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WATER SOLUBLE PHENOLICS IN FIVE WINTER SEASON LEGUMINOUS WEEDS AND THEIR PHYTOTOXICITY AGAINST WHEAT

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ABSTRACT

Weeds cause losses to crops by exerting their allelopathic effect through the release of leachates and decomposition of their residues in field. The present study was conducted to assess the allelopathic effect of aqueous extracts at different concentrations [2.5% (w/v) and 5% (w/v)] and residues with different decomposition durations (0, 15 and 30 days) at 2% (w/w) concentration of five winter season leguminous weeds viz. Medicago polymorpha, Lathyrus aphaca, Melilotus indica, Trigonella polycerata and Vicia sativa against germination and seedling growth of wheat. Aqueous extracts and residues of weeds suppressed wheat germination/emergence and seedling growth in differential pattern. Compared with control, maximum reduction in germination percentage (78.4%), germination index (86.2%), root length (88.6%) and seedling vigor index (SVI) (97.4%) of wheat was shown by 5% aqueous extracts of M. indica. However, 5% aqueous extracts of L. aphaca, M. polymorpha and V. sativa produced significantly lower shoot length (9.53 cm), seedling dry weight (14.72 mg) and higher mean germination time (3.98 d), respectively. Among weed residues, T. polycerata, V. sativa and M. polymorpha showed lowest emergence percentage (35%), emergence index (0.73), SVI (398.5); root (15.72 cm) and shoot length (7.25 cm); and seedling dry weight (16.2 mg) of wheat, respectively. The differential suppressive phytotoxic ability of weeds tested seem to be due to presence of phenolic compounds namely 4-hydroxy-3-methoxy benzoic, chlorogenic, caffeic, gallic, ferulic, p-coumaric, m-coumaric, syringic and vanillic acids in different concentrations as detected by HPLC analysis of their aqueous extracts.

Key words: Allelopathy, leguminous weeds, water extract, weed residues, wheat, germination, seedling growth.

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INTRODUCTION

Bur clover (*Medicago polymorpha* L.), yellow vetchling (*Lathyrus aphaca* L.), Indian sweet clover (*Melilotus indica* L.), trefoil (*Trigonella polycerata* L.) and common vetch (*Vicia sativa* L.) are winter annual leguminous weeds belonging to family Fabaceae. All of these are widely distributed in temperate as well as tropical regions of world including Europe, Australia, the United States, Africa and many Asian countries including India and Pakistan. These are associated with wheat, barley, lentil, gram and many winter season vegetables. However, these are abundantly observed in wheat fields (Hussain *et al.*, 1985). These weeds cause considerable losses in wheat. Reports have demonstrated allelopathic influence of *M. polymorpha* (Khan *et al.*, 2012), *L. aphaca* (Om *et al.*, 2002), *M. indica* (Alam *et al.*, 2001) and *V. sativa* (Mukhtar and Bajwa, 2011; Zohaib *et al*., 2014a) and *T. polycerata* (Zohaib *et al*., 2014b) on germination, growth and development of different plants.

Weeds interact with crops though direct or indirect allelopathic interactions. Allelopathy is any process whereby secondary metabolites produced by plants, microorganisms, viruses, and fungi influence the growth and development of agricultural and biological systems, including positive and negative effects (Torres *et al*., 1996). The nature of allelopathic interaction depends on the concentration of allelochemicals (Afridi and Khan, 2014). Higher concentrations of allelopathic compounds pose inhibitory (Afridi and Khan, 2015) and lower concentrations exert stimulatory allelopathic influence on germination and plant growth attributes (Hossain and Alam, 2010). Similarly, allelopathic effect exerted by weeds differs with difference in weed species (Verma and Rao, 2006). Plants liberate allelochemicals from leaves, shoots, roots and rhizomes mainly through leaching, decomposition of residues, exudation and volatilization (Zohaib *et al*., 2016). Allelochemicals released from the plants affect recipient plants mainly at germination and seedling growth (Oyerinde *et al*., 2009). Allelopathic effects of water extracts of numerous weeds have been observed on crops (Sayed *et al*., 2012; Yasin *et al*., 2012; Zohaib *et al*., 2014a,b). Similarly, allelopathic effect of decomposing plant materials on recipient plants has been documented by many reports (Ismail and Siddique, 2012; Omezzine *et al.*, 2011). In field conditions, the release of allelochemicals mostly occurs from plant residues (Singh *et al.*, 2001). The release and effectiveness of

allelochemicals is affected by the duration of decomposition of plant residues in soil (Teerarak *et al.*, 2010).

Allelopathic effect of a large number of weeds has been documented on wheat (Khan *et al.* 2009; Tanveer *et al.*, 2010; Majeed *et al.*, 2012) however; no information is available regarding the allelopathic effect of winter leguminous weeds on wheat. Therefore, the present study was conducted to determine the individual allelopathic effect of water extracts of *M*. *polymorpha*, *L*. *aphaca*, *M*. *indica*, *T*. *polycerata* and *V*. *sativa* at different concentrations, and soil residues of same weeds with varying decomposition durations on germination/emergence and seedling growth of wheat.

MATERIALS AND METHODS

Two year studies were carried out to ascertain allelopathic effect of five winter weeds *viz*., *M*. *polymorpha*, *L*. *aphaca*, *M*. *indica*, *T*. *polycerata* and *V*. *sativa* on the germination and seedling growth of wheat in Department of Agronomy, University of Agriculture Faisalabad, Pakistan during winter season of 2012 and 2013. Whole plants of *L*. *aphaca*, *M*. *polymorpha*, *M*. *indica*, *V*. *sativa* and *T*. *polycerata* were uprooted at maturity from wheat fields. The uprooted plants were first dried at room temperature and then in oven at 70°C for 24 hours. The dried plants were cut into small pieces ranging in size of 1-3 cm. These small pieces of weeds' plants were soaked in the distilled water for 24 hours in ratio of 1:20 (w/v) at room temperature to get their aqueous extracts. The water extracts were filtered with the help of sieve and then with Whattman No. 1 filter paper to get pure water extracts of whole weeds' plants. The water extracts obtained were poured in separate bottles, tagged and used for bioassays studies. These extracts were taken as stock solutions and further dilutions were made to conduct experiment at different concentrations.

Experiment 1: Influence of water extracts of some leguminous weeds on germination and seedling growth of wheat

During each year, experiment was conducted using water extracts of *M*. *polymorpha*, *L*. *aphaca*, *M*. *indica*, *T*. *polycerata* and *V*. *sativa* at 2.5% (1:40 w/v) and 5% (1:20 w/v) concentrations on wheat. Twenty five seeds of wheat were placed in Petri dishes (9 cm diameter) lined with double layer of filter papers, separately. In each Petri dish, 7 ml of water extracts and distilled water as control was poured pertaining to the treatments. Petri dishes were placed on laboratory shelves. Experiment was laid out in completely randomized design (CRD) with factorial arrangement and each treatment was replicated four times. The ambient laboratory temperature during the course of study ranged from 21.4-22.8° C. The experiment was carried

out for 16 days and the Petri dishes were kept moist during entire time span of study.

Experiment 2: Effect of weed residues of some leguminous weeds on emergence and seedling growth of wheat

During each year, residues of *M*. *polymorpha*, *L*. *aphaca*, *M*. *indica*, *T*. *polycerata* and *V*. *sativa* at 2% (w/v) concentration were incorporated in the soil with varying durations of decomposition *viz*. 0, 15 and 30 days. Soil without weeds residues was taken as control. Sowing of twenty five seeds of wheat was done in soil filled pots. Distilled water was used to moisten the soil during whole duration of experiment. Pots (18 cm diameter and 9 cm depth) were placed on laboratory shelves. Experiment was laid out in completely randomized design (CRD) with factorial arrangement and each treatment was replicated four times. The ambient laboratory temperature during the course of study ranged from 19.1-21.0° C. Emergence and seedling growth was noticed for 16 days.

Number of seeds germinated/emerged was counted daily by using the procedure of seedling evaluation in Handbook of Association of Official Seed Analysts (AOSA, 1990). When the radical was found to be 2 mm in length, the seed was counted as germinated. The germination/emergence percentage was calculated by using the formula:

$$GP = [NT \times 100]/N$$

where NT is the proportion of seeds germinated in each treatment for final measurement, and N is the total number of seeds used in bioassay.

The mean germination/emergence time was calculated using the formula of Ellis and Roberts (1981):

$$MGT = \Sigma Dn/\Sigma n$$

where N is the number of seeds which were germinated on day D, and D is the number of days counted from the beginning of germination.

The germination/emergence index was determined as per Association of Official Seed Analysts (AOSA, 1990) by using the formula:

$$GI = N1 / D1 + \ldots\ldots\ldots\ldots\ldots\ldots\ldots + NL / DL$$

Where N1 is the number of seeds germinated on 1st count, D1 is days to 1st count, NL is number of seeds germinated on last count, and DL is the days to last count.

At the end of experiment seedlings from each replication were uprooted, washed with water and dried using filter paper. The root and shoot length of seedlings from each replication was measured in centimeters. Oven drying of seedlings was done at 70°C and weighed till constant weight. The dry weight was expressed in millimeters. The

equation of Abdul-Baki and Anderson (1973) was used to calculate the seedling vigour index:

SVI = radicle length × germination percentage

Phenolics determination

For identification and quantification of suspected phytotoxins in tissues of all aforesaid weeds, their water extracts were analyzed chemically on the Shimadzu HPLC system (Model SCL-10A, Tokyo, Japan). The peaks were detected by UV detector. Standards of the suspected phytotoxins (Aldrich, St Louis, USA) were run similarly for their identification and quantification. The identified phenolics are listed in the Table-1 along with their concentrations. Concentrations of the isolated compounds were determined in parts per million (ppm) by the following equation and then converted to micro molar (µM):

$$\text{Concentration} = A_{sample}/A_{standard} \times C_{standard} \times \text{Dilution Factor}$$

where A_{sample} is area of sample, $A_{standard}$ is area of standard and $C_{standard}$ is concentration of standard.

Table-1. Types of phenolics identified and quantified in weeds

Phenolics	Weeds				
	M. polymorpha	*L. aphaca*	*M. indica*	*T. polycerata*	*V. sativa*
4-Hydroxy-3 -Methoxybenzoic acid	111.27 µM	-	-	173.18	418.26
Caffeic acid	-	98.36	71.99 µM	-	-
Chlorogenic acid		-	73.47 µM	-	-
Ferulic acid	-	-	69.16 µM	-	273.72
Gallic acid	-	29.63	-	-	-
m-Coumaric acid	20.65 µM	20.83	-	16.26 µM	-
p-Coumaric acid	27.47 µM	-	-	-	47.64
Syringic acid	-	22.24	-	32.95 µM	-
Vanillic acid	65.06 µM	-	-	-	-

Statistical analysis

A similar trend was observed during both the years of study for all the parameters therefore the data were pooled before statistical analysis and presented as mean of both the years. After pooling the data was analyzed using Fisher's analysis of variance technique and the least significant difference at 0.05 probability was used for the comparison of treatments' means (Steel *et al.*, 1997). Graphical representation of the data was carried out using MS-Excel.

RESULTS AND DISCUSSION

Germination bioassay

Wheat seed germination and seedling growth were depressed in a differential fashion by application of aqueous extracts of all weeds (Fig. 1). In comparison with distilled water treated control (DWTC), the highest decline in germination percentage (78.4%), germination index (86.2%), seedling root length (88.6%) and seedling vigor index (97.4%) of wheat was produced by 5% water extract of *M*. *indica* having values of 20%, 0.96, 1.81 cm and 38.45, respectively. However, germination index remained statistically similar with *V*. *sativa*, *M*. *polymorpha* and *L*. *aphaca*. Maximum delay in percent germination was observed in 5% aqueous extract of *V*. *sativa* (Figure 1). Maximum suppression of wheat seedling shoot was caused by 5% aqueous extract of *L*. *aphaca*. Significantly lower wheat seedling dry weight was recorded in treatment receiving 5% aqueous extract of *M*. *polymorpha* (Fig. 1).

Results indicate that germination and seedling growth of wheat was inhibited by weeds' aqueous extracts as compared to control. In present study it was noticed that germination and seedling growth of wheat was inhibited by extracts of all weeds; however, maximum inhibition was found by *M. indica* and *V. sativa* extracts. This might be due to the presence of phenolics in higher concentrations in these weeds as compared to others, as indicated by HPLC analysis (Table-2). Inhibition of germination by phenolics is attributed to the inhibition of respiration by interruption of respiratory enzymes and enzymes involved in pentose phosphate pathway in the germinating seeds (Muscolo *et al.*, 2001). The decrease in seedling growth results from reduced cell division and photosynthesis due to disruption of chlorophyll (Shao-Lin *et al.*, 2004). It has been revealed that phenolic compounds are responsible for inhibition of germination as well as plant growth (Yukiko *et al.*, 2001). In our study many phenolics were detected by HPLC analysis of water extracts of leguminous weeds (Table-6). The germination and growth hindering effect by plant phenolics *viz*. chlorogenic acid, caffeic acid, ferulic acid, vanillic acid, 4-hydroxy-3-methoxybenzoic acid, gallic acid and p-coumaric acid has

been described in literature (Rodzynkiewicz *et al.*, 2006; Muzaffar *et al.*, 2012). The results of our experiment are supported by Joshi *et al.* (2009) who observed inhibition of germination, shoot and root length and seedling dry weight of *T. aestivum* by water extracts of *Ageratum conyzoides*, *Chenopodium album*, *Cynodon dactylon* and *Parthenium hysterophorus*. Studies were conducted to assess the effect of root and shoot water extracts of wild barley on wheat with a result of reduction in percent germination, mean germination time, root length and shoot length of wheat (Hamidi and Ghadiri, 2011).

Inhibition of germination as well as seedling growth attributes of wheat was observed at both concentrations of water extracts of all weeds however; shoot length and seedling dry weight was promoted at lower extract concentration of some weeds *viz*. *M. polymorpha* and *V. sativa*. This might be due to the stimulatory effect of phenolics present in extracts of these weeds at lower concentrations as indicated by HPLC analysis (Table-1). These results are supported by Katoch *et al.* (2012a) who reported that water extracts of *Eupatorium adenophorum* and *A. conyzoides* posed inhibitory effect on germination attributes, radical length, plumule length and the seedling vigour index of *T. aestivum*, *O. sativa* and *Zea mays*. However, lower extract concentration of *E. adenophorum* stimulated the plumule and radical length of *T. aestivum* and *Z. mays*.

Pot study

Data relevant to various emergence and seedling growth attributes of wheat as influenced by soil incorporated residues of different weed species are presented in figure 2. It is shown by the data that residues of all weed species significantly suppressed wheat emergence and seedling growth when compared with control (Figure 2). *Trigonella polycerata* and *M. polymorpha* residues decomposed for 30 days after soil incorporation exerted highest inhibitory action on wheat seed emergence. Significantly lower emergence percentage (35%) and emergence index (0.73) were recorded with *T. polycerata* whereas significantly higher mean emergence time (7.9 days) was observed in *M. polymorpha* residues (Fig. 2). Regarding seedling growth of wheat, among weed species, *V. sativa*, *M. polymorpha* and *T. polycerata* residues showed maximum suppressive effect (Fig. 2). However, a significant increase in shoot and root lengths was noted with *L. aphaca* and *M. indica* residue incorporates decomposed for 15 days. Significantly lower shoot length (15.7 cm) and root length (7.3 cm) was noted in seedlings grown on soil incorporated residues of *V. sativa* decomposed for 15 and 0 days, respectively which were statistically at par with those observed in *M. polymorpha* decomposed for 30 and 0 days, respectively. However, significantly lower wheat seedling dry weight (16.2 mg) and seedling vigor index (398.5) were

produced by *M. polymorpha* and *T. polycerata* decomposed for 0 and 30 days, respectively (Fig. 2).

A significant reduction in emergence and growth of wheat seedlings was caused by soil incorporated weeds' residues. The inhibition of emergence and seedling growth of wheat might be due to release of allelochemicals from weeds' residues upon decomposition in soil. The results of our study are supported by Jalageri *et al.* (2010) who reported that residues of *Commelina benghalensis*, *P. hysterophorus*, *Cyperus rotundus* and *Prosopis juliflora* posed inhibitory influence on seedling length and seedling dry biomass of sorghum, groundnut and soybean. Katoch *et al.* (2012b) stated that residues of *E. adenophorum*, *A. conyzoides* and *Lantana camara* caused inhibition of germination attributes, seedling length and seedling weight of *T*. *aestivum*, *O*. *sativa* and *Z*. *mays*.

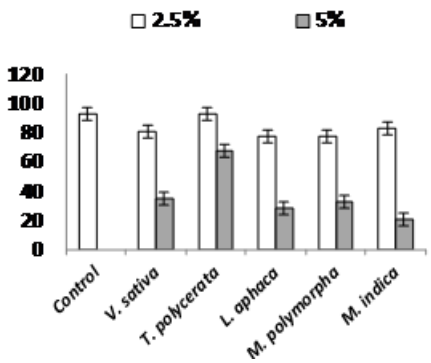
It was observed that maximum inhibition of wheat germination was caused by residues of *T. polycerata* and *M. polymorpha* while seedling growth was inhibited most by *V. sativa* which was at par with *T. polycerata* and *M. polymorpha*. This might be attributed to differential plant structure and residues decomposition pattern of different plant species (Alam and Shereen, 2002; Bonanomi *et al*., 2011). In present study, the seedling length and seedling dry weight of wheat was promoted by *M*. *indica*, *M*. *polymorpha* and *L*. *aphaca* residues with lower decomposition durations. This increase in growth attributes might be attributed to the release of plant growth promoting substances from the residues of these weeds that lead to the growth promotion of test crop (Salam *et al.*, 2011). The highest inhibition of emergence and growth of wheat seedlings was observed by residues of all weeds with highest decomposition durations. It was observed that the inhibition of germination and seedling growth of wheat was reduced by weed's residues without decomposition and then the inhibition was lowered followed by again inhibition by decomposition of residues up to 30 days. It might be due to the fact that in soil, the decomposition of allelochemicals takes place by microorganisms after their release from plant body with reduction in their activity with increasing duration but in some instances the decomposition products of allelochemicals get more toxic and hinder the growth and development with higher intensity with increasing duration of decomposition (Albuquerque *et al*., 2010). This might be due to enhanced accumulation of allelochemicals in the soil with increase in duration of decomposition which caused a decrease in germination and plant growth (Narwal *et al.*, 2005). Chou *et al.* (1976) reported 18 compounds released from decomposing residues of corn and 9 from rye residues in soil after 30 days of decomposition. Some of those allelochemicals were moderately toxic while others were highly toxic to

the growth of lettuce. An *et al.* (2001) also reported an increase in allelochemicals content from decaying *Vulpia myuros* residues with an increase in decomposition duration.

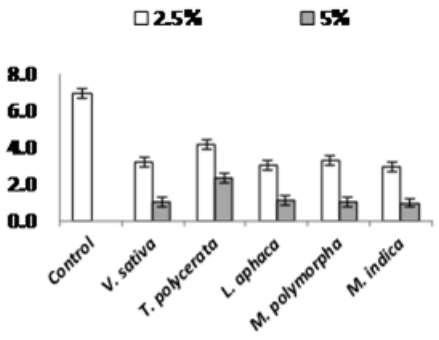
There was a differential allelopathic influence of weeds' aqueous extracts and residues on germination/emergence and growth of wheat seedlings. This might be due to the differential contact and uptake of allelochemicals by wheat seedlings from different weeds in both cases. A direct contact of allelochemicals with plants occur when applied as water extract while allelochemicals-plant interaction from decomposing residues depends upon various factors such as binding of allelochemicals to organic matter and clay particles in soil, leaching, physiochemical processes and microbial breakdown of allelochemicals (Albuquerque *et al.*, 2010).

CONCLUSION

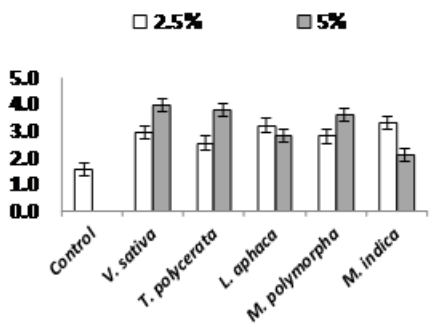
It is concluded from study that allelochemicals present in these weeds caused the allelopathic effect on crops and may cause losses to the associated crops through allelopathy. Therefore, these leguminous weeds should be eradicated from field at initial stages of crop growth to elude their harmful effect.



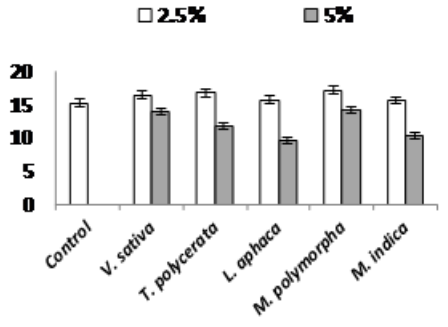
(a) Germination Percentage



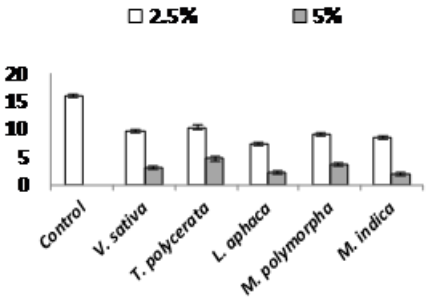
(b) Germination Index



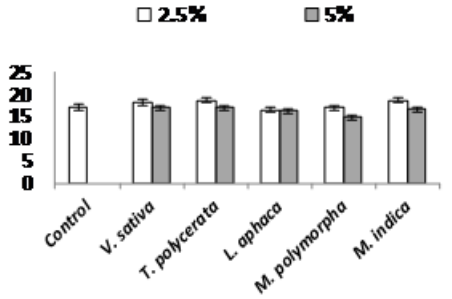
(c) Mean Germination time (days)



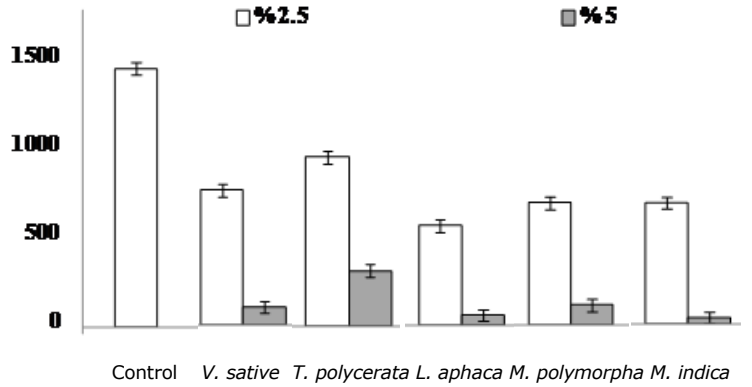
(d) Shoot Length (cm)



(e) Shoot Length (cm)

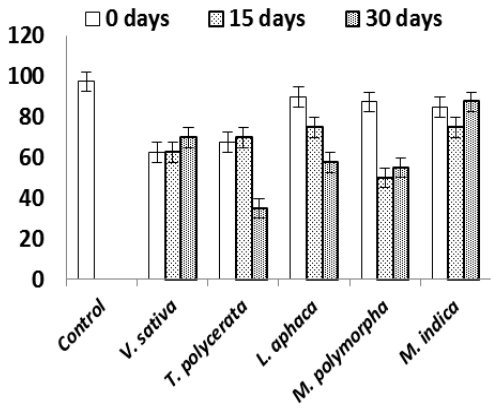


(f) Seedling dry weight (g)

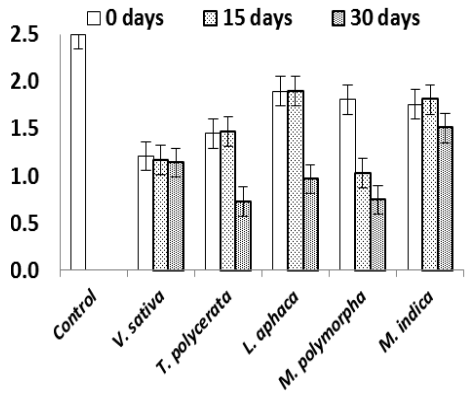


(g) Seedling vigour Index

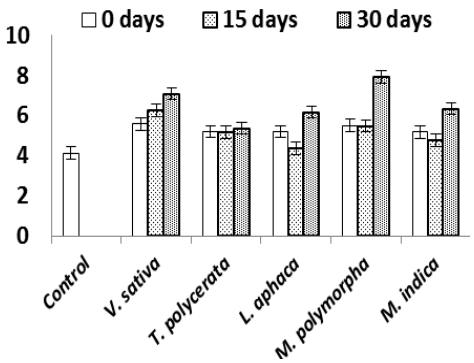
Figure 1 (a-g). Germination and seedling growth of wheat as effected by water extracts of five winter leguminous weeds



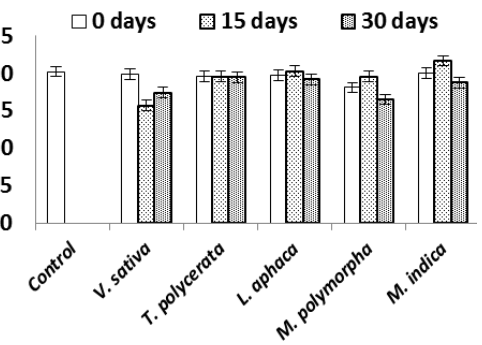
(a) Emergence Percentage



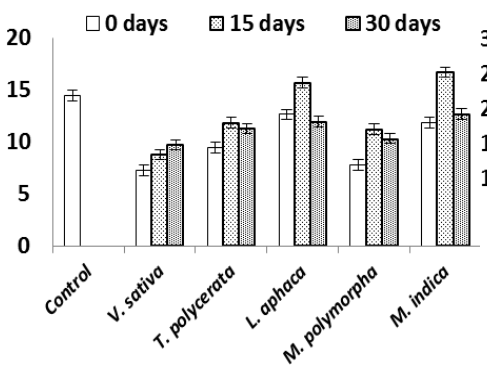
(b) Emergence Index



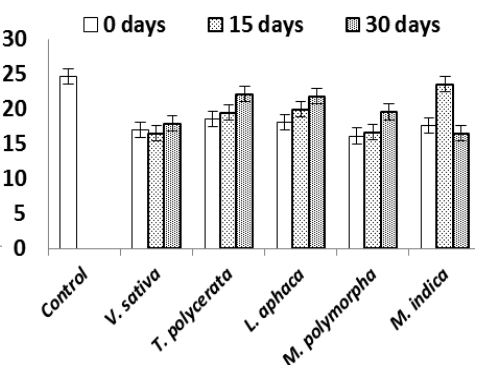
(c) Mean emergence time (days)



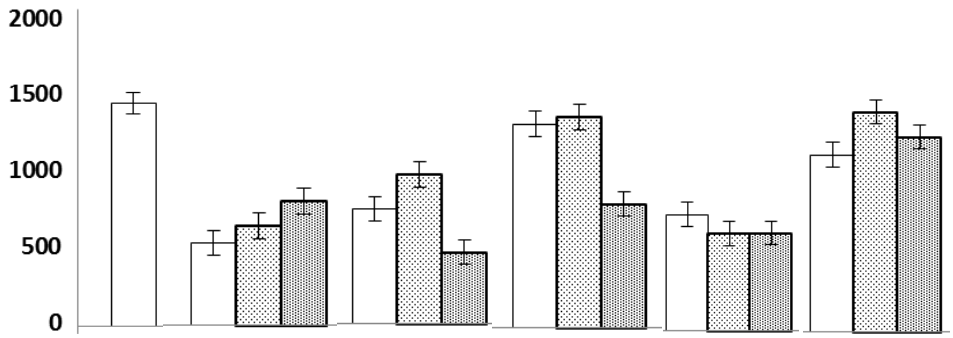
(d) Shoot Length (cm)



(e) Shoot Length (cm)



(f) Seedling dry weight (g)



(g) Seedling vigor index

Figure 2 (a-g). Germination and seedling growth of wheat as effected by soil residues of five winter leguminous weeds

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