Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2022.154.162

Research Article
Herbicidal Potential and Identification of Allelochemicals from *Moringa oleifera*

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Abstract

Background and Objective: Sunflower is one of the most important oilseed crops in the world due to the high oil contents in its seeds. However, its production is less as compared to potential use due to weed competition as one of the most factors affecting production. This study aimed to raise seed production and consequently oil production through overcoming weed problems using natural extract of *Moringa oleifera.* **Materials and Methods:** Sunflower plants and associated *Econocloa colonum* grassy weed in pot experiment were sprayed with Root, leaf and seeds water extracts of *Moringa oleifera* at the two concentrations (5 and 10%). **Results:** The results indicated a significant reduction in *Econocloa colonum* growth by all types of extracts as compared to the control. The higher concentration of leaf extract induced Maximum inhibition. In contrast, the reduction in weed growth was accompanied by an increase in sunflower growth and yield which was attained more increase by a higher concentration of leaf extract (10%). The results indicated that total polyphenol contents in the leaf extract were more than double their correspondence in both root and seed extract. Flavonoids were found in leaf extract only. The total reducing sugar in both leaf and seed extract was more than that in the root extract. The antioxidant activity in leaf extract was more than double of their correspondence in seed and root extract. **Conclusion:** Results are promising for controlling weeds as well as growth enhancement of the target plants especially by leaf extract of *Moringa oleifera.*

Key words: Water seed extract, water leaf extract, water root extract, sunflower, weed, allelopathic, metabolism

Citation: El-Rokiek, K.G., A.N. Shehata, S.A. Saad El-Din and R.A. Eid, 2022. Herbicidal potential and identification of allelochemicals from *Moringa oleifera*. Asian J. Plant Sci., 21: 154-162.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sunflower is considered the most important oilseed crop in the world because of the high oil content of its seeds (38-50%). Due to its significance in vegetable oil production, sunflower has been classified as an economically important crop[1]. Sunflower oil seed is considered better for health. So, the crop is grown for its oil. However, its production is less as compared to potential use due to several factors. Weed competition is one of these factors. Sunflower plants are usually planted at lower densities than many other crops and grows slowly during the first two to three weeks. Weeds that appear and establish during this time can be very competitive and reduce sunflower yield potential; however, sunflower is a strong competitor with weeds that appear three or more weeks after sunflower emergence. Therefore, maintaining sunflower weeds free for the first three to four weeks after planting will minimize yield losses from weeds[2].

Moringa oleifera which is related to the Moringaceae family considered an important vegetable in many tropical and sub-tropical countries. Its annual production is higher than many other secondary vegetables. It has multipurpose; Leaves, flowers and unripe fruits are used as vegetables and roots and barks are used for medicinal purposes[3]. *Moringa oleifera* possesses great allelopathic potential either to inhibit[4] or stimulates the other plants[5]. Both effects are either inhibiting or stimulating depending on concentration as well as all allelopathic materials[6]. Allelopathic plants are known to produce allelochemicals that affect germination, growth, metabolism, development and reproduction of neighbouring plants[7]. The allelopathic effects of *M. oleifera* have been documented by several workers[4-5,8-9]. *Moringa oleifera* increased growth, resulting in plant development and consequently resistance against pests and diseases[8]. *Moringa oleifera* leaf extract increased the capacity of metabolic materials in plants[10]. It increased cell elongation and division[11]. *Moringa oleifera* extract is used as growth regulators[12]. On the other hand, Awasthi *et al.*[13] reported that the leaf extract of *M. oleifera* reduced shoot length, root length, number of roots/plants, number of leaves/plant of wheat and mustard as compared to the control. Hossain *et al.*[4] reported similar results on mung bean by different plant parts of *M. oleifera* at concentrations from 2.5-15%. Sarmin[14] recorded a reduction in germination, seedling growth of the wheat by different parts of *M. oleifera.*

This work aimed to evaluate the allelopathic potential of leaf, root and seed extracts of *Moringa oleifera* on the growth and yield of sunflower (cv. Giza 102) and the associated grassy weed *Econocloa colonum.*

MATERIALS AND METHODS

Study area: This study was carried out at the greenhouse of the National Research Centre, Dokki, Egypt, during the two successive growing summer seasons of 2018 and 2019.

Plant materials and growth conditions: The leaves, roots and seeds of *Moringa oleifera* were collected from the farm of the Egyptian Scientific Society of Moringa. The plant was identified by Prof. Dr. Aboelfetoh Mohammed Abdalla, National Research Center, Giza, Egypt. The collected leaves, roots and seeds were air-dried, powdered and kept for extraction.

Very fine powder of leaves, roots and seeds of *Moringa oleifera* (200 g of each) were transferred to labelled beakers. Two litres of distilled water were added and the extracts were collected and filtered through a fine mesh and pressed carefully for complete extraction. The concentrations of the produced leaf, root and seed extracts (stalk) were 10%. The other concentration for each extract (5%) was prepared by dilution of the previous extracts with distilled water. The extraction process was repeated when needing so, the extracts were always fresh.

Treatments: The pots in the greenhouse are 30 cm in diameter and 30 cm in height, contained equal amounts of sieved soil (2:1 v/v clay and sand). Sunflower seeds (cv. Giza 102) were sown 2 cm deep and allowed to germinate. Seeds of the grassy weed were planted in each pot (0.02 g) at the same time 2 cm depth in the soil. Sunflower seedlings were thinned two weeks after sowing so that two homogeneous seedlings were left per pot (for measuring vegetative growth) and one seedling (for measuring yield). Before sowing Superphosphate was added to each pot and Ammonium nitrate was added during plant growth (2:1 w/w). The experiment was arranged a complete randomized design and consisted of eight treatments including two untreated controls, sunflower only, sunflower with *Echinochloa colonum* (unweeded treatment). The other six treatments were leaf, root and seed extract of the prepared extracts of *Moringa oleifera* at 5 and 10% for each. The extracts were sprayed on sunflower plants and *Echinochloa colonum* at the rate of 50 mL pot^{-1}. The treatments were applied three times for three weeks starting from a 15 days old plant. The data were taken ten days after the last spray and at harvest.

Following data were recorded: Associated weed *Echinochloa colonum* was collected from each pot at 40 Days After Sowing (DAS), fresh and dry weight were recorded and at the end of the season the dry weight of grown weed was also recorded. Data on Sunflower were recorded for each plant

at 40 days after sowing, including plant height, number of leaves/plants, fresh and dry weight/plant. At harvest, sunflower plants were taken to measure the head diameter and head weight. Heads were air-dried and threshed to determine seeds weight/head and 100-seeds weight.

Chemical analysis of *Moringa oleifera* extracts

Plant material and preparation of extract: Roots, leaves and seeds of *Moringa oleifera* were dried then ground to a fine powder in a mechanical blender. Dried powders (20 g) were extracted with distilled water. The extract of each was filtered through filter paper Whatman No. 1 Whatman® qualitative filter paper, Grade 1 circles, diam. 45 mm, USA.

Total phenolic content: The total phenolic content of the extract was determined by the Folin-Ciocalteu method[15]. A Total of 200 µL of crude extract were made up to 3 mL with distilled water, then thoroughly mixed with 0.5 mL of Folin-Ciocalteu reagent for 3 min, followed by the addition of 2 mL of 20% (w/v) sodium carbonate. The combination was let to sit for another 60 min in the dark and absorbance was measured at 650 nm. The calibration curve was used to compute the total phenolic content and the results were compared and expressed as mg of gallic acid equivalent per g dry weight.

Total flavonoid content: The total flavonoid content of the aqueous extract was determined by the aluminium chloride colorimetric method[16]. Shortly, 50 µL of crude extract were diluted up to 1 mL with methanol, then combined with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 min of incubation and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 M NaOH solution was added and the final volume of the mixture was brought to 10 mL with distilled water. After allowing the mixture to stand for 15 min and absorbance was measured at 510 nm. A calibration curve was used to calculate the total flavonoid content which was represented as mg rutin equivalent per g dry weight.

Total glucosinolates (µmol g^{-1} DW): Total glucosinolates were extracted from dry samples. Glucosinolates were measured by determining the liberated glucose released during hydrolysis by myrosinase enzyme[17]. The resulting glucose was determined colorimetrically according to the methods defined by Nasirullah and Krishnamurthy[18].

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl assay: The antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay as described earlier with some modifications[19]. About 200 µL of each extract were mixed with 3.8 mL DPPH solution and incubated in the dark at room temperature for 1 hr. The mixture's absorbance was then measured at 517 nm. Positive control was employed which was ascorbic acid. The ability of the sample to scavenge DPPH radical was determined from the equation:

$$\text{DPPH scavenging effect} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Detection of various phenolic acids and flavonoids in leaves, seeds and roots of *Moringa oleifera* plant

Plant materials and chemicals: All chemicals and solvents were of analytical grade. Standards for phenolic acids (Chlorogenic, caffeic, cinnamic, tannic, gallic and ferulic acids). Standards of flavonoids (Quercetin, diosmin, naringenin, naringin, rutin and hesperidin) were obtained from Sigma-Aldrich Company.

Methods

Preparation of extracts and standards solution for TLC analysis

Extract solutions: Air-dried ten grams powdered leaves, roots and seeds were extracted with methanol for 30 min and filtered.

Standards solutions: Flavonoids and phenolic acids standards were prepared as 0.05% solution in methanol[20-21].

Thin-layer chromatography of flavonoids and phenolic acids: TLC was performed on glass sheets coated with 0.25 mm layers of silica gel 60 F_{254}. After application of extracts and standards solution (10 µL) The first glass sheet of phenolic acids put in a glass jar contains mobile phase ethyl acetate: formic acid: water(8:1:1) (v:v:v)[15]. Visualization of the phenolic acids was achieved by spraying the sheet with 2% methanolic ferric chloride. The second glass sheet of flavonoids put in a glass jar contains mobile phase butanol: Acetic acid: Water (4:1:5) (v:v:v)[16]. Visualization of flavonoids was achieved by spraying the sheets with 1% methanolic aluminium chloride. The plate chromatogram was evaluated UV light at λ = 366 nm (flavonoids appeared as orange-yellow bands).

Statistical analysis: All data were statistically analyzed according to Snedecor and Cochran[22] and the treatment means were compared by using LSD at a 5% level of probability.

RESULTS AND DISCUSSION

Weed: The results in Table 1 reveal significant inhibition in both fresh and dry weight of *Echinochloa colonum* 40 Days After Sowing (DAS) as well as at the end of the season with spraying *Moringa oleifera* leaf, root and seed extracts as compared to the unweeded control. The degree of inhibition was different according to the type and concentration of the extract. Maximum growth inhibition was realized by the leaf extract especially the higher concentration (10%). The growth inhibition of *E. colonum* was persistent during the experimental period. Dry weight reduction of *E. colonum* reduced to 83.38% (reduced from 2.739-0.455 g) of that in the untreated pots 40 DAS with spraying leaf extract at 10%. This inhibition increased to 89.24% (reduced from 107.200-11.533 g) of the untreated weed by the same treatment at the end of the season as maximum inhibition.

Sunflower growth: The different growth parameters like plant height, the number of leaves/plants as well as fresh and dry weight of sunflower were increased significantly over the unweeded plants (sunflower+*E. colonum*) by *Moringa oleifera* leaf, root and seed extract in Table 2. The results show that the enhancement effect of *M. oleifera* root extract on the different growth parameters was more than both leaf and seed extracts as compared to the unweeded plants. A great significant increase in dry weight over unweeded plants was recorded in sunflower by the higher concentration (10%) of *M. oleifera* root extract (Table 2). The root water extract not only enhanced growth but also increased the branches from one branch in the control to more than three branches (miracle tree) by both concentrations (5 and 10%).

Sunflower yield and yield components: The responses of the sunflower yield parameters to different *M. oleifera* extracts were positive (Table 3). Significant increases in different

Table 1: Effect of leaf, root and seed extracts of *Moringa oleifera* on the growth of *Echinochloa colonum* associated sunflower plants (average of the two seasons)

		40 DAS		
Treatments	Concentration (%)	Fresh weight (g)	Dry weight (g)	At the end of the season (g)
Weed free	0	0.000	0.000	0.000
Unweeded	0	7.270	2.739	107.200
Leaf extract	5	2.361	0.750	16.500
	10	2.084	0.455	11.533
Root extract	5	3.633	1.738	45.000
	10	3.696	0.975	34.000
Seed extract	5	5.290	1.101	31.333
	10	3.246	0.782	32.500
LSD at 5%		0.354	0.137	2.452

Table 2: Effect of leaf, root and seed extracts of *Moringa oleifera* on different growth parameters of sunflower 40 days after sowing (average of the two seasons)

Treatments	Concentration (%)	Plant height	Number of leaves	Fresh weight (g)	Dry weight (g)
Weed free	0	58.0	11.0	28.35	3.250
Unweeded	0	38.0	10.0	10.00	1.272
Leaf extract	5	47.0	10.0	26.25	3.334
	10	53.0	16.0	33.30	3.486
Root extract	5	46.5	10.0	18.86	3.248
	10	59.0	17.2	44.25	4.545
Seed extract	5	45.0	9.5	11.60	2.782
	10	45.5	11.0	15.10	2.595
LSD at 5%		0.89	0.768	1.59	0.259

Table 3: Effect of leaf, root and seed extracts of *Moringa oleifera* on sunflower yield (average of the two seasons)

Treatments	Concentration (%)	Head diameter	Head fresh weight	Head dry weight	Weight of seeds/head	Weight of 100 seeds
Weed free	0	7.53	22.31	9.36	5.29	2.43
Unweeded	0	3.50	7.49	2.59	2.03	1.73
Leaf extract	5	6.33	19.56	6.08	4.05	2.49
	10	11.00	22.73	10.16	5.43	2.23
Root extract	5	6.33	13.76	7.69	3.56	2.18
	10	13.00	26.50	13.09	7.45	3.50
Seed extract	5	5.33	11.33	4.61	4.02	1.91
	10	7.00	11.50	7.12	4.36	2.71
LSD at 5%		0.45	1.98	0.92	0.25	0.19

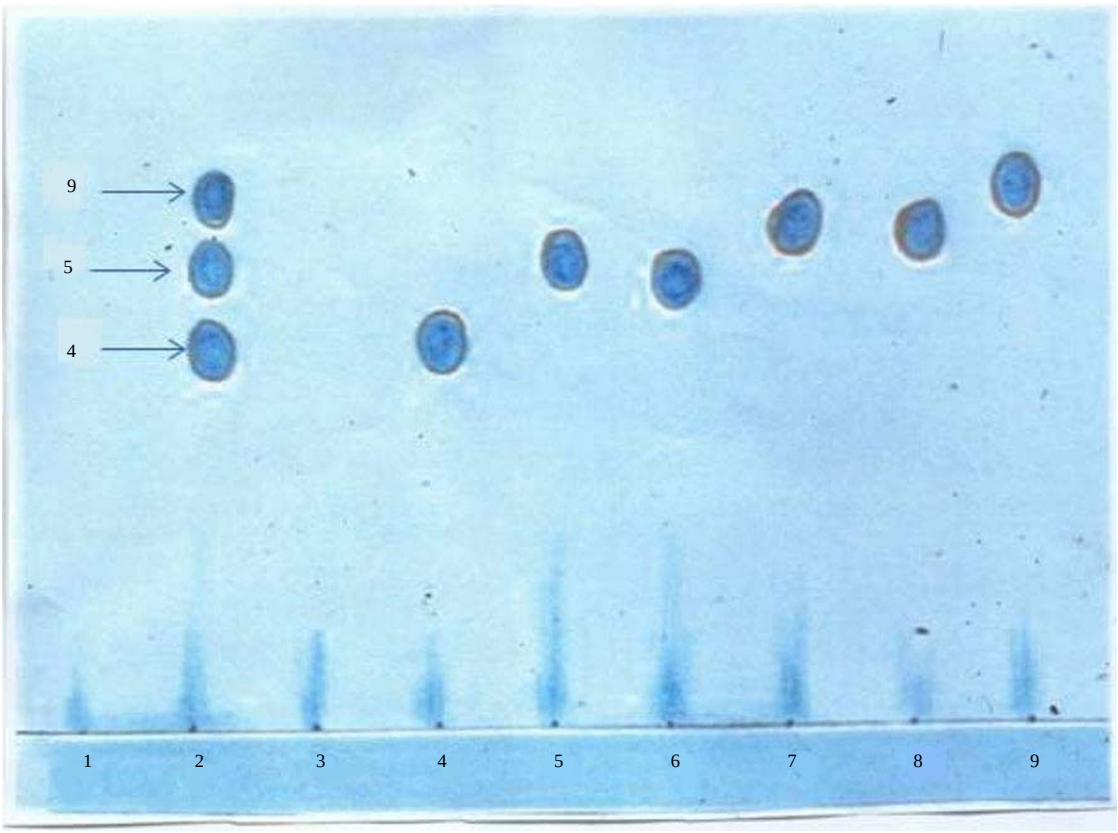

Fig. 1: Schematic TLC analysis of phenolic acids on silica gel
1: Methanolic extract of seeds, 2: Methanolic extract of leaves 3: Methanolic extract of stems and roots, 4-9 standards 4: Chlorogenic acid, 5: Tannic acid, 6: Ferulic acid, 7: Cinnamic acid, 8: Caffeic acid, 9: Gallic acid

Table 4: Total polyphenol (mg gallic acid/g dry weight), Flavonoids (mg rutin/g dry weight) and glucosinolates (µmol g^{-1} dry weight) in *Moringa oleifera*

Part of plant		Leaves	Roots	Seeds
Total polyphenol	Water extract	8.3	3.7	3.4
	Methanol extract	7.9	3.0	2.8
Flavonoids	Water extract	41.5	0.0	0.0
	Methanol extract	46.8	0.0	0.0
Glucosinolates		742.9	660.7	701.5

Table 5: Reducing sugars concentration (mg glucose/g dry weight) and antioxidant activity by 1,1-diphenyl-2- picryl-hydrazyl (DPPH) assay (%)

Part of plant		Leaves	Roots	Seeds
Reducing sugars	Water extract	5.6	3.9	5.8
	Methanol extract	5.0	3.8	4.0
Antioxidant activity	Water extract	80.0	33.0	17.0
	Methanol extract	80.0	30.0	15.0

growth parameters (Table 2) due to spraying with *M. oleifera* leaf, root and seed extracts was accompanied by a development in sunflower yield and yield components like head diameter, head fresh weight, head dry weight, the weight of seeds/head and weight of 100 seeds (Table 3) in comparison to that of unweeded control. The root extract was more effective than those extracts of leaves or seeds. The treatment of sunflower plants with the root extract at 10% caused a marked significant increase in weight of seeds/ head reaching more than 260% (from 2.03-7.45 g) over unweeded plants. The corresponding result in the weight of 100 seeds reached more than 100% (from 1.73-3.50 g).

Results of analysis of leaf, root and seed extracts of *Moringa oleifera*: The results indicate that the total polyphenol in the leaf water extract of *M. oleifera* was is more than two-fold their correspondence in both root and seed water extract. However, estimating flavonoids in the root and seed water extract show no results while were found in the leaf water extract in Table 4. In addition, estimating glucosinolates in leaf, root and seeds of *M. oleifera* showed that glucosinolates in the leaf were superior in comparison to their correspondence in both root and seed in Table 4.

The result of Table 5 reveals that reducing sugars in leaf and seed water extract of *M. oleifera* were more than root water extract, while antioxidant activity in leaf water extract

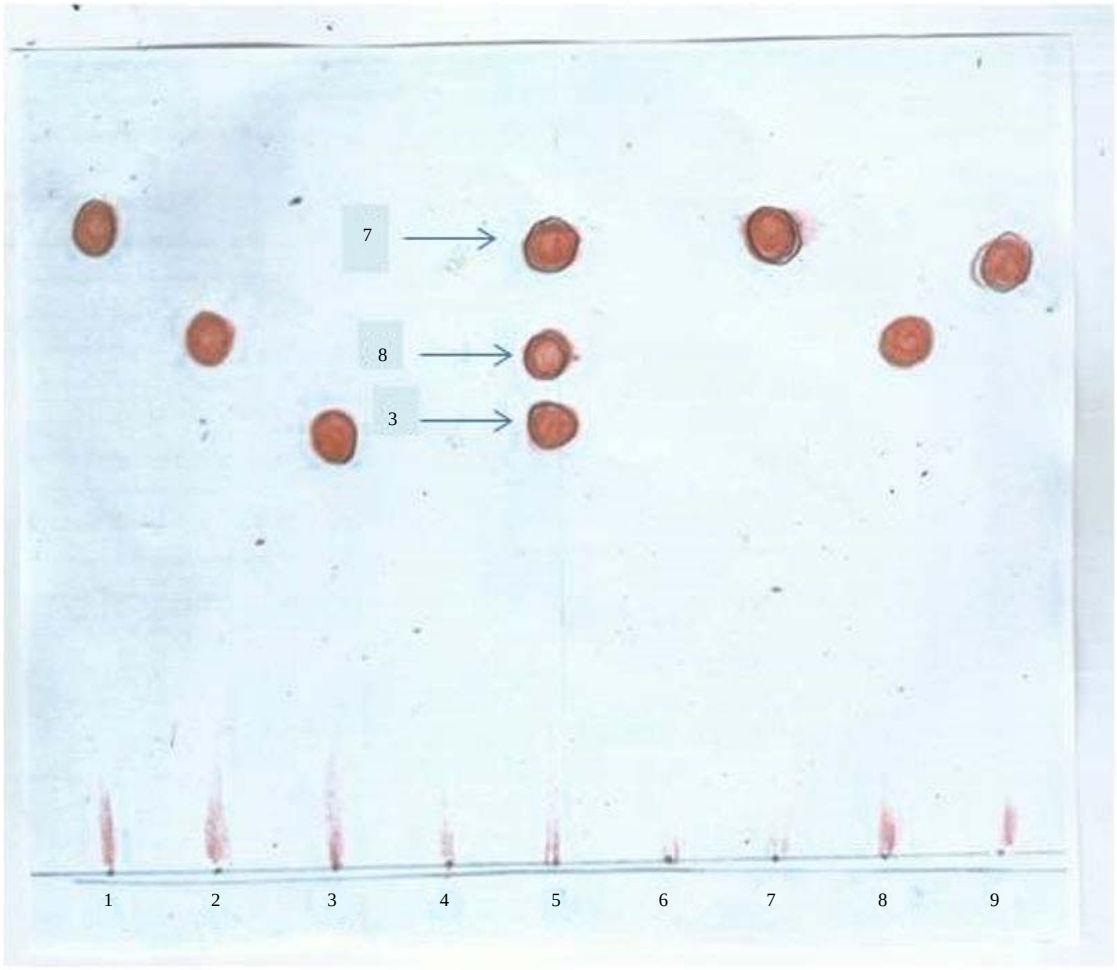

Fig. 2: Schematic TLC analysis of flavonoids on silica gel

1: Quercetin, 2: Naringin, 3: Diosmin, 4: Methanolic extract of seeds, 5: Methanolic extract of leaves, 6: Methanolic extract of stem and roots,7: Naringenin, 8: Rutin, 9: Hesperidin

Table 6: TLC analysis of phenolic acids and flavonoids on silica gel, methanolic extract of leaves, methanolic extract of roots and seeds methanolic extract

Part of plant		Leaves		Roots	Seeds
Phenolic acids	Gallic acid	Tannic acid	Chlorogenic acid	Non	Non
Flavonoids	Diosmin	Rutin	Naringenin	Non	Non

exceeded their correspondence in water extracts of root and seeds. In addition, the results obtained from TLC (Thin Layer Chromatography) analysis of phenolic acids in Table 6 and Fig. 1 showed only the methanolic extract of *M. oleifera* leaves contain gallic, tannic and chlorogenic acids. Also, for flavonoids, only the methanolic extract of *M. oleifera* leaves contains diosmin, rutin and naringenin in Table 6 and Fig. 2.

Moringa oleifera as many allelopathic plants contains secondary metabolites which are stimulators or inhibitors according to the concentration used. When used in lower concentration enhanced the yield[23], while inhibited germination and growth when applied at higher concentrations[4].

Herein in the present study, leaf, root and seed water extract were used to find out their effect on the aggressive grassy weed, *Echinochloa colonum* associated sunflower. The results indicated significant inhibition in both fresh and dry weight of *E. colonum* 40 Days After Sowing (DAS) as well as at the end of the season with spraying *M. oleifera* leaf, root and seed extracts as compared to the unweeded control. Using *M. oleifera* leaf extract at 10% controlled more than 89% of *E. colonum.* In this connection, Awasthi *et al.*[13] recorded a reduction in seedling growth of mustard and wheat.

Hossain *et al.*[4] cited Similar results on mung bean growth. In addition, the seedling growth of wheat was reduced by water extracts of different parts of *M. oleifera*[14]. Furthermore, Hordeum vulgare and *Trigonella foenum-graecum* germination and seedling growth were reduced by leaf extract of *M. oleifera*[24]. Analysis of leaf, root and seed water extracts of *M. oleifera* revealed that polyphenols in the leaf extract were more than two-fold compared to its correspondence in root and seed extracts. In addition, the leaf extract of *M. oleifera* contains a high amount of flavonoids while the results recorded none of the flavonoids present in both root and seed extracts. These results confirmed that phenols or flavonoids or both may be the cause of allelopathic inhibition. Different documented results confirmed these obtained results[25-27]. The results of TLC analysis (Thin Layer chromatography) indicated that total polyphenol contains tannin, gallic and chlorogenic acids in Table 6 and Fig. 1 and flavonoids contain rutin and diosmin and naringenin in Table 6 and Fig. 2. These results are similar to those obtained by Ahmad *et al.*[28] in *Teucrium royleanum* (Labiatae). A recent study documented that fresh and dry weight of common weeds; *Commelina communis*, *Artemisia princeps* var. orientalis, *Bidens frondosa* and *Oenothera biennis* were reduced by *Miscanthus* sacchariferous water extract[29]. The authors attributed the reduction in weeds to the presence of different phenolic compounds which contain chlorogenic acids and rutin. This suggestion confirmed our foundation. It is worthy to mention that the analysis of the extracts was carried out in both methanolic and water extracts and the difference between the two amounts was minor.

In addition, *M. oleifera* is related to order brassicales, this order contains glucosinolates that have a role against weed suppression[30-31]. So, glucosinolates in *M. oleifera* leaves, roots and seeds were estimated and the results illustrated in Table 5. The results showed that glucosinolates in the leaf extract were superior in comparison to their correspondence in both root and seed extract. This added another explanation that is why leaf extract exhibited more inhibiting activities against *E. colonum*. According to Price *et al.*[32] glucosinolates contain sulfur and nitrogen, are enzymatically hydrolyzed by myrosinase in the presence of water to form isothiocyanates, the active allelochemicals that may act against weed growth.

Although *M. oleifera* leaf extract-controlled *E. colonum* more than root and seed extract, the root extract resulted in an unexpected increase in growth of sunflower plant and yield (unique branching) compared to the corresponding control. This means that the increase in sunflower growth and consequently yield is not only attributed to controlling weeds but also to growth stimulation. The enhancement in growth increased the competitive ability of sunflower against *E. colonum*. Similar results were found by Phiri[5] and Jahangeer[33]. In this connection, Cheema *et al.*[23] attributed the promotive effect of moringa to the regulation of different physiological effects. The data in Table 6 show the percentage of anti-oxidant activity. In general, Antioxidants compounds may protect cells from damage caused by free radicals[34]. They are secondary metabolites as total phenol that can directly react with superoxide anions and lipid peroxyl radicals and consequently inhibit or break the chain of lipid peroxidation[35]. They induce better root and shoot growth[36]. Also, Table 4 show that total phenol in the root extract of moringa was lower than leaf extract and this may be the reason for promoting the activity of root according to El-Awadi *et al.*[37] who reported that phenolic acids have promotive effect at lower concentrations.

Thus, an increase in sunflower growth and yield may result from *E. colonum* control by different extracts of *M. oleifera* or enhancement effect. Several workers proved that increase in crop growth and yield was concomitant with controlling weeds[27,38-39]. This study indicated inhibition in weed growth by the extracts of different parts of *Moringa oleifera*. This means that *M. oleifera* possesses allelopathic property i.e., contain allelochemicals. So, based on these results, *M. oleifera* is considered a source of non-toxic natural materials that can be recommended to use as an alternative to chemical herbicides at concentrations started from 5% up to 10%.

CONCLUSION

The biochemical constituents in leaf, root and seed extract of *M. oleifera* in the current study indicates that can be used as bioherbicides against *E. colonum* and enhancement of *M. oleifera* growth and yield. The leaf extract of *M. oleifera* as well as root extract, increased sunflower yield. Results are confident for controlling weeds as well as the development of growth and yield of the target plants by *Moringa oleifera*.

SIGNIFICANCE STATEMENT

The results of this study revealed controlling grassy weed *Avena fatua* by leaf, root and seed extracts of *Moringa oleifera* and in turn, increasing seed yield and consequently increasing oil production. This investigation demonstrated the possibility of using natural extracts as bioherbicides alternative to chemical herbicides.

ACKNOWLEDGMENTS

The authors are thankful to the Agricultural Research Centre, Egypt, for providing the seeds of this experiment. The authors are grateful to the National Research Centre, Egypt, for providing materials, laboratories and facilitating this work.

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