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Optimization of *Moringa oleifera Lam*. Leaf Extract as a Priming Tool and Plant Nutrient Supplement for *Helianthus annuus* L

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ABSTRACT

To evaluate the comparative efficacy of different concentrations of moringa leaf extract on germination, seedling growth and seed yield of sunflower, two laboratory experiments in Allelopathy laboratory of Department of Agronomy and a field trial at Agronomic Research Area of University of Agriculture, Faisalabad, Pakistan, were conducted during spring, 2011. The experimental design of two laboratory experiments was CRD, while that of field trial was RCBD with factorial arrangement. Different concentrations of 5%, 10%, 15% and 20% of moringa leaf extract were used as treatments that were applied as foliar sprays at different time. The result showed that all concentration of moringa suppressed the sunflower germination, however moringa leaf extract in 5% concentration was effective in increasing the seedling growth and development. Results from field experiment showed that moringa leaf extract sprays in all concentrations at 45 and 60 DAS increased the sunflower seed yield. Thus foliar application of moringa leaf extract has the potential to increase the seed yield of sunflower and that too in an economic way.

Key words: Achene yield, Allelopathy, Foliar sprays, Growth regulators, Zeatin

INTRODUCTION

Sunflower is an oil seed crop grown for its oil which is deemed better for health. However its production is less as compared to potential. A variety of factors such as water deficiency, poor farming practices, seed of low yielding varieties and poor nutrition management are responsible for lower yield [1-5]. Moringa is the sole genus of *Moringaceae* family [6]. *Moringa* oleifera L. is widely distributed in the Pacific region, subtropical regions and in West Africa [7]. The charisma of moringa leaf juice is a substance called zeatin which is natural plant hormone [8]. Moringa leaf extract enhanced the growth of young plants, strengthened plants, improved resistance against pathogens, increased lifetime, prolonged the roots and number of roots, stems and leaves, produced more fruits and generally enhanced he yield by around 20-35% [9-12]. Moringa leaf extract contain Zeatin: a plant growth hormone from the Cytokinins group improves crop growth and yield of plants [13-16]. MLE proved an ideal plant growth enhancer in many experiments. Plant height, leaf area, chlorophyll a and b contents subjected to severe drought stress were increased significantly due to moringa leaf extract [17-22]. The objective of the these laboratory trials was to assess the comparative efficacy of different concentrations of moringa leaf extract as a seed priming tool and to evaluate the effect of different concentration levels and times of application of moringa leaf extract on growth and development of sunflower seedling along with sunflower yield as a part of nutritional management of sunflower.

MATERIALS AND METHODS

Laboratory Trial # 1: Germination of sunflower (*Halianthus annus* L.) as affected by different concentrations of moringa (*Moringa oleifera* L.) extracts

This laboratory trial was designed to assess the comparative efficacy of different concentrations of moringa leaf extract on germination of sunflower and was executed in the Allelopathy laboratory, department of Agronomy, University of Agriculture Faisalabad, Pakistan during spring 2011.

Bioassay for Moringa Leaf extract Preparation:

Moringa leaf extract (MLE) was prepared by collecting young and disease free leaves from moringa tree. Theses leaves were washed and then frozen for two days in refrigerator at 4°C. Leaves were grinded in a manual juicer to extract the leaf juice. The juice was collected and filtered by passing through a muslin cloth to remove all the green matter. Aqueous *Moringa oleifera* leaf extract (100 %) was diluted with distilled water to prepare solution of different concentrations like 5%, 10%, 15% and 20 % (v/v) as experimental treatments. The leaf extract of moringa was stored at room temperature for future use in both laboratory and field experiments.

Execution of Experiment

Ten seeds of sunflower (*Helianthus annuus* L.) were placed evenly between two layers of moist Whitman No. 42 filter paper in 9 cm diameter sterilized Petri dishes. 10 ml extract of 5%, 10%, 15% and 20 % concentration was added to Petri dishes while same volume of distilled water was applied to the control treatment. Half of the solution was used as moisture for

the filter paper receiving the seeds, while remaining was applied to the covering filter paper. During the course of experiment 3 ml distilled water was used in all the petri dishes. Germination was determined by counting and removing the germinated seeds every day over a period of ten days from each petri dish. Seed was considered germinated when radical length was over 2 mm.

Observations

Number of seeds germinated each day was recorded and final germination percentage, mean germination time and germination index were calculated. Final germination percentage was taken at the end of experiment. It represents the ratio in percentage of number of seeds germinated to the total number of seeds planted. The mean germination time (MGT) was calculated according to the formula:

 $MGT = \sum n \times g/N$

Where n is number of germinated seeds on day g and N is the total number of germinated seeds.

Germination index (GI) was recorded by the following formula:

 $GI = \Sigma Gt/Dt$

Where Gt is the number of seeds with radical at day t and Dt (day t) is the number of days since the beginning of the experiment.

Data collected were analyzed statistically using MSTAT-C, a computer package for statistical analysis and difference among treatments means were compared by employing least significant difference (LSD) test at 5% probability level [23].

Laboratory Trial # 2: Seedling growth of sunflower as influenced by different concentrations of moringa (*Moringa oleifera* L.) extract and time of application

The effect of M. oleifera on growth of sunflower seedling was studied in Allelopathy laboratory, department of Agronomy, University of Agriculture Faisalabad, Pakistan. Experiment was conducted in germinator at control temperature. Aqueous Moringa oleifera leaf extract (100 %) was diluted with distilled water to prepare solution of different concentrations as 5%, 10%, 15% and 20 % (v/v) and applied to Petri dishes at 5 DAS and 10 DAS respectively. Petri dishes 9 cm in diameter were filled with sand. Ten seeds were sown in each Petri dish. The experiment was conducted using a completely randomized design (CRD) with split arrangement having four replications. Distilled water was used as a control at the start of experiment. 10 ml extract of 5%, 10%, 15% and 20 % concentration was added once in each petri dish at 5 DAS and 10 DAS separately as experimental treatments. During the course of experiment 3 ml distilled water was added to each Petri dish. The petri dishes were placed in germinators at 25°C for a period of 15 days.

Observations:

The shoot length was measured with the help of scale in centimeters. The length was measured from the point where the root and shoot joins together at the end of the root and to the top of the shoot. The lengths of all plants in a pot were averaged to get mean length of a replication. Roots and shoots of all seedlings were separated and fresh weight of roots and shoots of all potted plants was taken immediately after the harvesting. For dry weight the samples were oven dried at 70 °C for 48 hours and then they were weighed separately. Data collected were analyzed statistically using MSTAT-C, a computer package for statistical analysis and difference among

treatments means were compared by employing least significant difference (LSD) test at 5% probability level.

Field Trial: Yield and quality of sunflower as influenced by different concentrations of moringa (*Moringa oleifera* L.) extract and time of application

A field experiment was conducted at Agronomic Research Area, University of Agriculture Faisalabad, during spring 2011. The climate of the region is subtropical to semi-arid. The experimental area is located at 73.09 ⁰East Longitude, 31.25 ⁰ North Latitude and at an altitude of 183 m above sea level. The experiment was laid out in Randomized Complete Design with split plot arrangement. Time of application (25, 40 and 55 DAS) of moringa leaf extract was kept in main plots, while different concentrations (5%, 10%, 15%, and 20%) of moringa leaf extract were placed in sub plots.

Statistical Analysis: The data collected were subjected to two way ANOVA with the help of computer program MSTAT-C and treatment means were compared at 5% probability level [24].

RESULTS AND DISCUSSION

Laboratory Trial # 1: Germination of sunflower (*Halianthus annus* L.) as affected by different concentrations of moringa (*Moringa oleifera* L.) extracts

Germination (%) and Germination index

The results for germination percentage are given in the Table 1.1. The results showed that the effect different concentrations of of *Moringa oleifera* leaf extracts for germinations are non-significant. The maximum value of germination percentage (100.00) was observed in control treatment, which shows that *Moringa oleifera* at each concentration suppressed the germination of sunflower. The results for germination index are given in Figure 1. The results showed that the effect of different levels of *Moringa oleifera* leaf extracts for germinations index was significant. The maximum value of germination index (6.1) was observed in 5 % *Moringa oleifera* leaf extract which showed that *Moringa oleifera* at 5% concentration enhanced the germination of sunflower. These results are in complete confirmation with results from many laboratory and field trials [25-28].

Table 1.1: Germination (%) of sunflower as influenced by time of application and concentration level of moringa leaf extract.

	Recordings		
Treatments			
	5 DAS	10 DAS	Means
T_1 = Control	100.00 A	97.50 AB	98.75 A
T ₂ = (5% Moringa	95.00 AB	87.50 BC	91.25 AB
leaf extract)			
$T_3 = (10\% \text{ Moringa})$	90.00 ABC	80.00 CD	85.00 BC
leaf extract)			
$T_5=(15\% \text{ Moringa})$	87.50 BC	80.00 CD	83.75 BC
leaf extract)			
T ₅ = (20% Moringa	90.00 ABC	72.50 D	81.25 C
leaf extract)			
Means	92.50 A	83.50 B	

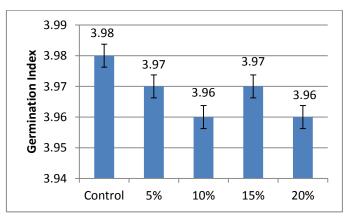


Fig. 1: Germination index of sunflower as influenced by time of application and concentration level of moringa leaf extract.

Mean germination time

The results for mean germination time are given in the Table 1.2. The results showed that the effect of different levels of *Moringa oleifera* leaf extracts for mean germinations time was significant. The maximum value of mean germination time (4.88) was observed in 15 % *Moringa oleifera* leaf extract which showed that *Moringa oleifera* at 15% concentration enhanced the germination of sunflower.

Table.1.2: Mean germination time of sunflower as influenced by time of application and concentration level of moringa leaf extract.

CAHUCI.			
Treatments	Recordings		
Treatments	5 DAS	10 DAS	Means
T_1 = Control	3.95 B	3.98 B	3.96 B
T ₂ = (5% Moringa	4.57 AB	4.42 AB	4.50 A
leaf extract)			
$T_3 = (10\% \text{ Moringa})$	4.39 AB	4.32 AB	4.35 AB
leaf extract)			
$T_5=(15\% \text{ Moringa})$	4.41 AB	4.88 A	4.64 A
leaf extract)			
$T_5=(20\% \text{ Moringa})$	4.34 AB	4.85 A	4.60 A
leaf extract)			
Means	4.33 A	4.49 A	

Laboratory Trial # 2: Seedling growth of sunflower as influenced by different concentrations of moringa (*Moringa oleifera* L.) extract and time of application Shoot Length (cm) and Root Length (cm)

The results for shoot length are given in the table 2.1. The results showed that the effect of both the time of application and concentrations of different levels of Moringa oleifera leaf extracts for shoot length was significant. As regards to the interactive effect of time of application and concentration levels, the results also revealed the significant effect. As regards to interactive effect of time of application and concentration of different levels of Moringa oleifera leaf extracts, the maximum value of shoot length (16.45cm) was observed in 5% Moringa oleifera leaf extract when applied at 5 days after sowing followed by 15.51cm and 15.15cm in case of 10% Moringa oleifera leaf extract when applied at 5 days after sowing and 20% Moringa oleifera leaf extract when applied at 5 days after sowing respectively. The lowest value of shoot length (9.50cm) was recorded in 15 % Moringa oleifera leaf extract when applied at 10 days after sowing. These findings

are also supported by the findings of Fuglie [14], who reported that *Moringa oleifera* leaf extract accelerate the growth of young plants, strengthen the plants, improve resistance to pests and diseases, increase leaf area duration and increase number of roots. The results for root length are given in the Table 2.2, which showed that the maximum value of root length (10.64cm) was observed in 5% *Moringa oleifera* leaf extract when applied at 5 days after sowing followed by 8.02cm and 7.03cm in case of 15% *Moringa oleifera* leaf extract when applied at 10 days after sowing and in control respectively. The lowest value of root length (4.88cm) was recorded in 20 % *Moringa oleifera* leaf extract when applied at 5 days after sowing.

Table 2.1: Shoot length (cm) of sunflower as influenced by the time of application and concentration level of moringa leaf extract.

Componentiana	Time of Application			
Concentrations	5 Days	10 Days	Means	
T_1 = Control	13.85 BC	11.47 D	12.66 BC	
$T_2 = (5\% \text{ Moringa})$	16.45 A	12.02 D	14.24 A	
leaf extract)				
$T_3 = (10\%)$	15.51 AB	12.45 CD	13.98 A	
Moringa leaf				
extract)				
$T_5 = (15\%)$	14.40 B	9.50 E	11.95 C	
Moringa leaf				
extract)				
$T_5 = (20\%)$	15.15 AB	11.30 D	13.22 AB	
Moringa leaf				
extract)				
Means	15.07 A	11.35 B		
		1		

Table 2.2: Root length (cm) of sunflower as influenced by the time of application and concentration level of moringa leaf extract.

Concentrations	Time of Application		
Concentrations	5 Days	10 Days	Means
Control (B ₀)	5.620 DE	7.035 CD	6.3275 BC
5 % (B ₁)	10.640 A	8.950 B	9.7950 A
10 % (B ₂)	6.440 CDE	6.900 CD	6.6700 B
15 % (B ₃)	6.625 CD	8.025 BC	7.3250 B
20 % (B ₄)	4.880 E	5.450 DE	5.1650 C

Shoot fresh weight (g) and Root fresh weight (g)

The results for shoot fresh weight are given in the Table 2.3. The results showed that the effect of both the time of application and concentrations of different levels of *Moringa oleifera* leaf extracts for shoot fresh weight was significant. As regards to the interactive effect of time of application and concentration levels of *Moringa oleifera* leaf extracts, the results also revealed the significant effect. As regards interactive effect time of application and concentration of different levels of *Moringa oleifera* leaf extracts, the maximum value of shoot fresh weight (2.68g) was observed in 5% *Moringa oleifera* leaf extract when applied at 5 days after

sowing followed by 2.03g and 2.01g in case of 20% Moringa oleifera leaf extract when applied at 5 days after sowing and 10% Moringa oleifera leaf extract when applied at 5 days after sowing respectively. The lowest value of shoot fresh weight (1.25g) was recorded in 20 % Moringa oleifera leaf extract when applied at 10 days after sowing. These findings are also supported by the findings of Fuglie [14] and Iqbal et al. [29], who reported that Moringa oleifera leaf extract accelerate the growth of young plants, strengthen plants, improve resistance to pests and diseases, increase leaf area duration and increase number of roots. The results for root fresh weight are given in Figure 2. The results showed that the maximum value of root fresh weight (0.89g) was observed in 5% Moringa oleifera leaf extract when applied at 5 days after sowing followed by 0.35g and 0.34g in case of 10% Moringa oleifera leaf extract when applied at 10 days after sowing and 15% Moringa oleifera leaf extract when applied at 10 days after sowing respectively. The lowest value of root fresh weight (0.20g) was observed in control treatment. These findings are also supported by the findings of Lee et al. [30], who reported that Moringa oleifera leaf extract accelerate the growth of young plants, strengthen plants, improve resistance to pests and diseases, increase leaf area duration, increase number of roots.

Table 2.3: Shoot fresh weight (g) of sunflower as influenced by the time of application and concentration level of moringa leaf extract.

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Componentiana	Time of Application			
Concentrations	5 Days	10 Days	Means	
Control (B ₀)	1.5675 bcd	1.6250 bcd	1.5962 B	
5 % (B ₁)	2.6850 a	1.8825 bc	2.2837 A	
10 % (B ₂)	2.0125 b	1.6250 bcd	1.8188 B	
15 % (B ₃)	1.9700 b	1.2500 d	1.6100 B	
20 % (B ₄)	2.0375 b	1.3500 cd	1.6937 B	
Means	2.0545 A	1.5465 B		

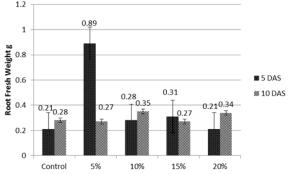


Fig. 2: Root fresh weight of sunflower as influenced by time of application and concentration level of Moringa oleifera leaf extract.

Shoot Dry Weight (g) and Root Dry Weight (g)

The results for shoot dry weight are given in the Table 2.4. The results showed that the effect of both the time of application and concentrations of different levels of Moringa oleifera leaf extracts for shoot dry weight is significant. As regards to the interactive effect of time of application and concentration levels of Moringa oleifera leaf extracts, the results also revealed the significant effect. As regards interactive effect time of application and concentration of different levels of Moringa oleifera leaf extracts, the maximum value of shoot dry weight (1.34g) was observed in 5% Moringa oleifera leaf extract when applied at 5 days after sowing followed by 1.00g and 0.98g in case of 10% Moringa oleifera leaf extract when applied at 5 days after sowing and 20% Moringa oleifera leaf extract when applied at 5 days after sowing respectively. The lowest value of shoot dry weight (0.78g) was recorded in 20 % Moringa oleifera leaf extract when applied at 5 days after sowing. These findings are also supported by the findings of Fuglie [14], who reported that Moringa oleifera leaf extract accelerate the growth of young plants, strengthen plants, improve resistance to pests and diseases, increase leaf area duration and increase number of roots. The results for root dry weight are given in Figure 3. The results showed that the maximum value of root dry weight (0.29g) was observed in 5% Moringa oleifera leaf extract when applied at 10 days after sowing control treatment followed by 0.172g and 0.17g in case of 5% Moringa oleifera leaf extract when applied at 5 days after sowing and 5% Moringa oleifera leaf extract when applied at 5 days after sowing respectively. The lowest value of root dry weight (0.10g) was in case of control. These findings are also supported by the findings of Fuglie [14], who reported that Moringa oleifera leaf extract accelerate the growth of young plants, strengthen plants and improve resistance to pests.

Table 2.4: Shoot dry weight of sunflower as influenced by time of application and concentration level of Moringa oleifera leaf extract.

	Time of Application		
Concentrations	5 Days	10 Days	Means
Control (B ₀)	0.78 BCD	0.81 BCD	0.79 B
5 % (B ₁)	1.34 A	0.94 BC	1.14 A
10 % (B ₂)	1.00 B	0.81 BCD	0.90 B
15 % (B ₃)	0.98 BC	0.62 D	0.80 B
20 % (B ₄)	0.90 BCD	0.67 CD	0.79 B
Means	1.00 A	0.77 B	

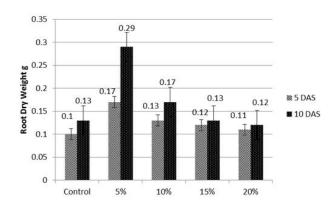


Fig. 3: Root dry weight (g) of sunflower as influenced by time of application and concentration level of Moringa oleifera leaf extract.

Field Trial: Yield and quality of sunflower as influenced by different concentrations of moringa (Moringa oleifera L.) extract and time of application

Plant height (cm)

Plant height is a key indicator of crops growth and yield as dwarf plants result in lower growth as well as yield. Moringa leaf extract in 20% concentration applied as a foliar spray at 40 DAS gave the maximum plant height of 187.97 cm and it was statistically at par with treatment in which 20% moringa leaf extract was sprayed at 55 DAS (Table 3). These results are in agreement with those of Jeyakumar *et al.* [28] and Iqbal *et al.* [29], who observed more growth and yield of crop plants when growth regulators were exogenously applied.

Table 3.1: Plant height (cm) of sunflower as influenced by time of application and concentration levels of moringa leaf extract.

Concentrations	Time of Application			
Concentrations	25 DAS (A ₁)	40 DAS (A ₂)	55 DAS (A ₃)	Mean
B ₀ = Control	149.17 H	157.47 FGH	162.03 EFG	156.22 E
B ₁₌ 5% moringa leaf	155.60 GH	170.07 CDE	166.43 CDE	164.03 D
extract				
B ₂₌ 10% moringa leaf	162.07 DEFG	173.20 BC	170.67 CD	168.64 C
extract				
B ₃₌ 15% moringa leaf	166.07 CDEF	180.57 AB	173.63 BC	173.42 B
extract				
B ₄₌ 20% moringa leaf	167.73 CDE	187.97 A	181.17 A	178.96 A
extract				
Mean	160.13 B	173.69 A	170.95 A	

Economical yield (t ha⁻¹)

The data pertaining to economical yield is given in the Table 8. The data showed that the effect of both the time of application and concentrations of different levels of *Moringa olifera* leaf extracts on economical yield was significant. As regards to the interactive effect of time of application and concentration levels, the results also revealed the significant effect. As regards to interactive effect of time of application and

concentration of different levels of *Moringa oleifera* leaf extracts, the maximum value of economical yield (2.96 t ha⁻¹) was observed in treatment combinations A_2B_4 (20% moringa leaf extract applied at 40 DAS). The lowest value of economical yield (2.19 t ha⁻¹) was recorded in control, in complete confirmation with Afzal *et al.*, [31], who reported more yields of a variety of crops with application of growth regulators and zeatin.

Table 8: Economic yield (t ha-1) of sunflower as influenced by time of application and concentration levels of moringa leaf extract.

Concentrations	Time of Application			
Concentrations	25 DAS (A ₁)	40 DAS (A ₂)	55 DAS (A ₃)	Mean
B_0 = Control	2.25 DEF	2.22 EF	2.19 F	2.22 C
B ₁₌ 5% moringa leaf extract	2.34 CDEF	2.66 ABCD	2.63 ABCDE	2.54 B
$B_{2=}$ 10% moringa leaf extract	2.46 BCDEF	2.74 AB	2.68 ABC	2.64 AB
$B_{3=}$ 15% moringa leaf extract	2.60 ABCDEF	2.79 AB	2.68 ABC	2.69 AB
$B_{4=}$ 20% moringa leaf extract	2.71 ABC	2.96 A	2.77 AB	2.81 A
Mean	2.4783 B	2.6858 A	2.59 AB	

CONCLUSION

Thus Moringa leaf extract in 5% concentration serves as a good seed priming tool as well as its foliar spray has the potential to

increase the seed yield of sunflower and that too in an economic way. Moring leaf extract is quite economical to prepare and has proved to be a rich source of growth promoting hormones.

However there is a need to conduct extensive research to optimize moringa leaf extract concentration for priming as well as for foliar spray to boost crops yield to feed the rising needs of skyrocketing population globally.

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