in *M. sativa*, which was statistically at par with *T. foenum-graecum* (44.38%). *M. indica* reduced the radicle length significantly of all test plants, followed by *M. polymorpha.* A significant interaction were observed between allelopathic potential of aqueous leaf extracts of donor species and radicle length of six test plants. *F. indica* had minimum allelopathic effects on radicle length of *Trifolium* spp. (90.84%) and *R. sativus* (80.03%), whereas *F. indica* exhibited strongest allelopathic effects on radicle growth (15.63) of *M. sativa* followed by *M. indica* in case of *Trifolium* spp. (17.33%).

Hypocotyl length: Significant difference was observed in hypocotyl growth of the test plants when subjected to 40% leaf aqueous extract of donor weeds (Table 12). Among the test plants, hypocotyl length of *T. foenum-graecum* (55.09%) was not significantly affected by aqueous extract of weeds followed by *M. sativa*, while *Trifolium* spp. was shown a sensitive crop under 40% aqueous extract of donor plants. On the other hand, *F. indica* displayed strong inhibitory effects on hypocotyl length (33.42%), whereas *M. indica* showed least allelopathic effects on hypocotyl length (49.33%) of test crops followed by *M. polymorpha*. A significant interaction was noticed between allelopathic potential of leaf aqueous extracts of donor species and hypocotyl length of test plants, as *F. indica* indicated strongest allelopathic potential on hypocotyl growth (19.61%) of *Trifolium* spp., whereas *M. indica* displayed lowest allelopathic effects on hypocotyl length (73.79%) of *T. foenum-graecum*.

Table 5. Effect of aqueous extract of four weed species (20%) on the radicle growth (% of control) of six crop plants.

Test plants	Donor plants				
	Melilotus indica	***Medicago polymorpha***	***Elusin indica***	***Fumaria indica***	**Means**
Triticum aestivum	43.92 gh	53.79 efg	82.88 bcd	60.86 e	60.36* AB
Hordeum vulgare	40.33 h	50.65 efgh	91.46 abc	46.96 gh	57.35 B
Medicago sativa	58.46 ef	27.81 i	86.47 abcd	21.49 i	48.55 C
Trifolium spp.	21.71 i	48.17 fgh	91.41 abc	95.23 a	64.13 A
Raphanus sativus	49.72 efgh	39.39 h	80.49 cd	92.38 ab	65.49 A
Trigonella foenum-graecum	21.73* i	43.20 gh	52.83 efg	78.78 d	49.14 C
Means	**39.31 C**	**43.83 C**	**80.92 A**	**65.95 B**	**-**

$LSD_{(0.05)}$ for Donor plants = 4.6, $LSD_{(0.05)}$ for Crop plants = 5.64, $LSD_{(0.05)}$ for Donor plants × Crops plants = 11.02
*Any two means carrying the same letter(s) in a column or row are non-significantly different at $p \leq 0.05$

Table 6. Effect of aqueous extract of four weed species (20%) on the hypocotyl growth (% of control) of six crop plants.

Test plants	Donor plants				
	Melilotus indica	***Medicago polymorpha***	***Elusin indica***	***Fumaria indica***	**Means**
Triticum aestivum	64.78 abcdef	80.31 abcd	63.37 abcdef	87.64 a	58.40* A
Hordeum vulgare	59.37 cdef	86.56 ab	68.76abcde	78.01 abcde	81.90 C
Medicago sativa	65.70 abcdef	80.36 abcd	72.49 abc	63.16 abcdef	74.6 B
Trifolium spp.	68.69 abcde	82.51 abcd	70.03 abcde	87.29 ab	79.20 B
Raphanus sativus	62.21 bcdef	72.59 abcde	77.99 abcde	74.77 abcde	78.43 BC
Trigonella foenum-graecum	53.04 *ef	68.56 abcde	59.16 def	77.37 ab	62.90 A
Means	**51.34 B**	**59.05 AB**	**60.57 AB**	**66.16 A**	**-**

$LSD_{(0.05)}$ for Donor plants = 1.70, $LSD_{(0.05)}$ for Crop plants = 2.08, $LSD_{(0.05)}$ for Donor plants × Crops plants = 4.16
*Any two means carrying the same letter(s) in a column or row are non-significantly different at $p \leq 0.05$

Table 7. Effect of aqueous extract of four weed species (30%) on the germination (% of control) of six crop plants.

Test plants	Donor plants				
	Melilotus indica	***Medicago polymorpha***	***Elusin indica***	***Fumaria indica***	**Means**
Triticum aestivum	73.64 ef	71.66 g	69.53 h	66.53 ij	70.34 * C
Hordeum vulgare	85.78 a	79.64 b	77.84 c	75.89 d	79.79 A
Medicago sativa	54.14 m	49.48 n	47.98 n	44.51 o	49.03 E
Trifolium spp.	73.23 fg	65.67 j	60.90 k	56.63 l	64.11 D
Raphanus sativus	86.81 a	80.33 b	75.04 de	67.35 i	77.38 B
Trigonella foenum-graecum	54.46* m	48.87 n	45.21 o	42.37 p	47.73 F
Means	**71.34 A**	**65.94 B**	**62.75 C**	**58.88 D**	**-**

$LSD_{(0.05)}$ for Donor plants = 0.69, $LSD_{(0.05)}$ for Crop plants = 0.84, $LSD_{(0.05)}$ for Donor plants × Crops plants = 1.68
*Any two means carrying the same letter(s) in a column or row are non-significantly different at $p \leq 0.05$

Table 8. Effect of aqueous extract of four weed species (30%) on the radicle growth (% of control) of six crop plants.

Test plants	Donor plants				
	Melilotus indica	***Medicago polymorpha***	***Elusin indica***	***Fumaria indica***	**Means**
Triticum aestivum	41.58 gh	51.45 efg	80.54 bcd	58.52 e	58.02* BC
Hordeum vulgare	37.36 h	47.68 efgh	86.15 abc	43.99 fgh	54.38 C
Medicago sativa	55.25 ef	24.60 i	83.26 abcd	18.28 i	45.34 D
Trifolium spp.	19.78 i	46.23 fgh	89.47 abc	93.29 a	62.19 AB
Raphanus sativus	47.93 efgh	37.60 h	78.70 cd	90.59 ab	63.70 A
Trigonella foenum-graecum	19.28* i	40.75 gh	50.38 efg	76.33 d	46.69 D
Means	36.86 C	41.38 C	78.47 A	63.50 B	-

$LSD_{(0.05)}$ for Donor plants = 4.60, $LSD_{(0.05)}$ for Crop plants = 5.64, $LSD_{(0.05)}$ for Donor plants × Crops plants = 11.27
*Any two means carrying the same letter(s) in a column or row are non-significantly different at $p \leq 0.05$

Table 9. Effect of aqueous extract of four weed species (30%) on the hypocotyl growth (% of control) of six crop plants.

Test plants	Donor plants				
	Melilotus indica	***Medicago polymorpha***	***Elusin indica***	***Fumaria indica***	**Means**
Triticum aestivum	37.63 ij	36.49 jk	33.37 kl	29.11 mn	34.15* C
Hordeum vulgare	51.42 de	47.19 f	42.79 gh	36.77 jk	44.54 B
Medicago sativa	62.13 bc	59.49 c	55.05 d	49.07 ef	56.43 A
Trifolium spp.	31.67 lm	28.75 mn	25.85 no	22.06 o	27.08 D
Raphanus sativus	51.78 de	47.22 f	41.20 hi	36.08 jk	44.07 B
Trigonella foenum-graecum	76.10* a	65.11 b	46.22 fg	42.17 gh	57.40 A
Means	**51.79 A**	**47.37 B**	**40.75 C**	**35.88 D**	**-**

$LSD_{(0.05)}$ for Donor plants = 1.70, $LSD_{(0.05)}$ for Crop plants = 2.08, $LSD_{(0.05)}$ for Donor plants × Crops plants = 4.16
*Any two means carrying the same letter(s) in a column or row are non-significantly different at $p \leq 0.05$

Table 10. Effect of aqueous extract of four weeds species (40%) on the germination (% of control) of six test plants.

Test plants	Donor plants				
	Melilotus indica	***Medicago polymorpha***	***Elusin indica***	***Fumaria indica***	***Melilotus indica***
Triticum aestivum	71.19 ef	69.21 g	67.08 h	64.08 i	67.89* C
Hordeum vulgare	83.44 a	77.30 b	75.50 c	73.55 d	77.45 A
Medicago sativa	51.49 l	46.83 m	45.33 m	41.86 n	46.38 E
Trifolium spp.	76.97 e	63.22 i	58.45 j	54.18 k	61.66 D
Raphanus sativus	70.78 fg	77.77 b	72.48 de	64.79 i	74.82 B
Trigonella foenum-graecum	52.15 l	46.56 m	42.90 n	40.06 o	45.42 F
Means	**68.88 A**	**63.48 B**	**60.29 C**	**56.42 D**	**-**

$LSD_{(0.05)}$ for Donor plants = 0.69, $LSD_{(0.05)}$ for Test plants = 0.84, $LSD_{(0.05)}$ for Donor plants × Tests plants = 1.68
*Any two means carrying the same letter(s) in a column or row are non-significantly different at $p \leq 0.05$

Table 11. Effect of aqueous extract of four weeds species (40%) on the radicle growth (% of control) of six test plants.

Test plants	Donor plants				
	Melilotus indica	***Medicago polymorpha***	***Elusin indica***	***Fumaria indica***	**Means**
Triticum aestivum	39.13 gh	49.00 efg	78.09 bcd	56.07 e	55.57* AB
Hordeum vulgare	35.02 h	45.34 efgh	86.15 abc	41.65 fgh	52.04 B
Medicago sativa	52.60 ef	21.95 i	80.61 abcd	15.63 i	42.69 C
Trifolium spp.	17.33 i	43.78 fgh	87.02 abc	90.84 a	59.74 A
Raphanus sativus	45.37 efgh	35.04 h	76.14 cd	88.03 ab	61.14 A
Trigonella foenum-graecum	16.97* i	38.44 gh	48.07 efg	74.02 d	44.38 C
Means	**34.40 C**	**38.92 C**	**76.01 A**	**61.04 B**	**-**

$LSD_{(0.05)}$ for Donor plants = 4.60, $LSD_{(0.05)}$ for Test plants = 5.64, $LSD_{(0.05)}$ for Donor plants × Tests plants = 11.07
*Any two means carrying the same letter(s) in a column or row are non-significantly different at $p \leq 0.05$

Table 12. Effect of aqueous extract of four weeds species (40%) on the hypocotyl growth (% of control) of six test plants.

Test plants	Donor plants				
	Mililotus indica	***Medicago polymorpha***	***Elusine indica***	***Fumaria indica***	**Means**
Triticum aestivum	35.183 ij	34.047 jk	30.927 kl	26.660 mn	31.70 C
Hordeum vulgare	49.087 de	44.857 f	40.457 gh	34.437 jk	42.20 B
Medicago sativa	59.487 bc	56.840 c	52.400 d	46.427 ef	53.78 A
Trifolium spp.	29.220 lm	26.307 mn	23.400 no	19.617 o	24.63 D
Raphanus sativus	49.227 de	44.663 f	38.643 hi	33.523 jk	41.51 B
Trigonella foenum-graecum	73.793 a	62.803 b	43.913 fg	39.863 gh	55.09 A
Means	**49.33 A**	**44.91 B**	**38.29 C**	**33.42 D**	**-**

$LSD_{(0.05)}$ for Donor plants = 1.70, $LSD_{(0.05)}$ for Test plants = 2.08, $LSD_{(0.05)}$ for Donor plants × Tests plants = 4.16
*Any two means carrying the same letter(s) in a column or row are non-significantly different at $p \leq 0.05$

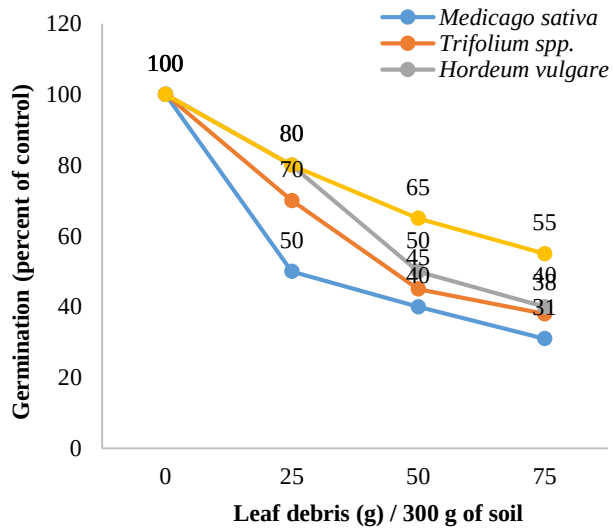

Fig. 1. Sensitivity of test plants to the incorporated leaf debris of *M. indica* in terms of their germination percentage.

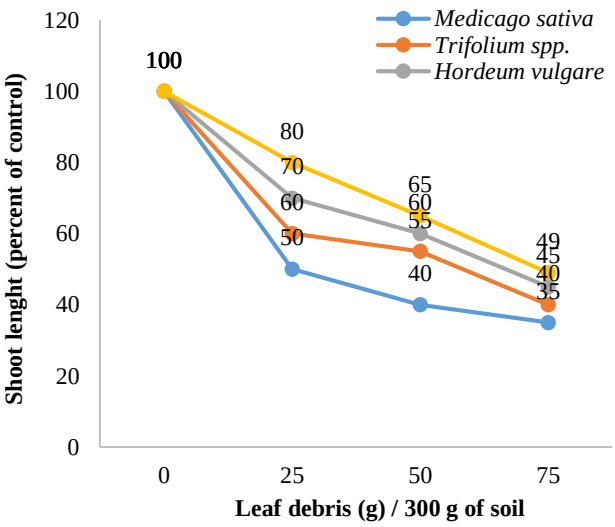

Fig. 2. Sensitivity of test plants to the incorporated leaf debris of *M. indica* in terms of their shoot length.

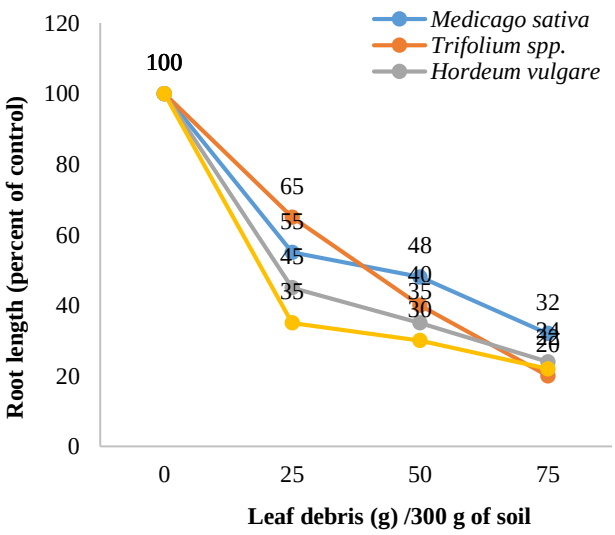

Fig. 3. Sensitivity of test plants to the incorporated leaf debris of *M. indica* in terms of their root length.

Leaf debris method

Germination percentage: Results presented in Fig. 1 show the effects of incorporated debris material of *M. indica* on germination percentage of four crop plants. The germination of all test crops was reduced when leaf debris of *M. indica* was mixed with clay loam soil. Generally, the rate of reduction in germination percentage was quite concentration dependent. Compared to control, germination percentage was recorded as 50~31% in *M. sativa*, 70~38% in *Trifolium* spp., 80~40% in *H. vulgare* and 80~55% in *T. aestivum*, when leaf debris was applied at the rate of 25~75 g per 300 g of soil. *M. sativa* and *Trifolium* spp. showed higher sensitivity on their germination percentage under leaf debris of *M. indica* compared to the *H. vulgare* and *T. aestivum*. The germination percentage was reduced between 50~40% in *M. sativa*, 70~45% in *Trifolium* spp., 80~50% in *H. vulgare* and 80~65% in *T. aestivum* when amount of applied leaf debris was raised from 25 to 50 g per 300 g of soil (Fig. 1). When 75 g of leaf debris was mixed with soil, highest reduction in germination (31%) was recorded in *M. sativa*, whereas *M. indica* showed minimum allelopathic effects on germination (55%) of *T. aestivum* among four species. Thus, overall leaf debris of *M. indica* exhibited selective behavior towards different crop species.

Shoot length: Results presented in Fig. 2 show the effects of incorporated leaf debris of *M. indica* on the shoot length of four test crops. The shoot length of all test crops was halted under all concentrations when soil was mixed with leaf debris of *M. indica*. Generally, the rate of reduction in shoot length was also concentration dependent. Compared to control, shoot length was recorded as 50~35% in *M. sativa*, 60~40% in *Trifolium* spp., 70~45% in *H. vulgare* and 80~49% in *T. aestivum* when leaf debris was applied at the rate of 25~75 g per 300 g of soil. *T. aestivum* and *Trifolium* spp. showed the highest sensitivity regarding shoot length growing with leaf debris of *M. indica* compared to *H. vulgare* and *M. sativa*. The shoot length reduced between 50~40% in *M. sativa*, and 60~55% in *Trifolium* spp., 70~60% in *H. vulgare* and 80~65% in *T. aestivum*, when amount of applied leaf debris was raised from 25 to 50 g per 300 g of soil (Fig. 3). When 75 g of leaf debris was mixed with soil, minimum shoot length (35%) was observed in *M. sativa*, whereas highest shoot length (49%) was recorded in *T. aestivum*. Thus, leaf debris of *M. indica* exhibited selective behavior over crop species.

Root length: Results presented in Figure 3 showed the effects of incorporated leaf material of *M. indica* on the root length of four test species. The root length of all species was inhibited under all concentrations when the soil was mixed with the leaf debris of *M. indica*. Generally, the rate of reduction in root length is also concentration dependent. Compared to control, root length was recorded as 55~32% in *M. sativa*, 65~20% in *Trifolium* spp., 45~24% in *H. vulgare* and 35~22% in *T. aestivum* when leaf debris was applied at the rate of 25~75 g per 300 g of soil. *T. aestivum* and *Hordeum vulgare* showed higher sensitivity regarding root length

growing with leaf debris of *M. indica* compared to *Trifolium* spp. and *M. sativa*. The root length was reduced between 55~48% in *M. sativa*, and 65~40% in *Trifolium* spp., 45~35% in *H. vulgare* and 35~30% in *T. aestivum* when amount of applied leaf debris was raised from 25 to 50 g per 300 g of soil. When 75 g of leaf debris was mixed with soil, the highest reduction in root length (20%) was recorded in *Trifolium* spp., whereas *M. sativa* showed the highest root length (32%) among four species.

Discussion

Aqueous extract method: In present experiment, leaf aqueous extract of donor weed species (*Melilotus indica, Medicago polymorpha, Elusine indica* and *Fumaria indica*) showed inhibitory effects on seed germination, radicle and hypocotyl growth of *Triticum aestivum, Hordeum vulgare, Medicago sativa, Trifolium* spp., *Raphanus sativus* and *Trigonella foenum-graecum*. Phenolics compounds (caffeic acid, ferulic acid, vanilic acid, chlorogenic acid, gallic acid) present in allelopathic weeds mainly contributed in the inhibition of metabolic processes during early growth stages of test plants (Kuiters, 1989; Muzaffar *et al.,* 2012). Present results are in accordance with the findings of Raza Ullah *et al.,* (2013), who concluded that leaf aqueous extract of *Fumaria indica* showed strong allelopathic effect on germination, root and shoot growth of wheat, lentil and chickpea. *Melilotus indica* showed strong allelopathic effect on wheat germination and initial growth as compared to *Medicago denticulata* and *Chenopodium album*; this might be due to the presence of higher amount of courmarin and metabolic compounds in *M. indica* (Einhelling & Suoza, 1992; Aaradhana *et al.,* 2017). *Melilotus indica* had strong allelopathic potential in suppression of radicle growth (Weston & Duke, 2003). Leaf aqueous extracts of *Medicago polymorpha* and *Portulacastrum* suppress the mitotic activity of newly born cells and inhibit the germination percentage, root, shoot and radicle length of *Triticum aestivum* (Mubeen *et al.,* 2011; Khan *et al.,* 2012). El-Darier *et al.,* (2014) studied the inhibitory effects of *Medicago polymorpha* on the biomass of *T. aestivum.* Present results agree with previous findings (Kumbhar & Shah, 2012; Anwar *et al.,* 2016).

Leaf debris method: Leaf debris of *Melilotus indica* exhibited selective behavior towards different crop species. Allelochemicals released from leaf debris of *M. indica* reduced the germination, shoot and root growth of *Medicago sativa, Trifolium* spp., *Hordeum vulgare* and *Triticum aestivum.* This inhibition might be attributed due to higher amount of water soluble phytoxins present in soil released from plant debris material, generally delaying or stopping the seed germination and cell enlargement of test plants (Rice, 1984; Basharat *et al*., 2017). However, the degree of inhibition was concentration dependent. Present results are in line with the finding of Alam *et al.,* (2011), who concluded that M*elilotus indica* contained leachable allelochemicals inhibiting seed germination, seedling growth and root length of associated crops. Debris material of *Melilotus indica* reduced the seedling growth and vigor of wheat, ultimately reducing yield (Farooq *et al.,* 2011; Mousavi *et al.,* 2013). Similar results were found by Shaukat & Siddiqui (2001), Jafariehyazdi & Javidfar (2011), Ullah *et al.,* (2013).

Conclusion

Present experiment demonstrated the allelopathic effects of *Melilotus indica, Medicago polymorpha, Elusine indica and Fumaria indica* on germination, radicle and hypocotyl growth of six crops, namely *Triticum aestivum, Hordeum vulgare, Medicago sativa, Trifolium* spp., *Raphanus sativus* and *Trigonella foenum-graecum.* All examined parameters showed pronounced poor growth under leaf aqueous extracts of different donor species, whereas *Melilotus indica* proved a stronger allelopathic weed species as its plant parts can also be used directly in assessing the allelopathic activity in weed control system. In present investigation, selected donor plants, especially *M. indica,* showed a promising future in terms of their allelopathic potential, which must be further studied for their selective behavior on particular crops for the development of bio-herbicides in weed management program.

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