Allelopathic Effect of Lemon Balm on Germination and Growth of Pea, Safflower and Wheat

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ABSTRACT: In order to investigate the likely allelopathic potential of an aqueous extract and powder of lemon balm (Melissa officinalis) on the germination and seedling growth of pea (Cicer arietinum), safflower (Carthamus tinctorius L.) and wheat (Triticum sativum), an experiment was carried out at the laboratory and greenhouse of agriculture faculty of Shahid Bahonar University of Kerman, Iran, from October 2009 to March 2010. A completely randomized design with three replicates using five extract concentrations of lemon balm plant including 0, 25, 50, 75, and 100 g l$^{-1}$ and four amounts of lemon balm powder including 0, 7.5, 15 and 30 g 2kg$^{-1}$ of soil was employed in laboratory and greenhouse experiments respectively. All extract concentrations of lemon balm except 25 g l$^{-1}$ inhibited pea and wheat seed germination significantly, but had no inhibitory effect on germination of safflower. The powder of mature lemon balm plants affected the fresh and dry weight and shoots elongation in these crops negatively compared with the control in all levels. Therefore use of this plant should be prevented in rotation or intercropping with these three crop plants. Further research conducted in the analytical laboratory as well as in the field is needed before a practical application of the extract and powder as weed inhibiting agent can be recommended.

Keywords: Allelopathy, lemon balm, germination, growth, pea, safflower, wheat

INTRODUCTION

Allelopathy arises from the release of chemicals by one plant species that affect other species in its vicinity, usually to their detriment. Defense agents, allelochemicals or allelopathins, are largely classified as secondary plant metabolites that play an important role in allelopathic interactions or plant defense and act as important ecological mechanisms (Rice, 1984). The effects of allelopathy on germination and growth of plants may occur through a variety of mechanisms including reduced mitotic activity in roots and hypocotyls, suppressed hormone activity, reduced rate of ion uptake, inhibited photosynthesis, respiration and protein formation, decreasing permeability of cell membranes and/or inhibition of enzyme action (Rice, 1984). The allelopathic characteristic of an allelochemical is defined as the biological property of the allelochemical as opposed to its physical or chemical properties (An et al., 1993). Allelochemicals are present in virtually all plant tissues, including leaves, flowers, fruits, stems, roots, rhizomes, seeds and pollen they may be released from plants into the environment by means of volatilization, leaching, root exudation, and decomposition of plant residues (Putnam and Tang, 1986). The avoidance of allelopathic effects between crops, or the
exploitation of beneficial interactions in a rotation or a mixed cropping system may have direct bearing on the crop yield. A number of plant species have been reported to possess allelopathic activity on the growth of other plant species (Duke et al., 2000). Lemon balm is widely used as aromatic, culinary and medicinal herb and much research has been conducted to evaluate the pharmacological effects of the plants (Tagashira and Ohtake, 1998; Van den Berg et al., 1997). The shoot powder of lemon balm inhibited the germination and growth of roots and shoots of *Amaranthus caudatus*, *Digitaria sanguinalis* and *Lactuca sativa* (Kato Noguchi, 2003). However, information about the allelopathic potential of this plant is limited. In many cases, allelochemicals move through soil and they may be transformed during movement, metabolized by soil microbes, or bound to soil organic matter. Allelochemicals toxicity may occur by microbes after entry into soil. Many studies on allelopathy, however, do not involve soil or involve an artificial soil substrate (Inderjit and Dakshini, 1995). Since the lemon balm as a medicinal crop is generally in rotation or intercropped with other crops in some parts of the country, assessment of its allelopathic potential seemed to be necessary. So the purpose of the present study was to elucidate the allelopathic potential of different levels of extract and powder of lemon balm on pea, wheat and safflower. Such information should be beneficial when planning to cultivate these crops with lemon balm as intercropping or having them in a rotational system.

**MATERIALS AND METHODS**

1. **Location:**
   Lemon balm plants were collected from Shahid Bahonar university fields (30˚ 17’ N, 57˚ 50’ E, 1754m altitude) in Kerman city, Iran, in July 2009. The experiment was carried out at the laboratory and greenhouse of the agricultural faculty, from October 2009 to March 2010.

2. **Experiment under laboratory condition:**
   The grown lemon balm was collected at maturity and dried under shade for a few days. The well dried plants were chopped into 1 cm long pieces and dried in an oven at 60˚C for 72 hours, then were pulverized. 100 g powder of lemon balm was extracted by soaking in 1 liter deionized water at 25˚C for 24 hours in a shaker to give a concentration of 100 g l⁻¹. The extract was filtered through four layers of cheesecloth to remove fiber debris and centrifuged at 3000 rpm for 4 hours (Chon et al., 2002). The supernatant was filtered again using a 0.2 mm filter ware unit. Fresh stock extract was kept in a refrigerator at 2˚C until used. Stock extract of lemon balm was diluted with sterile distilled water to give a final concentration of 0, 25, 50, 75 and 100 g l⁻¹. Seed germination tests were conducted with the extract as follows: 35 seeds of wheat (*Triticum sativum*) cultivar “Roshan”, 20 seeds of pea (*Cicer arietinum*) cultivar “Irani” and 50 seeds of safflower (*Carthamus tinctorius L.*) cultivar “IL-111” were surface sterilized with 5.25% (w/v) sodium hypochlorite solution for 15 min, rinsed three time with distilled water and were evenly placed on two-layer filter paper in sterilized 9-cm Petri dishes separately. The filter paper did not contain inhibitory compounds, because maximum seed germination was recorded when seed assayed using deionized water. 10 and 20 ml of each concentrations of the test plant were added to Petri dishes containing wheat and pea seeds respectively. The extract volume added to the Petri dishes containing safflower was equal to that of wheat. All Petri dishes were sealed to prevent the loss of moisture and avoid contamination, and then placed in a dark room at 25˚C. Treatments were arranged in a completely randomized design with three replications. Germination was determined by counting the number of germinated seeds at 24 hour intervals over a 6 day period and expressed as total percent germination. Germination was deemed to occur only after the radicle had protruded beyond the seed coat by at least 1 mm. Radicle length (RL), hypocotyl length (HL), mean germination time (MGT) and seed stamina index (SSI) of all three crops were measured 6 days after germination. Seedling dry weight (SDW) was determined by drying the plant material in an oven at 60˚C for 24 hours prior to weighting.

3. **Experiment under greenhouse condition:**
   Powder of the lemon balm plant was used in this pot experiment. The soil content of each pot was 2 kg. Additionally, four amounts of this plant powder including; 0, 7.5, 15 and 30 g/kg were mixed with the soil of each pot. The amount of 7.5 g powder per kg of soil applied was based on the lemon balm residue left on the field and obviously incorporated into the soil (depth of 25-30 cm) before cultivation of next crop. The other treatments were considered as the higher and lower amounts to be investigated. To ensure the decomposing and release the chemical substances of the plant powder into the soil, the moisture content of incorporated soil and powder was kept at the F.C. (Field Capacity) level for one month. Three seeds of each
crop (wheat, pea and safflower) were sown in each pot separately and the pots were irrigated based on the soil F.C. All three seedlings were kept until the end of the experiment. In addition to plant height, the shoot fresh and dry weights were measured at the end of experiment, 30 days after cultivation.

4. Data analysis:

Germination rate, mean germination time, inhibition percentage of germination and seed stamina index calculated for wheat, pea and safflower by following equations that were previously reported by others.

\[
G = \left( \frac{n}{N} \right) \times 100
\]

(Jefferson and Penachchio, 2003)

\[
RG = \frac{\sum (Ni/\text{Di})}{\text{MGT} = \frac{\sum (Ni \times \text{Di})}{\sum Ni}}
\]

(Jefferson and Penachchio, 2003)

(Khaled et al, 2007)

\[
SSI = \frac{[G \times (HL+RL)]}{100}
\]

(Abdul-baki and Anderson, 1970)

G: germination percentage, n: number of seeds germinated, N: number of seed planted, RG: rate of germination (seed day\(^{-1}\)), Ni: germinated seeds in each numeration, Di: day of each numeration, MGT: mean germination time (day), SSI: seed stamina index, HL: average of hypocotyls length (mm), RL: average of Radicles length (mm). The data processed using the GLM procedure of the Statistical Analysis System (SAS), (SAS Institute, 1990).

RESULTS AND DISCUSSION

Aqueous extract of lemon balm at concentration of 25 g l\(^{-1}\) did not exhibit any inhibitory effect on pea, wheat and safflower seed germination percentage compared to the control group. This trait decreased with increasing in extract concentration so that the highest concentration, 100 g l\(^{-1}\), resulted in a 93.34 and 51.43 percentage reduction in the germination of pea and wheat respectively. The two higher levels of extract (75 and 100 g l\(^{-1}\)) reduced germination percentage of wheat significantly (p<0.0001) compared with the control. None of the levels of lemon balm extract affected the germination percentage of safflower (p=0.3088) (Figure 1).

The germination rate of pea, wheat and safflower at the level of control were 18.62, 34.83 and 50 seed day\(^{-1}\), respectively. Application of 25 g l\(^{-1}\) of extract affected the germination rate of pea significantly and it decreased with higher concentrations (p<0.0001). Significant reduction in germination rate of wheat and safflower started with 50 (p=0.0008) and 75 g l\(^{-1}\) (p=0.0123) of extract, respectively. In general, the lowest germination rate was recorded at the highest extract concentration for these three crops (Figure 2).

The lowest mean germination time was recorded for the control group in all three crops. Rising levels of concentration resulted in a significant increase in this trait in pea (p=0.0103), wheat (p=0.0009) and safflower (p=0.0454). The amount of increase in MGT was highest in pea crop, fallowed by wheat and safflower. The effects of the first three levels of extract (25, 50 and 75 g l\(^{-1}\)) on MGT were the same in pea and were statistically-categorized in one group. MGT of wheat and safflower did not significantly differ at the level of 25 g l\(^{-1}\) compared with control (Figure 3).

In all three crops, the lowest and highest seed stamina index was belonged to the highest concentration of extract and control, respectively. Application of extract in all levels affected this trait significantly (p<0.0001) in test crops. Seed stamina index was almost zero at the highest level of concentration in all three crops. Compared with control treatment, the application of the first level of extract resulted in a reduction of 56.1%, 77.7% and 94.9% in SSI in pea, wheat and safflower, respectively (Figure 4).

The allelopathic effects of lemon balm extract on HL and RL of test crops are shown in Figure 5 and 6. The response of HL and RL to the applied levels of extract was almost the same. HL and RL of safflower was significantly affected (p<0.0001) by the extract application and appeared to stunt the growth of HL and RL of germinating so that the HL recorded for all concentrations used was zero. HL and RL of wheat and pea decreased with increasing concentration levels (p<0.0001).

SDW were affected significantly by the application of all the levels of extract in the test crops. The SDW of pea was reduced with increasing levels of extract (p<0.0001). The highest and lowest SDW was recorded for control and 100 g l\(^{-1}\) of extract, respectively. SDW of wheat was also significantly reduced (p<0.0001) by all the levels of extract. Application of 25 g l\(^{-1}\) of extract resulted in a reduction of 55.84% in SDW compared to the control. In 75 and 100 g l\(^{-1}\) of extract, no value was recorded for SDW of wheat. SDW of safflower was reduced abruptly even with 25 g l\(^{-1}\) (p<0.0001) of extract and no significant difference was observed among the various levels of extract (Figure 7).
The response of fresh and dry weight to the applied levels of powder was almost the same. The fresh weight at the levels of control for pea, wheat and safflower were 1.390, 1.658 and 2.407 g, respectively. Applied levels of powder resulted in a significant reduction (p<0.0001) in fresh and dry weights of test crops. No seedling development of pea was observed in the higher levels of powder applied (15 and 30 g kg\(^{-1}\) soil). Fresh and dry weight of wheat did not significantly differ at the first two levels of powder and were in the same statistical group, but were significantly (p<0.0001) different compared with the control. All the levels of powder had the same reductive effects on fresh and dry weight of safflower (Figure 8 and 9).

The allelopathic effects of lemon balm powder on shoot length of test crops are shown in Figure 10. Shoot length of pea, wheat and safflower decreased significantly (p<0.0001) with increasing amount of powder. The first applied level of powder on pea resulted in a 48.8 % reduction in shoot length, but the reduction value for the other levels was 100%. The inhibitory effect of the highest level of powder on shoot length of wheat was complete. The effects of all three levels of powder on this trait of safflower were statistically the same, but differed significantly from the control (p<0.0001) (Figure 10).

The effect of allelochemicals may play an important role in determining the growth and yield production of crops. The results of this study strongly suggest that allelopathy may be a possible mechanism controlling the timing of pea, wheat and safflower germination and seedling establishment. The aqueous extracts and powder of lemon balm have an allelopathic compound influencing these test crops. The germination of pea and wheat seeds was inhibited when treated with an aqueous extract of lemon balm, whereas germination of safflower was not significantly inhibited. The inhibitory effects of lemon balm aqueous extract differed with receptor plants and such a result has been reported by other researchers (Qian et al., 2010). The degree of inhibition largely depends on the concentration of the extracts and powder being tested, and thus the inhibitory effect increased with increasing extract concentration. This finding is congruent with the results of Chung and Miller (1995) and Shajie and Saffari (2007) who found the same response. In addition to the inhibition of seed germination of pea and wheat, the radicle and hypocotyl growth of these latter crops was negatively affected. Safflower seeds germinated well in all the levels of extract which possibly resulted from having a harder seed coat compared to other two test crops, but the growth of its hypocotyl and radicle was adversely affected. Our findings are consistent with those reported elsewhere for other species in a variety of plant families (Chiapusio et al., 1997; Escudero et al., 2000; Macias et al., 1999; Macias et al., 2000).
Figure 2. Allelopathic effect of lemon balm extract on germination rate of pea, wheat and safflower

Figure 3. Allelopathic effect of lemon balm extract on mean germination time of pea, wheat and safflower
Figure 4. Allelopathic effect of lemon balm extract on SSI of pea, wheat and safflower

Figure 5. Allelopathic effect of lemon balm extract on HL of pea, wheat and safflower
Figure 6. Allelopathic effect of lemon balm extract on RL of pea, wheat and safflower

Figure 7. Allelopathic effect of lemon balm extract on SDW of pea, wheat and safflower
Figure 8. Allelopathic effect of lemon balm powder on shoot fresh weight of pea, wheat and safflower

Crop plant

Figure 9. Allelopathic effect of lemon balm powder on shoot dry weight of pea, wheat and safflower

Crop plant
The results demonstrated that lemon balm plant powders release allelopathic substances that adversely affect measured traits of pea, wheat and safflower. A number of studies have suggested that plant residues (especially weed species) affect the growth and development of other plants including crops by releasing allelochemicals into the immediate soil environment (Batish et al., 2006a, b; Singh et al., 2003a, b; Singh et al., 2005). Hegde and Miller (1992) reported the adverse effects of phytotoxins from plant residues on the seedling growth of succeeding crops.

In fact soil treatment experiment led us to establish a clear connection between lab experiment and field condition. Allelochemicals compounds are in fact released into the soil and accumulated to levels of toxicity similar to that of the aqueous extracts. The present bioassays conducted under laboratory and realistic soil conditions indicate the presence of some water-soluble phytotoxins in lemon balm that leach from the debris into the soil solution and suppress the shoot length, fresh and dry weight of pea, wheat and safflower that is the same as the response observed visually in the field. Therefore, lemon balm must be considered as an allelopathic species posing risk in a rotation or an intercropping with pea, wheat and safflower. With the aim of alleviating its adverse effects on intercropping or subsequent crops, removing residues of lemon balm from the agricultural land could be beneficial to some extent. However, field experiments seem to be necessary before any special recommendation.

REFERENCES


Figure 10. Allelopathic effect of lemon balm powder on shoot length of pea, wheat and safflower

![Figure 10. Allelopathic effect of lemon balm powder on shoot length of pea, wheat and safflower]


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