

Evaluation of using *Moringa oleifera* on controlling weeds. i. Effect of leaf and seed water extracts of *Moringa oleifera* on broad and grassy weed associated *Narcissus tazetta* L.

Kowthar Gad El-Rokiek^{1*}, Rawia A Eid², Abeer Nasr Shehata³,
Samia Amin Saad El-Din¹

(1. Botany Department, National Research Centre, Dokki, Cairo, Egypt;

2. Ornamental Plants and Woody Trees Department, National Research Centre Dokki, Cairo, Egypt;

3. Biochemistry Department, National Research Centre, Dokki, Egypt, El Buhouth Street., Postal code, 12622, Dokki, cairo, Egypt)

Abstract: This study was conducted to evaluate the effect of leaf and seed water extracts of *Moringa oleifera* at different concentrations (0, 1.25%, 2.5% and 5%) on the growth and flowering of *Narcissus tazetta* L. as well as the growth of associated weeds. This study was achieved under greenhouse conditions at the National Research Center, Egypt, in the two winter seasons of the years 2014/2015 and 2015/2016. Leaf and seeds water extract of *Moringa oleifera* reduced the growth of both *Beta vulgaris* (broad weed) and *Phalaris minor* (grassy weed) as compared to the control. On the other hand, fresh and dry weights of leaves of *Narcissus tazetta* L., bulbs as well as flowering increased significantly by leaf and seed water extract over untreated control. Growth and flowering were increased with increasing concentration of the two extracts up to 5%. This increase in the growth was accompanied by increasing in both macro and micronutrient contents as well as chlorophyll and carbohydrate contents in *Narcissus tazetta* leaves. Total polyphenol and flavonoids contents in water extract were determined using Folin-Ciocalteu reagent and AlCl₃ method and their amount were calculated as mg gallic acid/g and mg rutin/g dry weight respectively. The results indicated that total polyphenol contents in the leaf extract was more than two fold of its correspondence in seed extract. Flavonoids were found in leaf extract only. Results are promising for controlling weeds as well as growth enhancement of the main plants especially by leaf extract of *Moringa oleifera*.

Keywords: *Moringa oleifera*, leaves and seeds water extract, broad and grassy weeds, total polyphenols and flavonoids

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1 Introduction

Narcissus tazetta L. belongs to the family Amaryllidaceae It is a herbaceous perennial growing from bulbs and seeds. It dominates over all spring flowers by its strong fragrance. There are about 50 species of *Narcissus*, all species are native to central Europe and Mediterranean region. It is grown as cut flowers and can

be planted in beds, in edging and borders along the paths or sides, in pots or bowls in gardens. Moreover, their volatile oils usually used in perfumes manufacturing (Hanks, 2002). *Narcissus* (*Narcissus tazetta*) is an onion plant of the Amaryllidaceae family flowering in the middle of winter and early in spring (Khalighi, 2003). This flower is one of the most important of garden plants that its species grow in any part of the world, except for tropical regions (Dole and Wilkins, 1996).

Allelopathic plants are characterized by the releasing of secondary metabolites (allelochemicals) in the environment. The produced allelochemicals have harmful or benefit effects (Yamagushi et al., 2011). These two

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* Corresponding author: El-Rokiek Kowthar Gad, Ph.D., Professor, Botany Department, National Research Centre, Dokki, Cairo, Egypt. Email: ahmed_ezat2000@yahoo.com, kowtharelrokiek@gmail.com. Tel: (+202)33371362, Fax: (+202)33370931C.

reverse effects were concentration dependent. So, the effects of these allelochemicals were promoters or inhibitors for neighboring plants according to concentrations of these released allelochemicals (Singh et al., 2005). Allelopathic plants produce allelochemicals that affect germination, growth, metabolism, development, distribution, behavior, and reproduction of other organisms (Duke et al., 1998; Batish et al., 2006; El-Rokiek, 2010). *Moringa oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. It has a wide range of medicinal uses and high nutritional value. The allelopathic effects of *M. oleifera* have been studied (Phiri and Mbewe, 2009). *Moringa oleifera* leaf extract was growth and yield promoters for number of agronomic crops when sprayed at low concentrations (Foidl et al., 2001). Phiri (2010) found that spraying leaf extract of *Moringa oleifera* at low concentrations increased both vegetative growth and grain yield of wheat and maize. On the other hand, the seedling growth parameters like shoot length, root length, number of roots/plant, number of leaves/plant of wheat and mustard were reduced by the leaf extract of *Moringa oleifera* as compared to control (Awasthi et al., 2008). Hossain et al. (2012) studied different plant parts of *Moringa oleifera* at concentrations from 2.5%-15% on the growth of mung bean. The authors reported that different concentrations of water extracts of *Moringa oleifera* reduced germination, number of leaves as well as dry weight of mung bean. The authors added that the rate of inhibition increased with increasing extract concentrations.

The present study was carried out to examine the effect of different concentrations of leaf and seed water extracts of *Moringa oleifera* on the growth and flowering of *Narcissus tazetta* L. as well as studying their effects on associated weeds.

2 Materials and methods

2.1 Preparation of leaf and seed water extracts

Very fine powder of leaves and seeds of *Moringa oleifera* (150 g of each) were transferred to labeled beakers. Three liters of distilled water were added, and allowed to soak for 24 h. Then the produced leaf and seed

extracts were collected and filtered through a fine mesh and pressed carefully for complete extraction. The concentrations of the produced leaf and seed extracts (stalk) were 5%. The other two concentrations (2.5% and 1.25%) were prepared by dilution of the previous extracts with distilled water. The extraction process was repeated when needed so, the extracts were fresh.

To check the allelopathic effect of leaf and seed water extracts of *M. oleifera*, series of concentrations (0, 1.5%, 3%, 6%, 9%, 12% and 15%) were tested on both germination and seedling growth of *Beta vulgaris* (broad leaved weed) and *Phalaris minor*. Seeds of *Beta vulgaris* and *Phalaris minor* were germinated on filter paper Whatman number 3 in 9 diameter Petri dishes. Seven milliliters of the extracts at different concentrations (from zero to 15%) were applied to the weed seeds. The Petri dishes were sealed and incubated at 30°C for 10 days. Five replicates were done for each treatment. After five days, 2 mL of the previous extracts was added. The number of germinating seeds was recorded, and the seedlings length of five randomly selected germinating seeds was measured. The experiment was repeated twice with one week interval.

Two pot experiments were carried out at the greenhouse of National Research Centre, Dokki, Egypt, during the two successive growing seasons of 2014/2015 and 2015/2016. The pots were 60 cm diameter and were filled with loamy sand soil infected with weeds. *Narcissus tazetta* L. corms were supplied by ornamental plants research, Ministry of Agriculture, Giza, Egypt. *Narcissus* mother bulbs, uniform in size (10-12 cm circumference) were used for planting. One bulb was planted in each pot at 5-6 cm depth. All the normal culture practices of growing *Narcissus* bulbs were applied as usual manner. Routine fertilizers were added as calcium super phosphate (15.5% P₂O₅) before planting at the rate of 6 g per pot, representing sources of P, ammonium sulfate (20% N) at the rate of 4 g per pot and potassium sulphate (48%, K₂O) at the rate of 2 g per pot, representing sources of N and K, respectively, were added 30 days after bulb planting. After 40 days from planting *Narcissus tazetta*, it was found that *Beta vulgaris* (broad weed) and *Phalaris minor* (grassy weed) were

dominated (the other weeds were removed if present). The prepared leaf and seed water extracts of *Moringa oleifera* at 1.25%, 2.5% and 5% were sprayed early in the morning 40 days after sowing at the rate of 250 mL pot⁻¹. The pots were sprayed three times during the three weeks. The pots were arranged in a complete block design with 8 treatments. Each treatment was represented by 6 pots.

2.2 Data recorded

2.2.1 Weeds

Associated weeds, *Beta vulgaris* (broad weed) and *Phalaris minor* (Narrow weed) were collected from each pot at 30 days after treatments (DAT) and at the end of the season (*Narcissus tazetta* flowering), the dry weight of grown weeds was recorded.

2.2.2 *Narcissus tazetta* L.

Data on *Narcissus tazetta* L. were recorded for each individual plant at the flowering stage, including plant height, number of leaves, fresh and dry weight of leaves, flowering date, number of flowers, spike length, fresh and dry weight of cormals.

2.3 Determination of some chemical constituents in *Narcissus tazetta* L.

2.3.1 Photosynthetic pigments

Chlorophylls a, b and carotenoids were extracted from fresh leaves of *Narcissus tazetta* and estimated, colorimetrically according to Moran (1982).

2.3.2 Total carbohydrate content

Total carbohydrate content was extracted from drying finely ground tissues (powdered). Drying was carried out in an electric oven at 60°C, until constant weight was achieved. Total carbohydrate content was extracted according to Herbert et al. (1971) and estimated colorimetrically by the phenolsulphoric acid method, as described by Montgomery (1961).

2.3.3 Macro- and micro-elements

Macro and micro elements were determined in dried leaves of *Narcissus tazetta* at flowering stage according to the official and modified methods of analysis (AOAC, 1984).

2.4 Chemical analysis of *Moringa oleifera* extracts

2.4.1 Plant material and preparation of extract

Powdered leaves and seeds of *Moringa oleifera* (20 g) were extracted with distilled water. The extracts were

filtered through filter paper Whatman No. 1.

2.4.2 Total phenolic content

The total phenolic content of the extract was determined by the Folin–Ciocalteu method (Kaur and Kapoor, 2002). 200 µL of crude extract were made up to 3 mL with distilled water, mixed thoroughly 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 mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight.

2.4.3 Total flavonoid content

The total flavonoid content of aqueous extract was determined by the aluminium chloride colorimetric method (Chang et al., 2002). In brief, 50 µL of crude extract were made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% NaNO₂ solution, 0.3 mL of 10% AlCl₃ 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. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per g dry weight.

2.5 Statistical analysis

The data obtained were submitted to standard analysis of variance, the least significant difference (LSD) values were obtained when *F* values were significant at 5% level (Snedecor and Cochran, 1980).

3 Results

3.1 Weeds

Laboratory test (data not showed). The leaf or seed water extract of *M. oleifera* at concentrations from 6% to 15% caused complete death to the tested weeds, *B. vulgaris* and *P. minor*. Results in Table 1 show that the leaf water extract of *M. oleifera* or seed water extract significantly suppressed the dry weights of both *B. vulgaris* (broad weed) and *P. minor* (narrow weed) after

30 days of treatments and at *N. tazetta* flowering. The reduction in weed growth was concentration dependent. The weed growth in pots sprayed with leaf water extract of *M. oleifera* recorded higher reduction than pots sprayed with seed water extract in comparison to the weeds in untreated pots. The reduction in *B. vulgaris* (broad weed) was higher than *P. minor* (narrow weed). This effect was true for both extracts at all concentrations (1.25%, 2.5% and 5%) used. At flowering the dry weight of *B. vulgaris*/pot recorded the highest reduction by spraying with leaf water extract at 5%, the reduction reached to 78% of that in untreated pots. Weight of *P. minor*/pot recorded 65% corresponding result.

3.2 *Narcissus tazetta* growth and flowering

The results in Table 2 reveal increasing in plant height of *N. tazetta* at flowering over untreated plants. The increase in plant height was significant with all applied concentrations of leaf or seed extracts (1.25%, 2.5% and 5%). Similarly, there are great significant increases in number of leaves as well as fresh and dry weight of leaves of *N. tazetta* as compared to the untreated plants. Spraying plants with 5% leaf extract

represent the most significant increase in dry weight of leaves (about 89% over untreated unweeded control).

The time required for flowering emergence varied significantly (60-80 days) in response to spraying treatments (Table 3) with *M. oleifera* leaf or seed water extracts as compared to that time required for the untreated unweeded plants to flower. The earliest first flower emergence was obtained in plants treated with *M. oleifera* leaf water extract at 5% (60 days). The maximum time required was observed in plants grown in unweeded pots (80 days). It is observed that both fresh and dry weight of *N. tazetta* cormals increased greatly over their correspondence in untreated unweeded pots (Table 3) with spraying leaf or seed water extract of *M. oleifera*, maximum significant increase in dry weight of cormals was recorded by spraying *N. tazetta* cormals with leaf water extract of *M. oleifera* at 5%, it exceeded 100% over unweeded untreated control. Spike length recorded high variation in response to different concentrations of leaf or seed water extracts in comparison to untreated unweeded control. Remarkable spike length was measured in that plants treated with 5% leaf extract (Table 3).

Table 1 Effect of leaf or seed water extract of *Moringa oleifera* on the growth of *Beta vulgaris* and *Phalaris minor* associated *Narcissus tazetta* L.

Treatments	Concentration	<i>Beta vulgaris</i>		<i>Phalaris minor</i>	
		Dry weight, g 30 DAT	At the end of the season, g	Dry weight, g 30 DAT	At the end of the season, g
<i>Narcissus tazetta</i>	0	-	-	-	-
<i>N. tazetta</i> + <i>B.vulgaris</i> + <i>P.mi nor</i>	0	2.525	30.523	3.924	26.657
<i>N. tazetta</i> + <i>B.vulgaris</i> + <i>P.mi nor</i> + Leaf extract	1.25%	1.519	12.233	2.146	15.000
	2.5%	0.461	7.360	1.743	11.527
	5%	0.414	6.773	1.108	9.443
<i>N. tazetta</i> + <i>B.vulgaris</i> + <i>P.mi nor</i> + Seed extract	1.25%	2.096	14.80	3.762	18.510
	2.5%	0.771	9.540	2.467	16.140
	5%	0.518	9.200	1.821	15.640
LSD at 5%		0.054	0.523	0.129	1.158

Table 2 Effect of leaf or seed water extract of *Moringa oleifera* on some growth criteria of *Narcissus tazetta* L.

Treatments	Concentration	Plant height	Number of leaves	Fresh weight of leaves	dry weight of leaves
<i>Narcissus tazetta</i>	0	71.33	17.33	93.89	35.10
<i>N. tazetta</i> + <i>B.vulgaris</i> + <i>P.mi nor</i>	0	46.33	9.67	58.87	21.15
<i>N. tazetta</i> + <i>B.vulgaris</i> + <i>P.mi nor</i> + Leaf extract	1.25%	69.50	11.33	69.31	29.85
	2.5%	73.33	16.00	76.75	31.90
	5%	78.33	17.33	112.77	40.05
<i>N. tazetta</i> + <i>B.vulgaris</i> + <i>P.mi nor</i> + Seed extract	1.25%	57.50	10.33	64.95	27.51
	2.5%	59.43	14.00	71.23	32.25
	5%	65.07	15.00	81.15	35.84
LSD at 5%		2.60	1.60	7.58	2.33

Table 3 Effect of leaf or seed water extract of *Moringa oleifera* on the yield production of *Narcissus tazetta* L.

Treatments	Concentration	Days from planting to Flowering	Number of flowers	Fw of cormals	Dw of cormals	Spike length
<i>Narcissus tazetta</i>	0	65.67	3.0	56.60	11.31	18.33
<i>N. tazetta</i> + <i>B.vulgaris</i> + <i>P.mi nor</i>	0	80.66	1.0	36.27	8.67	13.00
<i>N. tazetta</i> + <i>B.vulgaris</i> + <i>P.mi nor</i> + Leaf extract	1.25%	68.66	2.0	62.10	11.31	17.00
	2.5%	65.66	3.0	63.25	15.03	20.33
	5%	60.00	5.0	89.74	18.78	27.00
<i>N. tazetta</i> + <i>B.vulgaris</i> + <i>P.mi nor</i> + Seed extract	1.25%	71.00	2.0	52.80	11.08	15.00
	2.5%	69.66	3.0	61.83	12.81	16.85
	5%	65.66	4.0	68.25	16.47	23.33
LSD at 5%		1.31	0.10	5.12	0.71	0.97

3.3 Photosynthetic pigments and carbohydrate contents

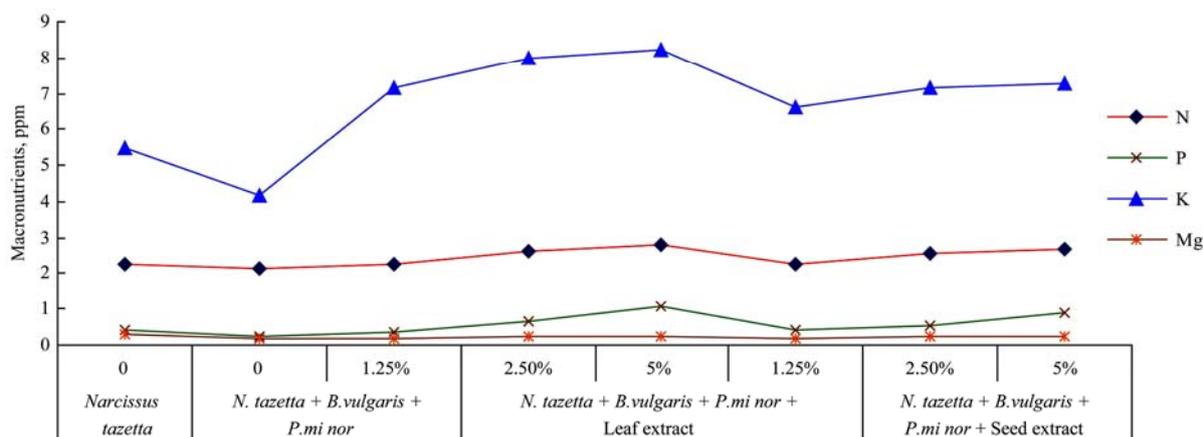
The results in Table 4 indicate increasing in the contents of chlorophyll a, chlorophyll b and carotenoids in leaves of *N. tazetta* by different concentrations of leaf or seed water extract over that of untreated unweeded control. Maximum increase was estimated in leaves of plants sprayed with 5% leaf water extract of *M. oleifera*. The response of carbohydrate content to spraying with 5% as well was the best indicating maximum increase in *N. tazetta* leaves in comparison to untreated unweeded control (Table 4).

3.4 Macro- and micronutrient contents,

Measuring the content of N, P, K and Mg in leaves of *N. tazetta* sprayed with leaf or seed water extract reveal higher content than that measured in unweeded untreated plants (Figure 1). The contents of macronutrients were more observable at the highest concentrations of leaf or seed water extracts (5%) as compared to the control. As have been expected spraying *N. tazetta* plants with leaf or seed water extract accumulated the content of Fe, Mn, Zn and Cu (Micronutrients) elements in leaves specially with using the extracts at 5% (Figure 2).

Table 4 Effect of leaf or seed water extract of *Moringa oleifera* on chlorophyll a, chlorophyll b, carotenoids and total carbohydrate contents in leaves of *Narcissus tazetta* L.

Treatments	Concentration	Pigment contents (mg/g fresh weight)			Carbohydrate percentage
		Chl. a	Chl. b	Carotenoids	
<i>Narcissus tazetta</i>	0	0.493	0.186	0.235	11.82
<i>N. tazetta</i> + <i>B.vulgaris</i> + <i>P.mi nor</i>	0	0.321	0.128	0.221	9.05
<i>N. tazetta</i> + <i>B.vulgaris</i> + <i>P.mi nor</i> + Leaf extract	1.25%	0.574	0.149	0.301	15.11
	2.5%	0.721	0.221	0.311	18.34
	5%	0.878	0.287	0.356	22.78
<i>N. tazetta</i> + <i>B.vulgaris</i> + <i>P.mi nor</i> + Seed extract	1.25%	0.385	0.132	0.228	10.43
	2.5%	0.440	0.165	0.239	14.84
	5%	0.581	0.243	0.286	17.22
LSD at 5%		0.021	0.03	0.001	1.23

Figure 1 Effect of *Moringa oleifera* leaf or seed water extract on macronutrient contents (ppm) in leaves of *Narcissus tazetta* L.

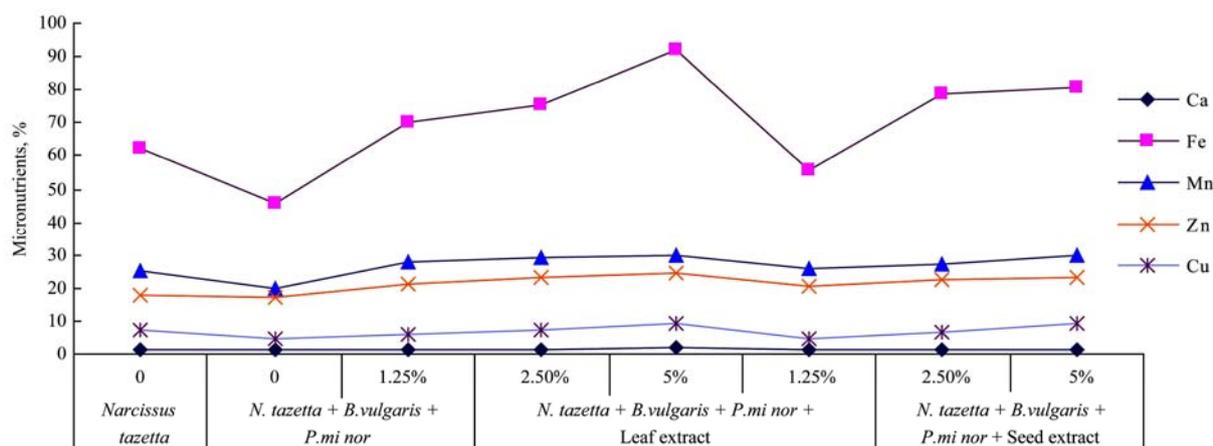


Figure 2 Effect of *Moringa oleifera* leaf or seed water extract on the percentage of micronutrient contents in leaves of *Narcissus tazetta* L.

The results indicate that the total polyphenol in the leaf water extract of *M. oleifera* was more than two fold its correspondence in seed water extract. However, estimating flavonoids in the seed extract show no results while were found in the leaf water extract (Table 5).

Table 5 Total polyphenols and total flavonoids in leaf and seed water extracts of *Moringa oleifera*

Part of plant	Total polyphenol, mg gallic acid/100 g dry weight	Total flavonoids, mg rutin/100 g dry weight
Leaves	8.3	41.5
Seeds	3.4	0.0

4 Discussion

Using allelopathic phenomenon in biological control of weeds has become more important in agricultural practices (Singh et al., 2005). Allelochemical-produced plants were become possible approach for using environmentally friendly bioherbicide (Batish et al., 2006). *Moringa oleifera*, the allelopathic plant has two reversible effects as many allelopathic plants, inhibitor or promoters depending on concentrations. Foliar spray of *moringa* water extracts enhanced the yield significantly when applied in very in minute amounts (Cheema et al., 2012). On the other hand, *Moringa oleifera* different extracts inhibited germination of *Vigna radiate* and the inhibition increased with increasing concentration (Hossain et al., 2012).

The results of the present study reveal inhibition in weed growth of both broad weed, *Beta vulgaris* and grassy weed *Phalaris minor* with different degrees of inhibition depending on the concentration of *M. oleifera* leaf or seed water extract. Spraying *M. oleifera* leaf extract at 5% caused the highest reduction in both weed

growth among other concentrations of leaf or seed extracts. The reduction in broad leaved weed *B. vulgaris* was higher than the reduction in a grassy weed *P. minor*. Allelopathic inhibition of water extracts of different parts of *M. oleifera* was documented by many workers (Awasthi et al., 2008; Phiri and Mbewe, 2009; Hossain et al., 2012; Aytah, 2017). So, the results of the present study came in accordance with Awasthi et al. (2008), Phiri and Mbewe (2009), Hossain et al. (2012) and confirmed by Oluwafemi (2014) who reported that leaf extract of *Moringa* suppressed both seed germination and some growth parameters of *Euphorbia heterophylla*. Additive confirming results obtained by Aytah (2017) on *Hordeum vulgare* and *Trigonella foenum-graecum*. The author cited that 2.5% of *Moringa* leaf extract represented the highest degree of allelopathic inhibition on seed germination and radicle length of *Hordium vulgare* and added that the inhibitions were concentration dependent. Several workers cited that the caused factors for allelopathic inhibition might be some allelochemicals that present in the extracts of allelopathic plants such as phenols, flavonoids and/or alkaloids (Chon et al., 2003; Hegazy and Farrag, 2007; El-Rokiek and Eid, 2009; El-Rokiek et al., 2014; Abou-Zeid and EL-Darier, 2014). More confirming data were recorded by El-Rokiek et al. (2016) that the extract of allelopathic plant extract contained polyphenols and flavonoids, so, the authors suggested that the inhibition in weed growth may be due to the presence of polyphenols or flavonoids. In the present study analysis of both leaf and seed water extract of *Moringa oleifera* indicated the presence of polyphenols and flavonoids (Table 5). These results refer

to that total polyphenol content in the leaf extract more than two fold its correspondence in the seed extract. On the other hand, the results of analysis of both leaf and seed extracts reveal no flavonoids in seed extract while present in leaf extract. Consequently, the presence of polyphenols in both leaf and seed extracts of *M. oleifera* may be the causative factors for weed growth inhibition. The higher amount of polyphenols and the presence of flavonoids in leaf water extract may be correlated with increasing in weed growth inhibition by spraying leaf water extract than its correspondence in seed water extract.

Data in Table 2 reveal increase in different growth parameters in *N. tazetta* plant in pots sprayed with leaf or seed extract of *M. oleifera* in comparison to *N. tazetta* grown in unweeded untreated pots. The increase in growth was reflected on increasing net return of yield represented by number of flowers, fresh and dry weight of cormals (Table 3) of *N. tazetta*, etc. The increase in growth was accompanied by increasing in photosynthetic pigments as well as carbohydrates (Table 4). The increase in growth also was conjugated with increasing macro and micronutrients in leaves of *N. tazetta* (Figure 1&2).

The increase in growth and flowering and consequently some metabolites may be attributed to controlling weeds as the competition of and weeds, both broad weed (*B. vulgaris*) and narrow weed (*P. minor*) against *N. tazetta* was reduced as have been reported by many workers (El-Rokiek and Eid, 2009; El-Rokiek et al., 2011; El-Nagdy et al., 2016).

It is worthy to mention that the increase in growth, flower yield of *N. tazetta* as well as photosynthetic pigments, carbohydrates and macro and micronutrients exceeded their correspondence in untreated weed free plants. These results may be attributed to hormonal activity in leaf or seed extract of *M. oleifera* at which *moringa* leaf juice may contain substances that promoted the vegetative growth and yield of many crops (Foidl et al., 2001).

5 Conclusion

The current work suggests that using the leaf or seed extract of *M. oleifera* has potential allelopathic inhibition

on weed growth, so, can be used as bioherbicide. There will be excess of study in the next paper for recognizing some more contents in the leaf and seed extracts of *M. oleifera*.

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