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Allelopathic Effect of *Moringa oleifera* Leaves Extract on Seed Germination and Early Seedling Growth of Faba Bean (*Vicia faba* L.)

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Abstract The allelopathic effects of *Moringa oleifera* leaves extract on seed germination and seedling growth of Faba Bean (*Vicia feba* L.) was investigated *in vitro*. Faba bean seeds were soaking in different concentration of *Moringa* leaf extract at 2.5%, 5.0%, 7.5% and 10% w/v. After 9 days of application, results revealed that significant germination reduction was observed in faba bean seeds with increase concentration of MLE except (86.67%) was noted in (2.5% MLE) treatment. MLE at high concentration (10% w/v) had inhibitory effect, it decreased seed germination and reduced seedling growth (lengths of plumule and radical root) compared to control. Moringa extract had no significant effect ($p>0.05$) on germination percentage, root length, plumule length and fresh root weight of faba bean plants. Moringa extract treatments were not significantly different where extracts exhibit allelopathy to exclude the associated faba bean seedling by reducing their regeneration. Based on the study results, moringa leaves showed negative allelopathic effects on faba bean growth and should be eliminated from the field before sowing such crops.

Keywords: *Moringa oleifera*, *Vicia faba*, leaf extract, Allelopathic effect, Germination

Introduction

Faba bean (*Vicia faba* L) is one of the oldest crops that rank sixth in production among the different legumes grown in the world. Faba bean is an annual legume which also known botanically as the broad bean, faba bean, field bean, bell bean, or tic bean, is a species of bean (Fabaceae). In developing countries, food legumes, including beans are an important component of the agricultural sectors due to their capacity to produce large quantities of protein-rich seed for human nutrition.

Moringa (*Moringa oleifera*) is an important plant of Moringaceae family having tremendous allelopathic potential. Foliar spray of leaf extracts

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of moringa accelerates the growth of plants, improves resistance to pests and diseases, and enhances the yield by 20–35 % in different crops (Fuglie 2000). *Moringa oleifera* leaf extract is low-cost and environmentally friendly (Noaman, *et al*., 2010). MOLAE is a plant bio-stimulant when applied singly as seed soaking and/or foliar spray modify plant growth and production with positive alterations in metabolic processes under salt stress conditions (Rady *et al*., 2013; Semida and Rady, 2014a). MOLAE was sprayed onto leaves of onions, bell pepper, soyabeans, sorghum, coffee, tea, chilli, melon and maize and was shown to increase yields of these crops (Fuglie, 2000). MOLAE application has proven its worth as an excellent source of plant growth-promoting substances. *Moringa oleifera* extract is either used as foliar spray or seed priming agent for growth promotion (Mehboob *et al.,* 2011; Nouman *et al.,* 2012). Extracts from moringa leaves and seeds may be thus used for seed treatment and foliar application. Moringa cultivation should therefore be propagated and its new uses and applications should be exploited.

Allelopathy is a mechanism in which chemicals produced by some plant species may increase or decrease the associated plant growth (Jabeen and Ahmed, 2009).Such positive or negative effects are due to release of active biomolecules commonly called as “Allelochemicals” (Albuquerque *et al.*, 2010). Allelochemicals usually are secondary metabolites, which are produced as byproducts during different physiological processes in plants (Farooq *et al*., 2011a; Bhadoria, 2011). Action of these compounds is concentration dependent (Einhellig, 1986) as these inhibit the plant growth at high concentrations and promote that at low concentrations (Narwal, 1994).These allelochemicals may thus be used as natural pesticides at high concentration (Farooq *et al.*, 2009a).

The present study was carried out to investigate the interaction level among *Moringa oleifera* and *Vicia faba* (broad bean) by determine the possible allelopathic effects (inhibitory or stimulatory effects) of *Moringa oleifera* leaves extract on seed germination and seedling growth of this economical crop.

Materials and methods

Present investigation was carried out under laboratory conditions at Department of Biology, Faculty of Science, Yanbu Branch, Girls Sections, Taibah University, Kingdom of Saudi Arabia; During February and March 2016. Seeds of one cultivar variety of Faba bean (*V. faba* L.) a recipient species were purchased from local markets in Yanbu, Kingdom of Saudi Arabia. The seeds were kept in glass jars at 5^{o}C until use in germination studies. Fresh *Moringa oleifera* leaves were collected from growing field of mature *Moringa oleifera* tree in Arid lands Cultivation Research Institute, City of Scientific research, Alexandria, Egypt.

Preparation of MLE

Fresh *M. oleifera* leaves were air-dried, then ground to keep in powder form. The crude powders were stored in paper bags at room temperature. Stock Moringa leaves extract was obtained by soaking100 g of the moringa leaves powder in one liter of distilled water at room temperature (20 ± 2^{o}C) for 24 hours with occasional shaking. The mixture was filtered through four layers of cheesecloth to remove the fiber debris, then Whatman No.1 filter paper and the purified extract was adjusted to pH 6.8 with NaOH 10%. Four different concentrations of MOLAE (i.e., 2.5, 5.0, 7.5 and 10%) were prepared from the stock solution, in addition to the control (distilled water).

Seed viability and seedling vigor evaluation

The seeds of faba bean were soaked in distilled water to test their viability, and then the precipitated seeds were air dried at room temperature. Healthy seeds were disinfected with 0.1% $HgCl_2$ solution for 5 minutes and washed 5-6 times with distilled water to remove its traces. This was done by dipping the 20 viable seeds of each accessions randomly selected into each treatment solution of MOLAE for 5 minutes and then transferred to moistened whattman filter paper which has been carefully layed into 85mm diameter petri dish. Each treatment was replicated 3 times using the completely randomized design (CRD).

Seed Germination Bioassay

The allelopathic potential effects of MLE upon faba bean seeds were studied using different aqueous extract concentrations (2.5, 5.0, 7.5, and 10.0%) as a substrate medium for the germinating seeds. Twenty healthy and nearly equal size of seeds were placed in each Petri dishes double laid with Whatman No1 filter paper. Seeds were allowed to germinate in the different dilutions under normal laboratory conditions with day temperature ranging from 20-22^{o}C and night temperature from 14-16^{o}C. Sterilized petri-dishes (150×20 mm diameter) were used for germination test. Fifteen to twenty ml of each level of individually concentration of moringa extract was added to each petri-dishes. Distilled water was used as a control. Petri dishes were incubated in a lit room at an average temperature of about 25^{o}C for 9 days. The experiment was replicated 3 times. The seeds were observed daily for germination count. Opening of the seeds with radicle appearance served as criterion for germination. Treatments were arranged in a complete randomized block design (CRD). Measurements of germination percentage (GP), plumule (PL), radicle (RL) lengths and weight of fresh radicle were recorded daily along 9 days.

Seed vigor index

Seed germination tests were conducted for each treatment according to the ISTA, 1985. Standard. Root length, plumule length and Vigour index determined following method of (Abdul Baki and Anderson,1973). Seedling vigour index was determined as the product of the germination percentage and that of seedling length. Seedling vigor index (SVI) was calculated by the following formula : SVI _ Seedling length cm x Germination percentage ⁄100 (Abdul-Baki and Anderson, 1973).

Germination inhibition or stimulation percentage (IP and SP)

Germination inhibition or stimulation percentage was calculated for each concentration treatment according to recommended methods by (ISTA, 1985). Number of germinated seeds was counted starting from the 1st day after germination when roots appeared till the 9^{th}day, which is the last day. Germination Percentage was calculated as follows:

$$\% \text{ Germination} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Determination of fresh matter

Remove seedlings from perti-dishes and wash off any filter paper remains. Blot seedlings gently with soft paper towel to remove any free surface moisture. Weigh immediately (plants have a high composition of water, so waiting to weigh them may lead to some drying and therefore produce inaccurate data).For dry matter determination, about 3 g of fresh samples (in triplicate) were dried in an electric drying oven at 70 °C for 3 days until a constant mass was achieved.

Statistical analysis

Data concerning the effect of different concentrations of *M. oleifera* leaf aqueous extract on faba bean seed germination and seedling growth was subjected to standard analysis of variance (ANOVA) using SPSS V.16 and with the least significant difference was used to compare means of traits ($p < 0.05$).

Results

Effect of MLE on Seed viability and Vigour index tests (SVI)

Effect of the four concentrations of MLE on vigour index of faba bean (Table1, Fig.1) showed significance decrease when compared with control. As concentration of MLE increased the vigour index significantly decreased. Maximum vigour index recorded in 2.5% MOLAE (2253.42 ±64.52) compared to control sets and minimum value recorded in MLE at 10% (320 ± 82.76).

Table 1. Effect of different concentrations of mle on the seedling vigour index of germinated faba bean seeds.

MLE Concentration %	Seed Vigour Index (SVI) /Days				
	1st day	3rd day	5th day	7th day	9th day
Control	0	106.68	319.98	733.48	1319.94
2.5 %	0	111.3	540	1026.62	2253.42
5.0 %	0	66.88	240	466.70	653.38
7.5 %	0	26.67	133.33	280	530
10.0 %	0	83.32	100	200	320

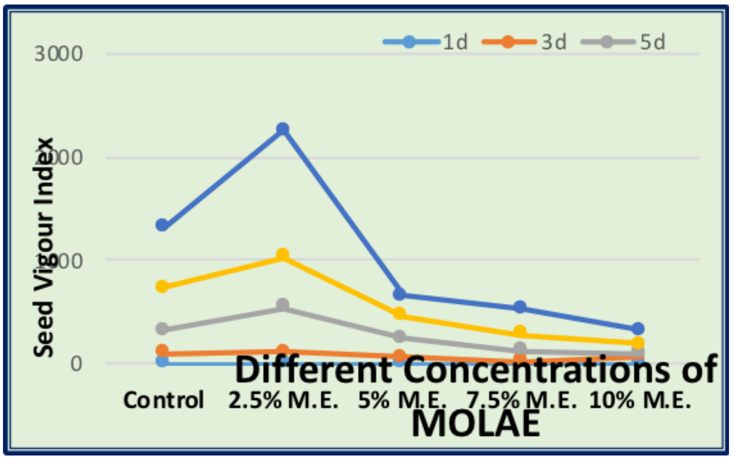

Figure 1. Effect of different concentrations of mle on the seedling vigour index of germinated faba bean seeds.

Germination percentage (GP)

The allelopathic effects of *Moringa oleifera* leaves extract on germination percentage (GP) of faba bean was represented in (Table2) GP of the recipient plant was significantly affected by applying the different concentrations MOLAE, where it decreased with increasing the extract concentration. The maximum percentage of germination in (Table 2 and Fig. 2) was reported in the concentration 2.5% of MLE, which was 86.33 ± 0.1833% compared to that of all the concentrations and control which was reported (73.3±0.7386 %). The maximum percentage of germination 86.33 ± 0.1833% and 73.33±0.7009 % recorded with 2.5% and 5% concentrations of MLE respectively. Whereas the minimum percentage of germination (Maximum inhibition) of seed germination was found on higher concentration of MLE (10 %) showing only 40.33± 0.6179 %, germination.

Table 2. Effect of different concentrations of mle on the germination percentage (gp) of faba bean seeds.

MLE Concentration %	**Germination Percentage (GP) /Days**				
	1st day	3rd day	5th day	7th day	9th day
Control	0	26.6	53.3	66.6	73.3
2.5 %	0	33.3	60	73.3	86.33
5.0 %	0	30.6	45.0	58.6	68.3
7.5 %	0	24.6	33.3	40.0	53.3
10 .0%	0	22.3	29.3	35.0	40.0

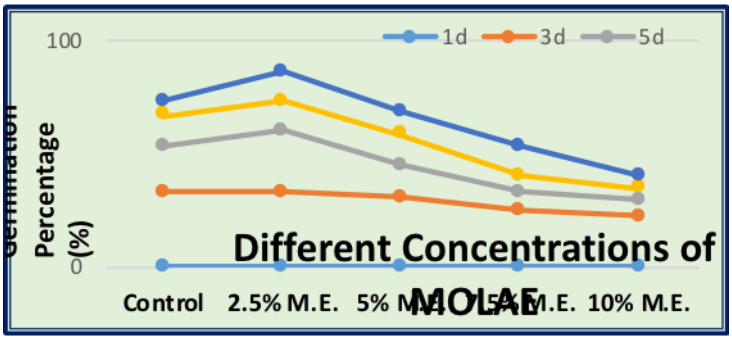

Figure 2. Effect of different concentrations of mle on the germination percentage (gp) of faba bean seeds.

Root Length (RL)

Results in Table 3 and fig 3 show that ,MLE at 2.5% led to increaseing the root length as compared to control (13.3 ±0.4485 cm). The increment of root lengths followed by increasing MLE to 5% compared to control (7.00±0.270cm). While a minimum root length was found at highest concentration of MLE 10% (4.00±0.5958 cm). As the concentration of aqueous extract decreased, there was an increment in root length.

Table 3. Effect of different concentrations of mle on root lengths (rl) (cm) of faba bean seedlings.

MLE Concentration %	Root Length (cm) /Days				
	1st day	3rd day	5th day	7th day	9th day
Control	0	1.5	3	5	9.38
2.5 %	0	2	5	7	13.3
5 .0%	0	1.5	4	6	7
7.5 %	0	1	3	4	5.01
10.0 %	0	1.5	2	3	4

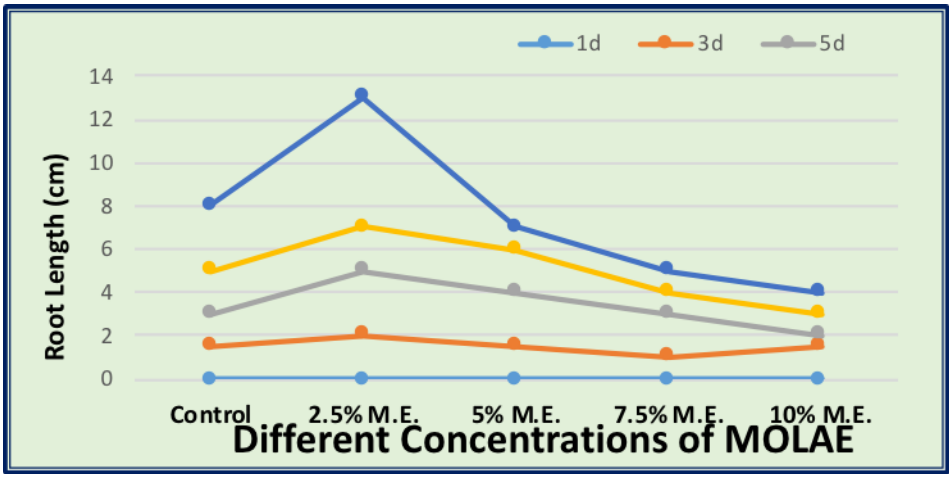

Figure 3. Effect of different concentrations of mle on root lengths (rl) (cm) of faba bean seedlings

Plumule Length (PL)

Results in (Table 4 and Fig.4)clearly show that all treatments of moringa leaf extract MLE decreased the plumule length as compared to control .Whereas the minimum root length was found at highest concentration 10% of MLE which was (4.00 ±0.591 cm) while the maximum was given by 2.5% MLE (13.00±0.114 cm). As the concentration of aqueous extract decreased, there was an increment in plumule length.

Root Fresh Weight

(Table5, Fig.5) reveals that the highest root fresh weight (7.416±0.068 gm) was recorded by 2.5% MLE in comparison with control priming and it was followed by 5%, 7.5% and 10% MLE (3.9±0.0432 , 3.3 ±0.063 gm) respectively while minimum root fresh weight was given by 10% Moringa leaf extract (2.8 ±0.0372 gm).

Table 4. Effect of different concentrations of mle on plumle lengths (pl) (cm) of faba bean seedlings**.**

MLE Concentration %	**Plumle Length (cm) /Days**				
	1st day	3rd day	5th day	7th day	9th day
Control	0	2	3	6	10
2.5 %	0	1	4	7	13
5.0 %	0	1	2	4	7
7.5 %	0	0	1	3	5
10.0 %	0	0	1	2	4

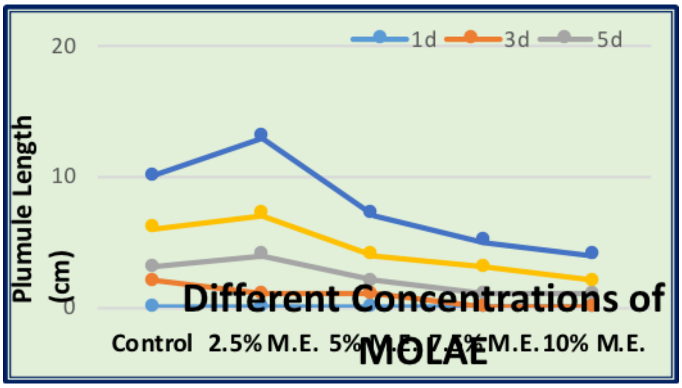

Figure 4. Effect of different concentrations of mle on plumle lengths (pl) (cm) of faba bean seedlings.

Table 5. Effect of different concentrations of mle on root fresh weight (gm) of faba bean seedlings.

MLE Concentration %	**Root Fresh Weight (gm) /Days**				
	1st day	3rd day	5th day	7th day	9th day
Control	0	1.0	2.0	3.0	5.6
2.5 %	0	1.5	2.5	4.0	7.4
5.0 %	0	1.1	1.8	2.0	3.9
7.5 %	0	1.0	1.3	2.0	3.3
10.0 %	0	1.2	1.4	2.0	2.8

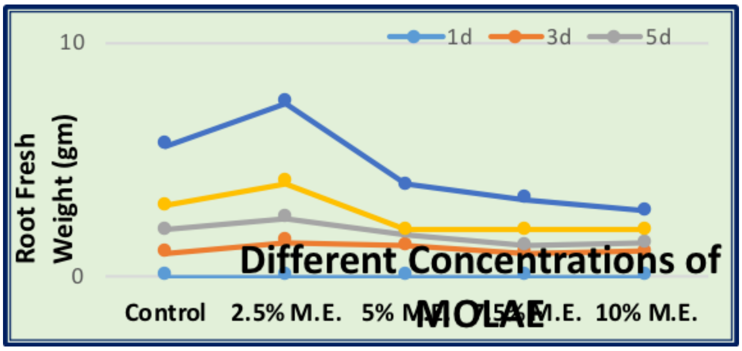

Figure 5. Effect of different concentrations of mle on root fresh weight (gm) of faba bean seedlings

Discussion

Some researchers reported that allelochemicals exhibited inhibitory effects on physiological processes during germination and growth of plants that may occur through a variety of mechanisms including reduced mitotic activity in roots and hypocotyls, suppressed hormone activity, reduced rate of on uptake, inhibited photosynthesis and respiration, inhibited protein formation, decreased permeability of cell membranes and/or inhibition of enzyme action (Rice, 1984; Singh, 2001).

The inhibitory effect of the donor plant is directly proportional to the increasing extract concentrations. The differences in the germination percentage between the different concentrations could be attributed to differences in the selective permeability of broad bean seeds to inhibitory substances (Zakaria and Razak, 1990). Therefore, effects of allelochemicals on seeds germination appear to be mediated through a disruption of normal cellular metabolism rather than through damage of organelles (Mohamadi and Rajaie, 2009). The inhibitory effect of MOLAE on seed germination and seedling growth of faba bean seeds as gradual increase in concentration may be related to the presence of allelochemicals including phenolic contents and volatile compounds in its foliage.

Furthermore, the toxicity might be due to synergistic effect rather than single one. Phenolic acids have been shown to be toxic to germination and plant growth processes (Enhilelling, 1995; Asghari and Tewari, 2007). As root membranes are a primary site of action for phenolics. The contact of phenolic acids with the root cell membrane leads to depolarization, an efflux of ions, and a reduction of hydrolic conductivity. Root growth is characterized by high metabolic rates and, for this reason; roots are highly susceptible to environmental stresses such as allelochemicals in soils (Cruz-Ortega *et al*., 1998).

Moreover, these allelochemicals may either have inhibitory or stimulatory effects on germination and growth of an adjacent or subsequent crop to varied extents depending upon their concentration and plant part

(Swain *et al.*, 2005 and Sinha *et al.*, 2012). In the present study, we have shown that allelopathic compounds of MOLAE reduced GP as compared to control treatments of *vicia Faba* seeds. On contrarily, Phiri (2010) reported that M. *oleifera* leaf extracts enhanced germination of sorghum, length of maize radicle and hypocotyl of wheat. Furthermore, El-Darier *et al*., (2014) and Chandra *et al.*, (2011) found a gradual increase of inhibition percentage in germination and some seedling growth parameters of *Vicia faba* as a response to the higher concentration levels of *Medicago sativa* and *A. aspera* aqueous extract, respectively .

Allelochemicals can affect actively for specific enzymes such as amylases and proteinases, which are necessary for seed germination (Rice, 1984). During germination, the action of gibberellic acid which induces the production of amylase is disrupted by the phytotoxic chemicals (Aghajanzadeh *et al.*, 2007). The nature of the inhibitory effect of allelochemical to seed germination could be attributed to inhibit water absorption which is a precursor to physiological processes that should occur in seed before germination is triggered (Oyerinde *et al.*, 2009). Similarly, the nature of the effect of the allelochemicals on seedling growth was likely to be that of inhibition to nutrient uptake by seeds thereby reducing growth parameters. The allelochemicals inhibit the growth of the radicle and plumule in various crops by blocking hydrolysis of nutrients and cell division (Oyerinde *et al.*, 2009).

On the other hand (El Awady 2003; Taiz and Zwiger;2006) pointed out that Moringa leaves have high zeatin content which plays an important role in cell division and cell elongation, this in agreement with (Price, 1985) who confirmed that zeatin influence the improvements in crop growth and yield. These findings have also been supported by Fuglie (2000) who reported that Moringa accelerate growth of young plants, strengthen plants, increase number of roots, improve resistance to pests and diseases, produce more and larger fruits and generally increase yield by 20 to 35.

In conclusion, the present study shows that the inhibitory effect of Moringa aqueous extract on germination and seedling growth of faba bean was greatly affected by increasing concentration of MOLAE .The results presented here; are in agreement with those of (Singh *et al.*, 1992; Nandal *et. al.*, 1999 a and b; Patel *et. al.*, 2002 and Odofin, 2010) who all observed reduction in germination percentage with extract leachates application to different crop species. In this study, the reduction in number of germinated seeds and subsequent reduction in seedling parameters of *Vicia faba* by higher concentration of *M. olifera* could be as a result of allelochemicals produced by *M. olifera* which might have retarded these growth parameters and due to possible allelopathic compounds contained in the leaves of *M. olifera* which became phytotoxic to the germination and seedling growth of target plant.

The research needs further investigation to determine the nature of the chemical components of MOLAE and then test their activities against the bimolecular and molecular behavior of the intercrops.

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