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RESEARCH ARTICLE

Biological interactions between *Moringa oleifera* Lam. and two common food intercrops: growth and some physiological attributes

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Abstract

The aim of the present study was to evaluate the possible allelopathic potential of *Moringa oleifera* Lam. on some growth and physiological attributes of the two economically important food crops; *Vicia faba* L. and *Zea mays* L. in mixed cropping system. Germination percentage (GP), plumule (PL) and radicle (RL) lengths as well as dry weights were significantly affected by applying the different concentrations (0, 10, 20 and 40%) of *M. oleifera* leaves aqueous extract (MOLAE) and *M. oleifera* leaves crude powder (MOLCP) where it decreased with increasing the extract concentration (*V. faba* > *Z. mays*). Additionally, it was found that MOLCP decreased the efficiency of chlorophyll fluorescence spectra, photosynthetic pigments and photosystem II photochemistry (Fv/Fm). The effect was more detected in *V. faba* compared to *Z. mays*.

In conclusion, the cultivation of *M. oleifera* accompanied with *V. faba* and *Z. mays* as intercrops is not recommended for its dramatic effects on germination efficiency and growth besides some photosynthetic activity.

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INTRODUCTION

Genus *Moringa* is the only genus in family Moringaceae and comprises 13 species from Africa, Madagascar, western Asia and the Indian subcontinent (Verdcourt, 1985). *Moringa oleifera* Lam. is a small to medium-sized deciduous tree that develops a swollen underground rootstock. It is now widely cultivated and naturalized throughout the tropical and subtropical world and is regarded as potentially invasive or moderately invasive. It is drought resistant, preferring regions with a wet/dry climate, but can grow in a wide range of habitats on a variety of soils. The tree loses its leaves from December to January, though during droughts it may also lose its leaves at other times of the year (HDRA, 2002). In Egypt, the tree did not consider being an agricultural weed, so it was not considered a problem in agricultural areas. Only it is an economic threat to agriculture in cropping areas for its probable allelopathic potentiality of their falling leaves.

Allelopathic potentiality under field conditions can be utilized in different ways. For example, surface mulch (Cheema and Khaliq, 2000), incorporation into the soil (Sati et al., 2004), aqueous extracts (Iqbal and Cheema, 2007a), rotation (Narwal, 2000), smothering (Singh et al., 2003) or mix cropping/intercropping (Iqbal and Cheema, 2007b).

Intercropping; the agricultural practice of cultivating two or more crops in the same space at the same time is an old and commonly used cropping practice aimed to match efficiently crop demands to the available growth resources and labor. The most common advantage of intercropping is the production of greater yield on a given piece of land by making more efficient use of the available growth resources using a mixture of crops of different rooting ability, canopy structure, height, and nutrient requirements based on the complementary utilization of growth resources by the component crops (Lithourgidis et al., 2011). The relatively recent move to large scale from

monoculture to polyculture practices has led to undesirable environmental effects in the form of chemical inputs that leach into the soil and contaminate water supplies (Broz, 2006). It was noticed that in polyculture, some crops give better yield, while others give lower yield (Jensen, 1996). For example, seeds of *Nigella sativa* adversely affect seed germination and seedling growth of *Lupinus termis* that are commonly intercropped with it (Belgasem, 2012).

One should be aware by the chemical interfering between the mixed crops in order to avoid undesirable potential effects of some crop on the others. Consequently, the main objective of the present study was to assess the probable allelopathic effects of *M. oleifera* on some growth and physiological attributes of the two economically important food crops; *V. faba* L. and *Z. mays* L. in mixed cropping system.

MATERIALS AND METHODS

Collection of plant materials

Leaves of *M. oleifera* (donor species) were newly harvested from polyculture fields distributed along the Alexandria-Cairo desert road during year 2014. On the other hand, *V. faba* seeds and *Z. mays* grains (recipient species) were purchased from the National Research Center (NRC), Dokki, Giza. The seeds were kept in glass jars at 5°C until use. The experiments were conducted at Department of Botany and Microbiology, Faculty of Science, Alexandria University, Alexandria, Egypt.

Preparation of plant materials and aqueous extracts

Samples of the fresh leaves of *M. oleifera* were air-dried then ground to keep in powder form. The crude powders were stored in paper bags at room temperature. Stock aqueous extract was obtained by soaking 100 g of the air-dried donor plant materials in one liter of distilled water at room temperature ($20 \pm 2^\circ\text{C}$) 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%. Different concentrations (10, 20, and 40%) were prepared from the stock solution, in addition to the control (distilled water).

Preparation of seeds for germination bioassay

The seeds of *V. faba* and *Z. mays* were soaked in distilled water to test their viability, and then the precipitated seeds were air dried at room temperature. Healthy seeds of the two recipient species were disinfected with 0.1% HgCl_2 solution for 5 minutes and washed 5-6 times with distilled water to remove its traces.

Germination bioassay

The potential allelopathic effects of *M. oleifera* leaf aqueous extract (MOLAE) upon *V. faba* and *Z. mays* were studied using different aqueous extract concentrations (0, 10, 20, and 40%) as a substrate medium for the germinating seeds. Ten healthy seeds (in three replicates) each for the two recipient species during the current year were allowed to germinate in the different dilutions of *M. oleifera* leaf aqueous extract (MOLAE) under normal laboratory conditions with day temperature ranging from 20-22°C and night temperature from 14-16°C. Ten ml of each level of the donor species extracts were added to each of the recipient species. Treatments were arranged in a complete randomized block design. Measurements of germination percentage (GP), plumule (PL) and radicle (RL) lengths were recorded daily along 7 days.

Calculations

1. **Germination inhibition percentage (IP)** was calculated for each concentration treatment according to the general equation:

$$\text{Inhibition percentage} = [1 - (\text{allelopathic} / \text{control}) 100]$$

Where:

Allelopathic = number of germinated seeds for each treatment

2. **Reduction in plumule and radicle lengths**

$$\text{Reduction percentage} = (\text{control-allelopathic}/\text{control}) 100$$

Growth experiment

Six soil samples from natural sites where the alleged allelopathic materials are not present were used to undergo the growth experiment. The samples were air-dried and passed through 2mm sieve to eliminate the gravels and debris, and finally analyzed for some of their chemical and physical properties according to Allen et al. (1984).

Growth experiment was carried out in three replicates to test the effect of *M. oleifera* leaves crude powder (MOLCP) mixed with sandy clay loam (w/w) on seedling dry weight and length as well as some physiological parameters. Two concentrations (20 and 40%) as well as the control (without crude powder) were applied. Ten seeds from the two recipient species were sown in plastic pots with 15cm diameter (1.1 kg soil). The pots were watered every two days on the average with normal tap water. The amount of water calculated from water loss over a 24-hours interval was applied. Homologous seedlings of the two recipient species were harvested 15 days after planting and washed three times with distilled water, then shoot and root lengths were measured. A part of the harvested samples (leaves) were taken to determine photosynthetic pigments and capacity as well as chlorophyll fluorescence. Another part was oven-dried separately at 70°C for 72 h to determine the dry weight of the seedlings.

Photosynthetic pigment contents and chlorophyll stability index (CSI %)

Leaf chlorophyll (Chl) and carotenoid contents were determined by the method described by **Lichtenthaler (1987)**. Leaf tissues (50 mg) were homogenized in 10 ml chilled acetone (80 %). The homogenate was centrifuged at 4,000 g for 12 min. Absorbance of the supernatant was recorded at 663, 647 and 470 nm for Chl. a, Chl. b and carotenoids, respectively. The contents were expressed as mg Chl or carotenoids g^{-1} fresh weight (FW). The Chl stability indices (CSI %) were measured using the formula:

$$(\text{Total Chl content in stressed leaves} / \text{total Chl content in control leaves}) \times 100.$$

Chlorophyll absorption spectra

The absorption spectra were recorded at room temperature (25 °C) with a UV-visible light spectrophotometer (T80+ UV-Vis spectrophotometer, double beam). Fluorescence emission spectra was recorded from 400-700 nm according to the method applied by (**Sudheer et al., 2011**)

Chlorophyll fluorescence Fv/Fm measurements

Measurements of Chl fluorescence was performed with OS-30P pulse modulated chlorophyll fluorimeter (Opti-sciences, Hudson, USA.). Before each measurement, leaves were dark- adapted for 30 min with leaf-clips. To determine the minimal fluorescence (F_0), the weak measuring light was turned on and F_0 was recorded. The leaves were then exposed to 0.1 s saturated flash of approximately 6000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to obtain the maximal fluorescence yield (F_m). The ratio of variable to maximal fluorescence (F_v/F_m) was calculated automatically according to F_0 and F_m measured [$F_v/F_m = (F_m - F_0)/F_m$].

Statistical analysis

Data concerning the effect of different concentrations of *M. oleifera* leaf aqueous extract (MOLAE) and crude powder (MOLCP) on some germination and physiological parameters of the recipient species were subjected to standard analysis of variance (ANOVA) (**Zar, 1984**) using the COSTAT 2.00 statistical analysis software manufactured by Co-Hort Software Company.

RESULTS

Germination bioassay

Germination percentage (GP)

The allelopathic effects of *Moringa oleifera* leaves aqueous extracts (MOLAE) on germination and inhibition percentage (GP) of *Vicia faba* and *Zea mays* are represented in Figure 1. GP of the two recipient plants was significantly affected by applying the different concentrations (0, 10, 20 and 40%) of MOLAE where it decreased with increasing the extract concentration (*V. faba* > *Z. mays*). The inhibitory effect of the donor plant is directly proportional to the increasing concentration. The inhibition percentage attained values of about 42 and 35% for *V. faba* and *Z. mays* respectively compared to the control. Simple linear regression obtained by plotting seed germination percentages of the test species versus the different concentrations of MOLAE achieved values of coefficient of determination (R^2) for data at the seventh of about 0.979 and 0.875 for *V. faba* and *Z. mays* respectively.

Plumule (PL) and radicle lengths (RL)

Compared to the control, a gradual decrease in plumule (PL) and radicle (RL) lengths of the two recipient species was noticed along the gradual MOLAE concentrations (Figure 2). Obviously, all concentration levels had significantly reduced PL and RL of the study species and the reduction was 84 and 77% for PL and 85 and 82% for RL at 40% with respect to *V. faba* and *Z. mays*, respectively. The application of the regression analysis resulting in a value of coefficient of determination of about 0.948 and 0.957 for PL and 0.845 and 0.887 for RL with respect to *V. faba* and *Z. mays*, respectively.

Growth experiment

Soil analysis

The routine analyses for soil applied in the current study are listed in Table 1. The soil type is sandy clay loam comprises a considerable content of organic matter and attained pH value of about 7.8. The electrical conductivity (EC) is about 2.72 dSm^{-1} . Nitrogen, phosphorous and potassium achieved values of about 1.1, 0.5 and 3.5 mg g^{-1} , respectively.

Table 1: Variation in some physical and chemical characteristics of the soil applied to carry out the pot experiment. Considering that, a: dsm^{-1} , b: % and c: mg g^{-1} .

Parameter	Range
Physical properties	
Texture	Sandy clay loam

Clay ^b	24.00 – 26.90
Sand ^b	58.00 – 61.28
Silt ^b	18.00 – 19.23
Chemical properties	
Electrical conductivity ^a	2.72 – 2.82
Organic matter ^b	9.08 – 10.00
pH	7.82 – 9.01
Free carbon ^b	1.50 – 1.53
N ^c	1.100 – 1.104
P ^c	0.520 – 0.527
K ^c	3.50 – 3.98
Ca ^c	15.40 – 17.34
Mg ^c	1.40 – 1.42
Cl ^c	15.25 – 16.45
CO ₃ ^c	35.00 – 37.21
SO ₄ ^c	18.70 – 19.38

Seedling dry weight and length

M. oleifera leaves crude powder (MOLCP) showed a gradual inhibition in shoot and root lengths as well as the dry weights of the two organs of the two recipient species. The inhibitory effect was concentration dependent (0, 20 and 40%) (Figure 3). The reduction in shoot and root lengths was gradual at all the concentration levels. Maximum reduction of 48% and 64% in shoot and root growth was recorded at 40% concentration for *V. faba*. Regarding *Z. mays* the relevant values were 55% and 54% at the same concentration. The dry weights of seedlings of the two recipient species follow the same tendency where the reduction percentage were 55% and 53% for *V. faba* and *Z. mays*, respectively at maximum concentration.

Chlorophyll absorption spectra

The absorption spectra of chlorophyll are presented in Figure 4. Three curves each with well defined two peaks were recognized. The intensity of the fluorescence peaks decreased as the *M. oleifera* leaves crude powder (MOLCP) increase. Chlorophyll spectra from control plants demonstrated main fluorescence emission peaks for both *V. faba* and *Z. mays*. Chlorophyll spectra from control plants presented main fluorescence emission peaks stabilized at blue (450 nm) and red (670 nm). The intensity and the shape of fluorescence spectra showed maximum decrease in intensity at 40% of MOLCP concentration.

Photosynthetic pigments

The results presented in Figure 5 proved that there was an inverse proportional relationship between increasing the severity of different percentages of MOLCP on one hand, and leaves content of chlorophyll a and b, carotenoids and total chlorophyll content on the other hand. Chlorophylls a, b, and carotenoid contents in leaves of *V. faba* and *Z. mays* seedlings were decreased significantly with increasing MOLCP concentration. Chlorophyll a, b, and carotenoids were decreased by 29%, 8%, and 18% for *V. faba* and 15%, 15, and 12% for *Z. mays* at 40% MOLCP, respectively compared to the control. Reduction percentages of about 22% and 15% in the total chlorophyll content (Chl.a + Chl.b) for the two recipient species at 40% MOLCP, respectively. The value of chlorophyll stability index (CSI %) was reduced markedly in both tested plants and attained the lowest values (77% and 85%) at 40% MOLCP for *V. faba* and *Z. mays*, respectively.

Chlorophyll fluorescence Fv/Fm measurements

The maximal photochemical efficiency of PSII (Fv/Fm) represented in Figure 6 showed that photosynthetic rate (Fv/Fm) was decreased by increasing of MOLCP concentrations and the maximum decrease was observed in both tested plants. The reduction was 53% and 31 % in *V. faba* and *Z. mays* respectively compared to control.

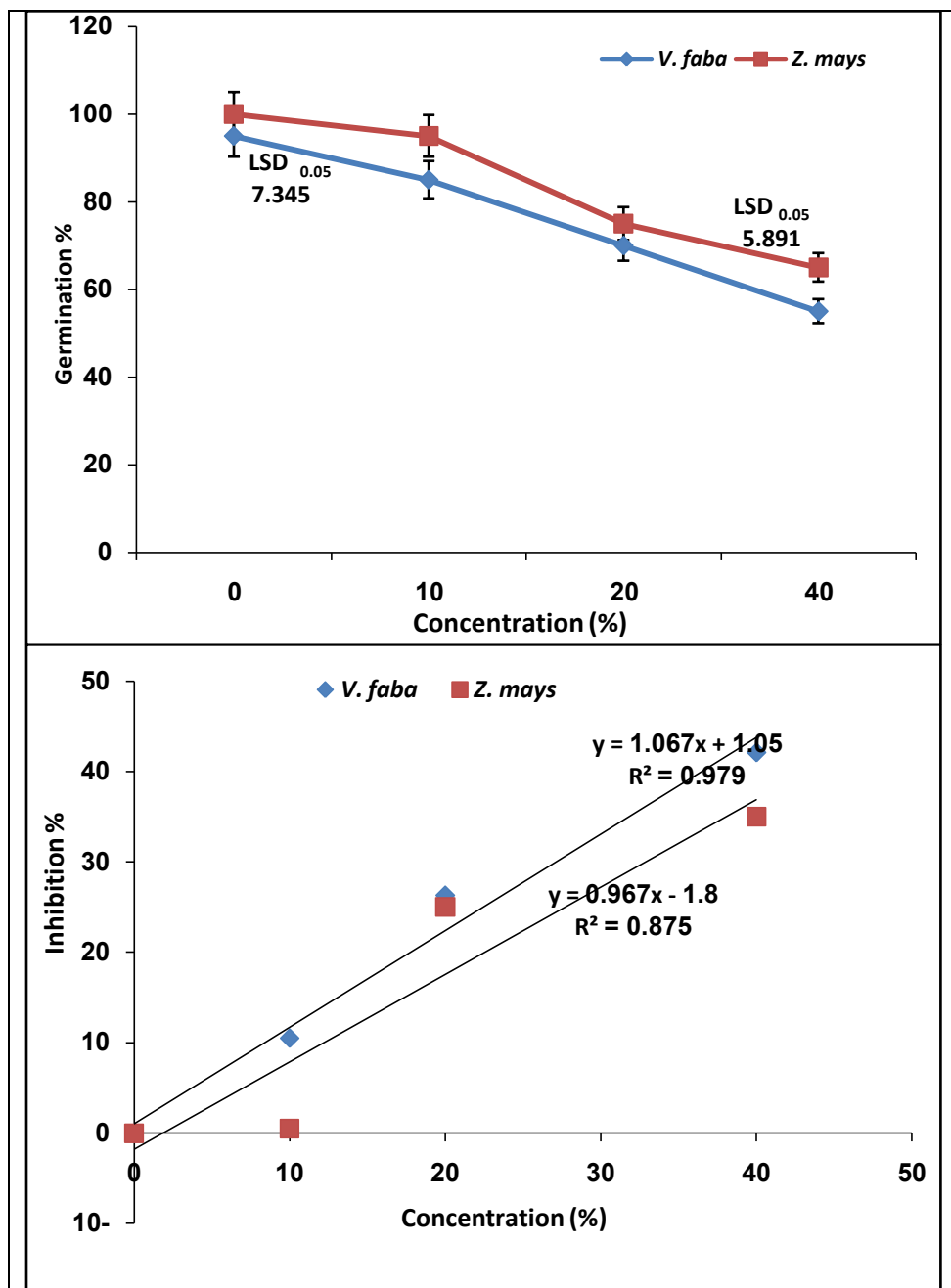


Figure 1: Allelopathic effect of different concentrations of *Moringa oleifera* leaves aqueous extract (MOLAE) on germination percentage (GP) of *Vicia faba* and *Zea mays* seeds. Each value is the mean of triplicates. Bars represent standard deviation.

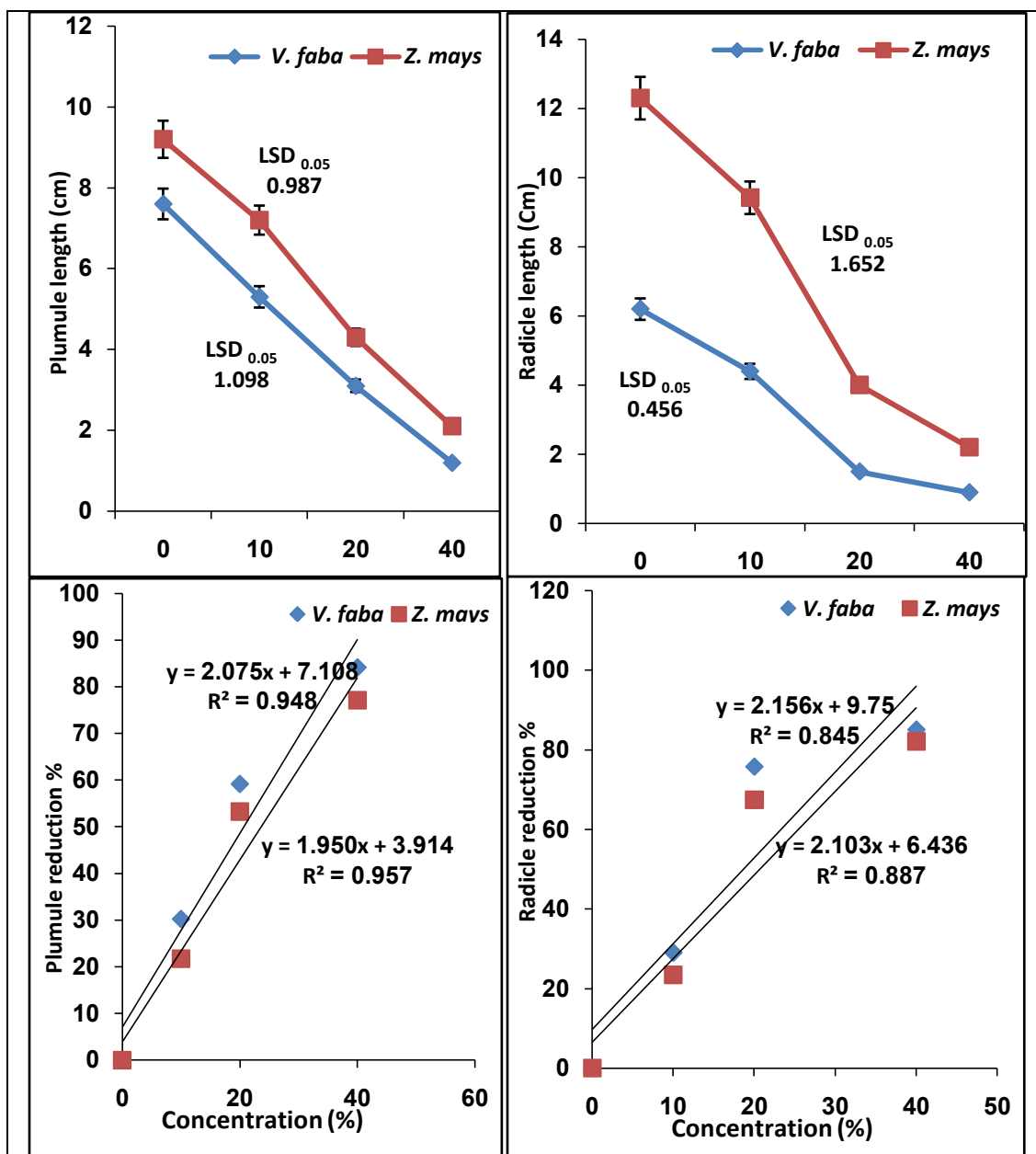


Figure 2: Allelopathic effect of different concentrations of Moringa oleifera leaves aqueous extract (MOLAE) on plumule length, radicle length, plumule inhibition %, and radicle inhibition % of Vicia faba and Zea mays seeds. Each value is the mean of triplicates. Bars represent standard deviation.

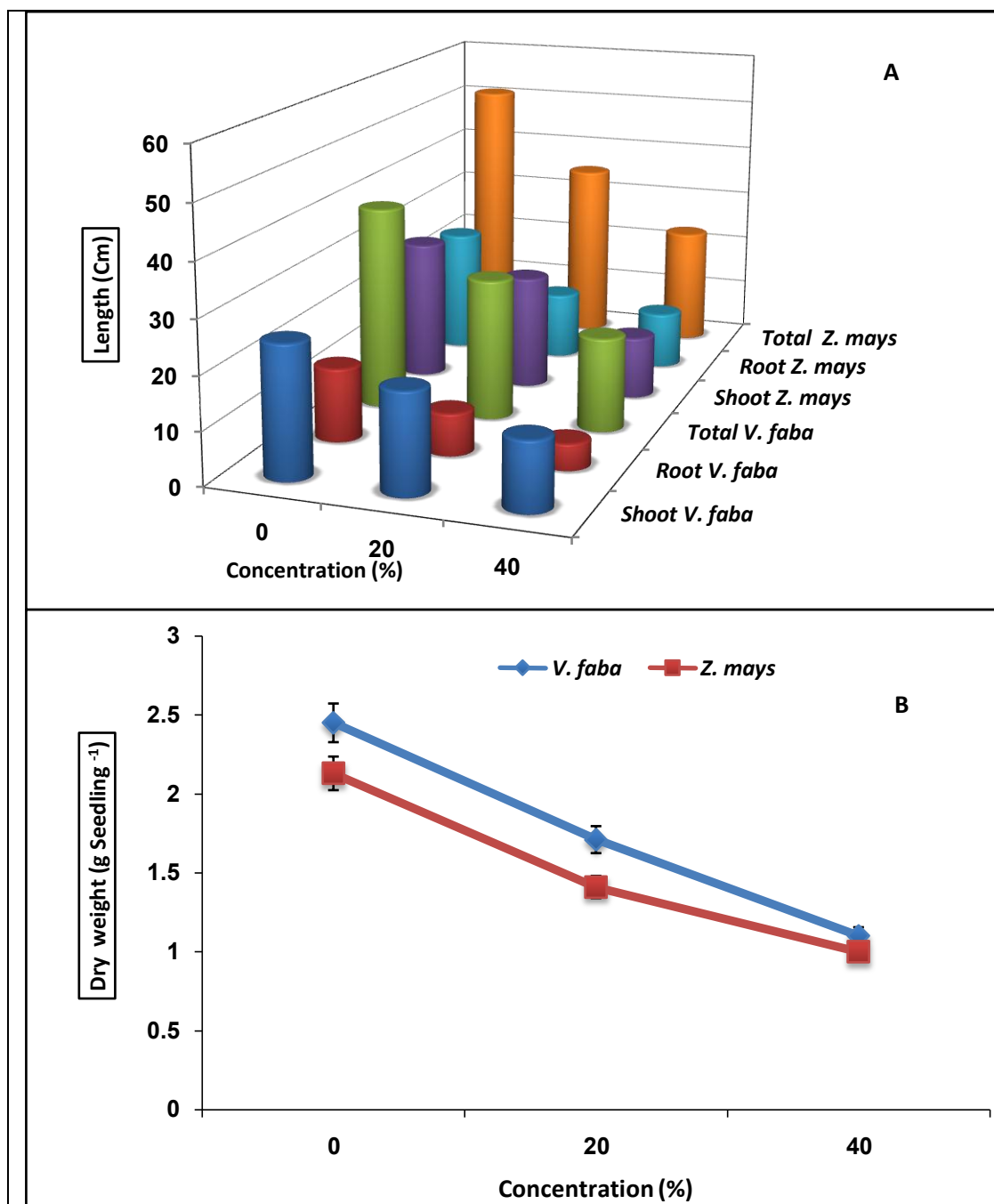


Figure 3: Allelopathic effect of different concentrations of Moringa oleifera leaves crude powder (MOLCP) on (A) shoot, root, and total seedling length and (B) dry weight of Vicia faba and Zea mays. Each value is the mean of triplicates. Bars represent standard deviation.

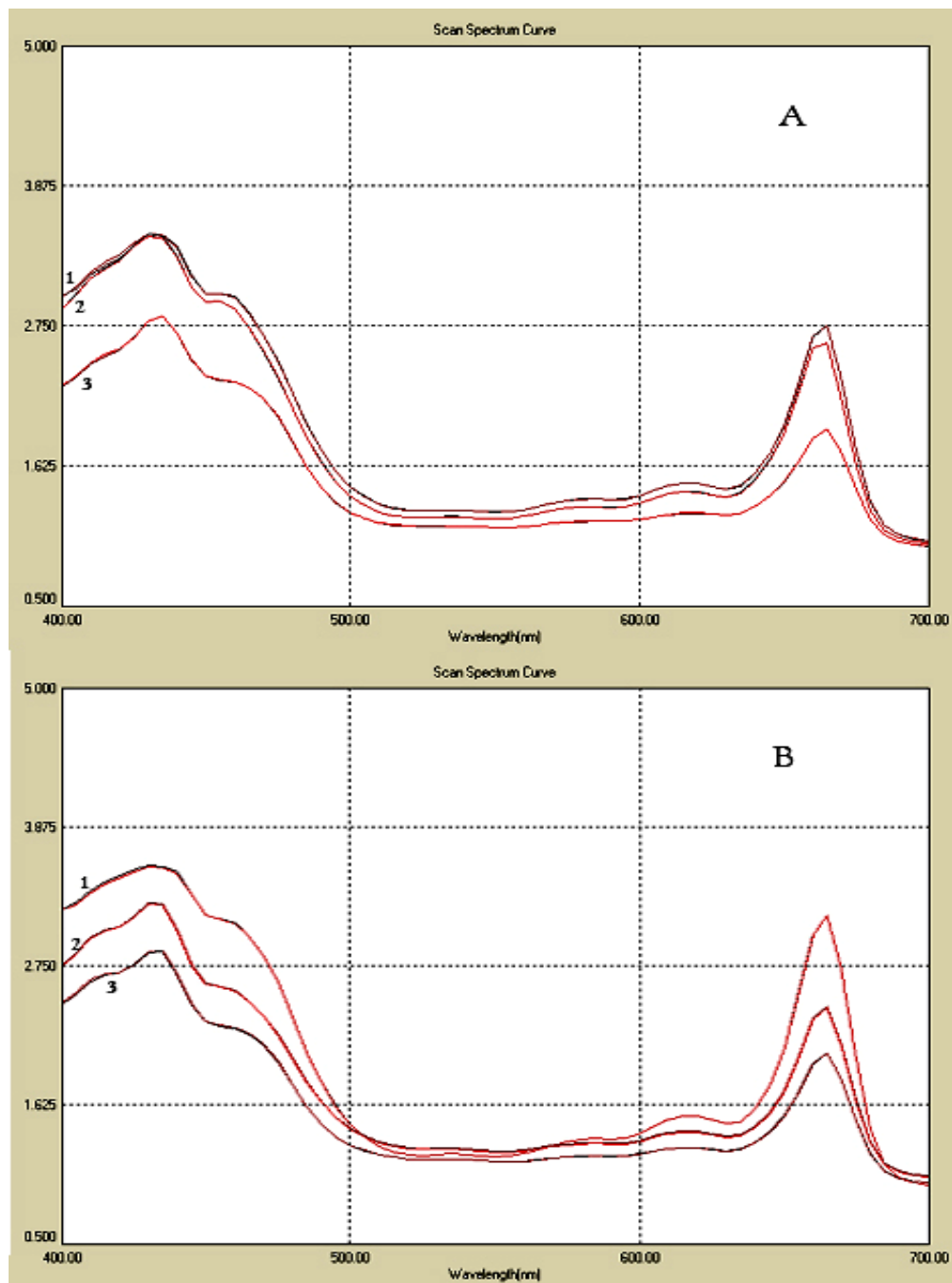


Figure 4: Allelopathic effect of different concentrations of *Moringa oleifera* leaves crude powder (MOLCP) on chlorophyll absorption spectra of acetone extract of (A) *Vicia faba* and (B) *Zea mays* leaves (1= 0%; 2= 20%; 3= 40%)

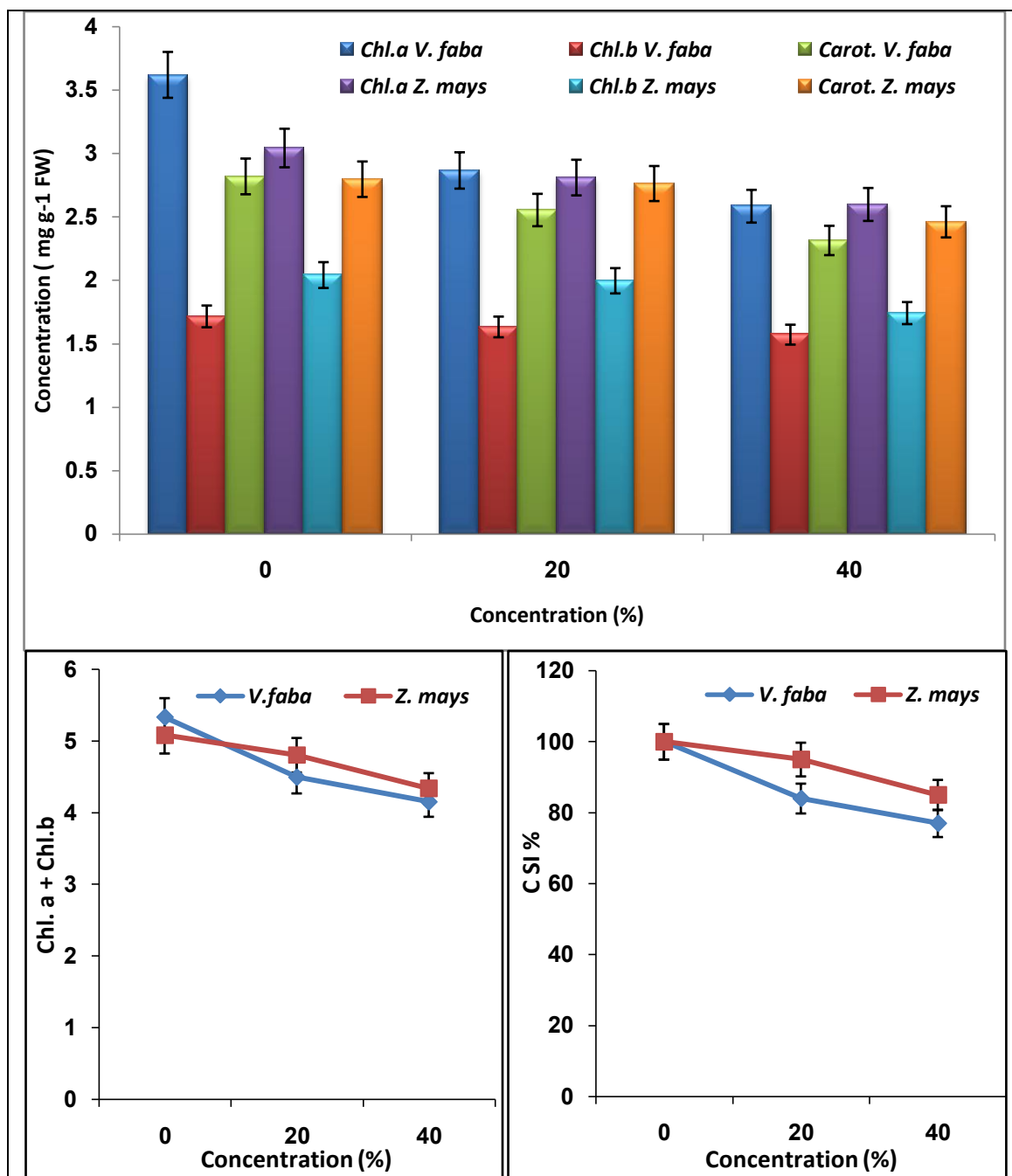


Figure 5: Allelopathic effect of different concentrations of *Moringa oleifera* leaves crude powder (MOLCP) on photosynthetic pigments, and chlorophyll stability index of *Vicia faba* and *Zea mays* leaves. Each value is the mean of triplicates. Bars represent standard deviation.

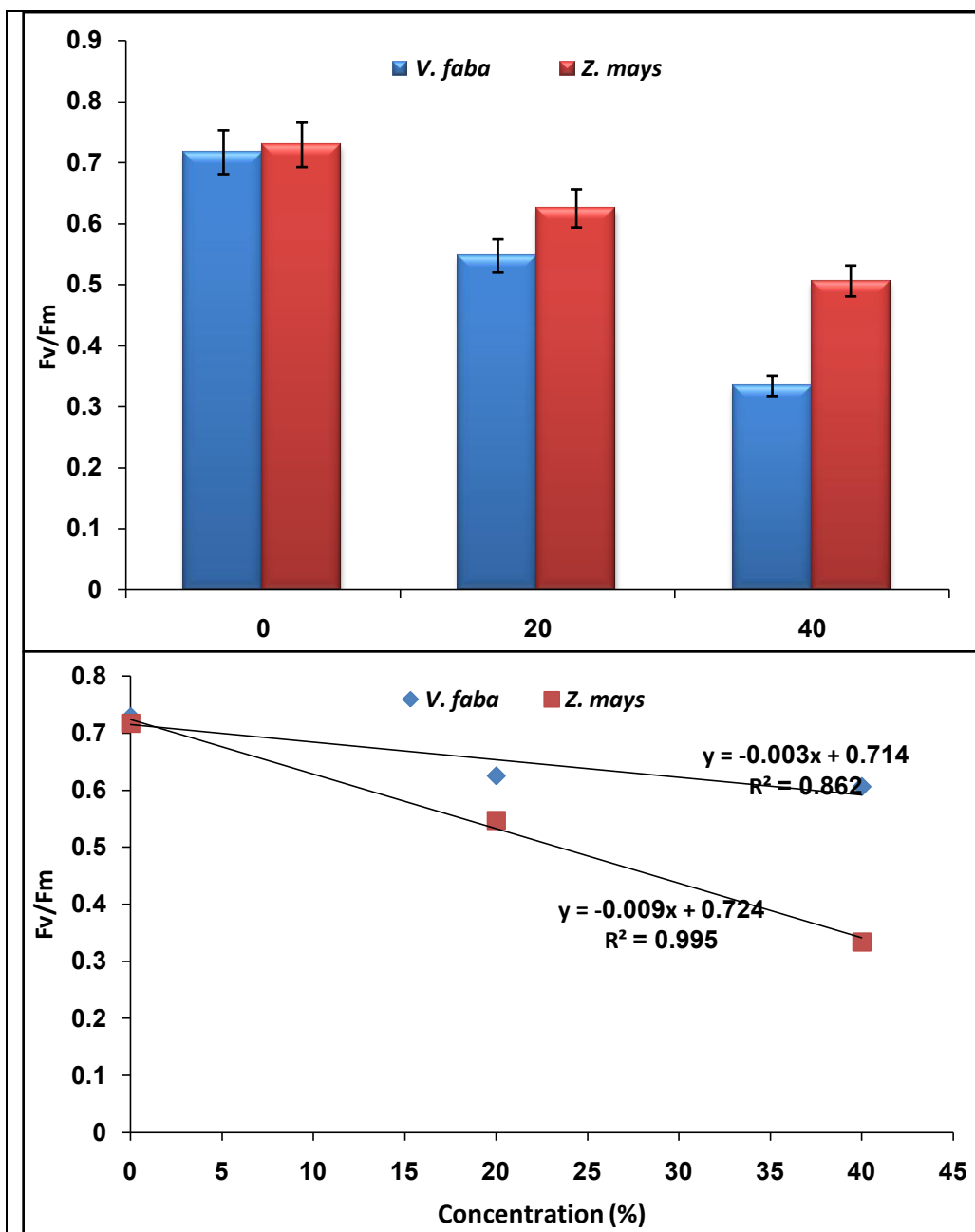


Figure 6: Allelopathic effect of different concentrations of Moringa oleifera leaves crude powder (MOLCP) on mean values of maximum quantum yield of photosystem II (Fv/Fm) of Vicia faba and Zea mays leaves. Each value is the mean of triplicates. Bars represent standard deviation.

DISCUSSION

Chemical constituents of aqueous extract of Moringa oleifera fresh leaves were found to be tannins, saponins, carbohydrates, flavonoids, cardiac glycosides, alkaloids, steroids and terpenes. On the other hand, the aqueous extract of the dried leaves contain the same constituents like that of the fresh leaves except for the absence of steroids and terpenes. The residual aqueous fraction of the extract contained tannins, carbohydrate, flavonoid, and phlobatannin (Furo and Ambali, 2012). In addition, Bamishaiye et al. (2011) and Sinha (2012) reported that leaves of M. oleifera contained a number of phytochemicals such as alkaloids, glycerides flavonoids, steroids, terpenoids, saponins, tannins and anthraquinone.

Multiple cropping has been experienced in many parts of the world as a technique to maximize land productivity in a specific area in a growing season. However, plant litter or fallen leaves upon rainfall or irrigation water leaches out several allelochemicals which influence the growth of the same or other intercrops.

The present study was conducted to investigate the biological interactions between *M. oleifera* leaves aqueous extracts (MOLAE) and crude powder (MOLCP) and the two recipient intercrops; *V. faba* and *Z. mays*. MOLAE showed inhibitory effects on seed germination, plumule and radicle lengths in the two recipient plants. The inhibitory effect of the donor plant is directly proportional to the increasing extract concentrations and the effect was more prominent in *V. faba* relative to *Z. mays*. To go through with this, the shoot and root growth as well as their dry weights were inhibited in all treatment when compared with the control upon applying MOLCP. **Rajangam (1984)** stated that the aqueous extracts of *Rhizophora apiculata* decreased the growth of *Capsicum annum* considerably only at higher concentration. Similar inhibition of shoot and root length of *Oryza sativa*, *Zea mays* was also documented by **Eyini et al. (1989)**. Specifically, **Hossain et al. (2012)** reported that any plant part whether leaf, root, bark, fruit kernel or fruit of *M. oleifera* irrespective of concentrations has inhibitory effect on the rate of germination of *Vigna radiata* in laboratory condition. **Phiri and Mbewe (2010)** reported that addition of *M. oleifera* leaves extracts reduced germination percentage of groundnut seed and caused lower seedling survival. Besides, it also reduced hypocotyl formation and length resulting in delayed crop emergence and low field establishment of some legume crops. In consistent with the present study the extracts reduced germination percentage, radicle and hypocotyl of rice and sorghum. 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)** found a gradual increase of inhibition percentage in some germination parameters of *Vicia faba* as a response to the higher concentration levels of *Medicago sativa* aqueous extract. The reduction in plumule and radicle lengths of *Chenopodium album* and *Portulaca oleracea* upon applying of *Eucalyptus rostrata* aqueous extract may be attributed to the presence of a diversity of allelochemicals in the extracts (**Abou-Zeid and El-Darier, 2014**). As well, the allelotoxic effects of *M. oleifera* had been studied by several authors (**Fuglie, 2000; Phiri and Mbewe, 2009 Phiri, 2010**). Alternatively, a recent trial suggested that *M. oleifera* possesses some bio-herbicidal properties that may be used to help suppress of *Euphorbia heterophylla*. Explicitly, the higher concentrations of *M. oleifera* fresh leaves extract suppress the seed germination and some growth parameters of *E. heterophylla* more than the lower and the control. Seedling survival got reduced as the concentration of extract increased which may suggest that *M. oleifera* possesses some bio-herbicidal properties (**Oluwafemi, 2014**).

Allelochemicals appear to alter a variety of physiological processes. Lately, **Kancheva et al. (2014)** reported that chlorophyll is a key biochemical component that is responsible for photosynthesis and is a physiological indicator of plant condition. Changes in plant pigment content can be used to assess the impact of environmental stresses. High correlations were observed and empirical relationships derived linking plant optical properties and chlorophyll content. These relationships were used for plant stress diagnosis in terms of chlorophyll synthesis inhibition. In the current experiment, it was found that MOLCP decreased the efficiency of chlorophyll fluorescence spectra, photosynthetic pigments and photosystem II photochemistry (Fv/Fm). The effect was more detected in *V. faba* compared to *Z. mays*. Chlorophyll fluorescence, as a measure of photosynthesis (Fv/Fm) provides insights into a plant's ability to tolerate environmental stresses. The reduction could be due to the disturbance or damages of photosynthetic apparatus (PS II) (**Niinemets, 2002**). It is also direct indicators of the photosynthetic activity (**Lichatenthaler and Babani, 2000**) which mainly may be due to stomatal process (**Hamidou et al., 2007**).

Yang et al. (2004) reported that phenolic allelochemicals influence both degradative and synthetic pathways of chlorophyll. Allelochemicals can affect the performance of the three main processes of photosynthesis: stomatal control of CO₂ supply, thylakoid electron transport (light reaction), and the carbon reduction cycle (dark reaction) (**Zhou and Yu, 2006**). Furthermore, it has been reported that the allelochemicals produced by invasive species affect the photosynthesis and plant growth by destroying the chlorophyll (**Peng et al., 2004**). Various studies have shown that, allelochemicals released by allelopathic plants do have negative effects on leaf chlorophyll content of neighboring plant species (**Oyerinde et al., 2009**). The action of allelochemicals affects large number of biochemical reactions of target species resulting in alteration of different physiological functions (**Gniazdowska and Bogatek, 2005**). The allelochemicals released to the environment by cohort plant species, have significant effects on neighboring plants by reducing the rate of photosynthesis and respiration processes and finally reduce yield (**Bogatek and Gniazdowska, 2007**).

In conclusion, the cultivation of the invasive species; *M. oleifera* accompanied with *V. faba* and *Z. mays* as intercrops is not recommended for its dramatic effects on germination efficiency and growth besides some photosynthetic activity of the two species. Therefore, *M. oleifera* must be considered as an allelopathic species posing risk in a rotation or an intercropping or mixed cropping system. With a view to alleviate its adverse effects on intercropping or subsequent crops, farmers should be conscious of leaves fallen from the mature trees and mixed

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

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