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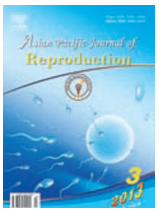

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Rapid bioassay for the study of growth promoting activity of *Morinda pubescens* leaf extract

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ABSTRACT

Objective: To study the influence of aqueous extracts of *Morinda pubescens* Smith. (M. *pubescens*) on the germination (%) and seedling growth (fresh and dry weight) of wheat and fenugreek. **Methods:** The various concentrations (0.05%, 0.1%, 0.15%, 0.25%, 0.50%, 1%, 1.25% and 2.5%) of these AE were prepared and used for the germination trials. Distilled water was used as control. **Results:** Aqueous extracts at the concentration of 0.15% and 0.25% shows significant stimulatory effect on seed germination and seedling growth of wheat and fenugreek which also found significant in the significant enhancement in root and shoot legth, vigour index and mobilization efficiency of wheat as compared to control, while fenugreek seeds exhibits stimulatory response at these concentrations. It was also noticed that seed germination and seedling growth is sensitive to higher concentrations of leaf extract showing its inhibitory allelopathic effect. **Conclusion:** These findings indicate that aqueous leaf extract of *M. pubescens* possess biotonic potential.

1. Introduction

Green plants are the receivers of major compounds having wide range of biological activities. They are having potent medicinal values, growth regulatory, herbicidal and pesticide effects and also some having toxic values. Plant based fertilizers are of beneficial to soil, including nutrient cycling, soil protection and soil improvement. Plant produces wide varieties of biologically active constituents. Germination generally incorporates the events which commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis [1] . Recent agrochemical-based farming has contaminated of surface and ground water resources by nutrients and herbicides[2]. To overcome the adverse effects of agrochemicals and to reduce chemical input in agricultural systems we must rely on the enhancing organic farming[3]. The plant based fertilizers are of beneficial to soil, including nutrient cycling, soil protection and soil improvement. Plant produces wide varieties of biologically active constituents.

The genus *Morinda pubescens* (*M. pubescens*) is a member of Family Rubiaceae and one of the important medicinal plants. The biotonic potential of *M. pubescens* fruits as a promising medicinal source is well known. But no work has been carried on the agronomical potential of leaf extract. The present investigation was therefore, conducted to envisage the biotonic potential of aqueous leaf extract of *M. pubescens* on germination and seedling growth of wheat and fenugreek.

2. Materials and methods

The fresh and mature leaves of *M. pubescens* were collected from Osmanabad. The leaves were washed thoroughly and blotted to dry. The certified wheat seeds of variety Ankur were collected from cooperative agriculture shop and the fenugreek seeds were from the local market.

2.1. Preparation of aqueous extract (AE) of M. pubescens leaves

Fresh leaves of *M. pubescens* (100 g) were harvested and cut into small chips of about 4 cm length and finely ground with mortal and pestle. The ground plant material was soaked in 500 mL of distilled water in a large beaker for 12 h. The solution was filtered through cheese cloth to remove debris and finally filtered using Whatman No. 1 filter paper. The final filtrate served as the pure aqueous extract (AE) of *M. pubescens*. Then the various concentrations (0.05%, 0.1%, 0.15%, 0.25%, 0.50%, 1%, 1.25% and 2.5%) of these AE were

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prepared and used for the germination trials. Distilled water was used as control.

2.2. Germination studies and seedling growth of wheat and fenugreek

Laboratory experiments in petridishes were done for wheat and fenugreek seeds for the observation of germination percentage; shoot growth and root growth, plant height etc. Clean petridishes with two sheets of filter papers were used. For the investigation of germination percentage, growth and development of both seeds, 15 mL of each aqueous extract was put in each petridish. In control, only distilled water was used and amount of distilled water was also same. Then 20 seeds of each crop were kept in each petridish and each treatment was replicated three times. The petridishes were kept in natural diffused light under laboratory conditions at (29±2) °C temperature and relative humidity of (85±5)% after placing. 5 mL of water was used per day per petridish to keep constant moisture. In control, only water was added if necessary per day per petridish. In this experiment, all subsequent observations were recorded and it was started from 1st day. After setting the experiment, the germination percentages, shoot length, root length and completion of germination were recorded. Effects of different treatments on morphology of seedlings were also recorded. The collected data were analyzed statistically and the co-efficient of variance and means were compared by using Duncan's New Multiple Range Test (DMRT). Vigour index (VI) was calculated [4]:

VI=germination% × length of embryonic axis

The mobilization efficiency (ME) of reserved food material present in seed during germination was calculated[5]:

ME= Dry weight of embryonic axis/Dry weight of residual grain×100

2.3. HPLC analysis of plant hormones

The HPLC used was a Shimadzu instrument (Shimadzu Corp., Kyoto, Japan) comprising a Rheodyne injector, a 250 mm-4.6 mm (i.d.) Nucleosil C18 stainless steel column (5 μm particle size, 300A° pore size) (Sigma-Aldrich, Inc., St. Louis, MO), 6AD pumps, an SPD-10A UV-Vis detector, and an LC-10 chromatography manager. Standards and organic extracts of sap were injected with a 20 μL syringe (Hamilton, Reno, NV). Isocratic elution was performed at 40 _C with MeOH/H_2O (3:7, v/v) mobile phase containing 0.1% acetic acid at a constant flow rate of 1.3 mL/min. UV detection was carried out at 254 nm except in the case of gibberellins, for which the detection wavelength was 205 nm. After their identification, concentrations of the different PGRs were estimated on the basis of HPLC peak are as measured against standards.

2.4. Statistical analysis

The collected data were analyzed statistically and the co-efficient of variance and means were compared by using Duncan's New Multiple Range Test (DMRT).

3. Results

3.1. Effect of aqueous extract of M. pubescens leaves on wheat growth

The highest germination percentage of wheat seeds were studied after 15 days. It was found that seeds treated with 0.05% of aqueous leaf extract of *M. pubescens* showed 100 % of seed germination which was higher as compared to control and statistically identical. Where the lowest germination percentage was found in seeds treated with 0.50% and that was 70%. The second highest germination percentage was obtained from seeds treated with 0.10% (95%), 0.15% (90%) which were statistically similar (Table 1). Similarly the Vigor index of wheat at 0.05% was higher as compared to control. The mobilization efficiency at the concentration 0.1% was also found higher. Shoot length of wheat at different days after sowing influenced significantly by the effects of different aqueous leaf extract (Table 2). At 15 days after sowing with 0.05% leaf extract showed the highest shoot length (9.54 cm) whereas the lowest shoot length (4.90 cm) at 15 days was recorded at 1% concentration. The second highest shoot length (9.43 cm) at same days was recorded at the concentration of 0.10%. Similar trend in increase in shoot length was recorded at concentration up to 0.25% with increase in days after sowing. But at the highest concentration (1%), interestingly reduction in shoot length is recorded. Root length of wheat seedling at different days after sowing was found lowest at 3 days and showed an increasing trend up to 15 days (Table 3). At 15 days after sowing seed treated with 0.1% showed highest root length (6.26 cm) as compared to control and other treatments, whereas at 1% reduction in root length (4.38 cm) was recorded. The second highest root length at studied days was recorded at the concentration of 0.1% (6.13 cm). The other treatments upto 0.25% showed quite interesting and increase in root length as compared to control.

3.2. Effect of aqueous extract of M. pubescens leaves on fenugreek growth

The highest germination of fenugreek seed was obtained in seed treated with 0.1% and 0.15% concentration. Consequently, lowest germination was recorded at the higher concentration (0.50% & 1%). The mobilization efficiency and vigor index was also higher at lower concentrations. The decrease in germination with increased concentration (0.50%) was quite noticeable (Table 1). Shoot length of fenugreek at different concentration of *M. pubescens* aqueous leaf extract and at different days was recorded in Table 2. The shoot length influenced significantly by the effect of *M. pubescens* leaf extract up to 0.25%. The highest shoot length (8.20 cm) at 15 days was obtained when seeds treated with 0.15%. The second higher shoot length recorded in seed treated with 0.10% (8.10 cm). On the other hand lowest shoot length 5.9 cm was obtained in seeds treated with concentrations of 1% at 15 days which was lower than the control too. Effect of leaf extract on root length of fenugreek varied significantly at different days after sowing (Table 3). The highest root length (8.18 cm) was obtained at the concentrations of 0.10% at 15 days. The second highest root length at the same days was recorded at the concentration of 0.15% (7.98 cm); while lowest root length (6.87 & 5.03 cm) was noticed at higher concentration 0.5% & 1% concentration.

3.3. HPLC analysis of growth regulators

High pressure liquid chromatography analysis of aqueous extract of *M. pubescens* leaves are shown in Figure 1. The HPLC spectral analysis revealed that *M. pubescens* leaf extract exhibits two peaks with one unknown peak. The identified growth hormones in *M. pubescens* leaves were NAA with 26.6% area and 1164.98 mg/kg concentration, and GA with 44.45% area showing 57.32 mg/kg concentration.

Table 1
Effect of *M. pubescens* leaf extract on percent germination of wheat and fenugreek seeds.

Treatment	Germination %					
	Wheat (Ankur)			Fenugreek		
	Germination %	Vigor Index	ME	Germination %	Vigor Index	ME
Control	90[a]	460.93	377.77	90[a]	260.83	522.22
0.05%	100[c]	633.46	522.22	90[c]	344.80	630.00
0.10%	95[ac]	607.49	650.00	100[bc]	461.59	546.00
0.15%	90[d]	604.22	550.00	95[d]	426.27	650.00
0.25%	80[ae]	551.10	420.00	85[ad]	375.23	625.00
0.50%	70[ca]	384.56	475.00	75[dc]	277.06	477.77

(Means followed by the same letter(s) did not differ significantly at 5% level by DMRT).

Table 2
Effect of *M. pubescens* leaf extract on shoot length of wheat and fenugreek seeds different days after sowing.

Treatment	Shoot Length (cm)									
	Wheat seeds					Fenugreek seeds				
	3 days	6 days	9 days	12 days	15 days	3 days	6 days	9 days	12 days	15 days
Control	4.49[a]	6.67[c]	7.23[d]	8.18d[c]	9.20[c]	5.41[d]	5.68[d]	6.41[e]	6.72[e]	7.18[d]
0.05%	5.24[c]	7.12[b]	7.60[bc]	8.74[ad]	9.54[cd]	6.15[b]	6.48[ab]	7.10[c]	7.38[de]	8.01[e]
0.10%	5.45[bc]	6.98[bc]	7.59[c]	8.61[c]	9.43[c]	6.45[d]	6.88[bc]	7.32[e]	7.98[dc]	8.10[eb]
0.15%	5.49[b]	6.94[b]	7.47[b]	8.42[b]	9.38[b]	6.49[a]	6.72[a]	7.19[cd]	7.88[ae]	8.20[ac]
0.25%	5.35[a]	6.82[a]	7.38[a]	8.29[a]	9.32[a]	6.14[c]	6.67[cb]	7.16[d]	7.35[ae]	7.88[e]
0.50%	4.32[b]	6.27[d]	7.18[e]	8.09[e]	8.98[d]	5.13[bc]	5.22[c]	5.78[ac]	6.01[de]	6.10[c]
1.00%	2.90[a]	4.14[cd]	6.38[ad]	5.54[b]	4.90[bc]	3.40[cb]	4.04[a]	4.78[ac]	5.07[b]	5.90[dc]

(Means followed by the same letter(s) did not differ significantly at 5% level by DMRT).

Table 3
Effect of *M. pubescens* leaf extract on root length of wheat and fenugreek seeds.

Treatment	Root length (cm)									
	Wheat seeds					Fenugreek seeds				
	3 days	6 days	9 days	12 days	15 days	3 days	6 days	9 days	12 days	15 days
Control	4.63[e]	4.71[ac]	5.19[b]	5.32[de]	5.90[e]	5.33[e]	5.64[e]	6.32[d]	6.88[e]	7.08[e]
0.05%	5.32[a]	5.66[d]	5.82[da]	6.02[e]	6.18[ac]	6.55[e]	6.76[ac]	7.01[bc]	7.28[d]	7.64[b]
0.10%	5.52[b]	5.76[de]	5.93[dc]	6.13[b]	6.26[c]	7.2[ab]	7.38[cd]	7.84[ae]	8.02[e]	8.18[a]
0.15%	5.50[b]	5.70[a]	5.88[ab]	6.08[ca]	6.20[e]	7.09[d]	7.22[ab]	7.69[bc]	7.82[bd]	7.98[e]
0.25%	5.47[e]	5.68[d]	5.83[ba]	6.03[ce]	6.18[ed]	6.82[a]	7.02[ba]	7.13[e]	7.32[cd]	7.72[d]
0.50%	4.32[b]	5.48[cd]	5.32[ab]	5.27[e]	5.14[c]	5.56[a]	6.02[c]	6.14[da]	6.63[ed]	6.87[d]
1.00%	3.18[a]	3.70[bd]	4.18[c]	4.32[ce]	4.38[d]	4.41[d]	4.53[cd]	4.53[ea]	4.88[d]	5.03[c]

(Means followed by the same letter(s) did not differ significantly at 5% level by DMRT).

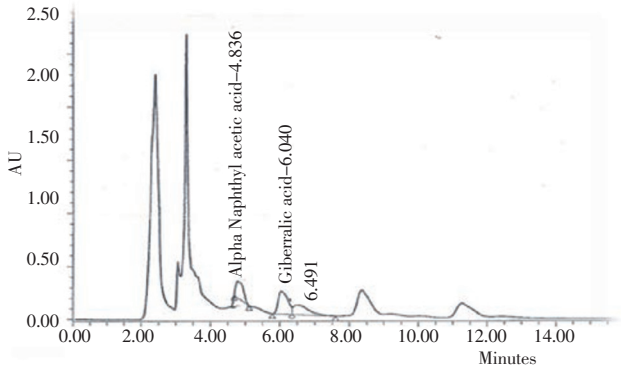

Figure 1. HPLC chromatogram of plant hormones detected in *M. pubescens* aqueous leaf extract.

4. Discussion

The effects of aqueous extract of *M. pubescens* on seed germination and seedling growth of wheat and fenugreek were evaluated. The distilled water used as the control. Data on the effect of the aqueous extract on seed germination of wheat showed in dose dependant manner. The germination percentage in the seed of wheat and fenugreek treated with lower concentrations of *M. pubescens* leaf extract shows higher germination percentage. At the concentration of 0.05% the germination percentage is higher (100%) where as in fenugreek the germination percentage is higher at 0.1% (100%). The root length and shoot length in both wheat and fenugreek is also significantly higher at the lower concentration of 0.1% & 0.15%. The vigour index and mobilization efficiency of wheat and fenugreek is also higher at lower concentrations. As the concentrations increases (at 0.5% and above) the leaf extract shows some inhibitory effect on seed germination and growth. The increase in germination as well as root length and shoot length in both the seeds due

to effect of aqueous of *M. pubescens* leaf extract possibly due to presence of growth regulators NAA and GA or may be other bioactive substances present in the extract.

The results of this study also indicated that the stimulatory effects of the extracts on germination, root and shoot growth of wheat and fenugreek, is significantly higher at lower doses as the extract concentration increased beyond 1% it shows decrease in the germination percentage as well as seedling growth, and hence were concentration dependent. Similarly lowest concentration of Beta vulgaris plant extract stimulated the germination and some metabolic activities associated with growth of wheat and at higher concentration (above 1%) germination was retarded[6]. Higher stimulatory effect of a weed *Cassia tora* on paddy cultivars was recorded on Abhilash followed by Chromolaena extract on variety 9926. Chromolaena extract have significant stimulatory effect on root length of Abhilash followed by Parthenium root extract on BPT and *Cassia tora* extract on paddy variety 9926 [7]. The *Acacia nilotica* leaf extracts shows stimulatory effect on paddy[8] this may be due to the presence of certain substances, which can induce germination. The lower concentration (10%-25%) of *Albizia lebbeck* (L.) Benth. leaf extracts showed stimulatory effect on germination and growth behavior of some popular agricultural crops such as *Cucumis sativus*, *Raphanus sativus*, *Vigna unguiculata*, *Phaseolus mungo* and *Brassica juncea* [9]. Sevarla workers also studied effect of leaf extract on economic plants. Tithonia diversifolia leaf extract on the germination and growth of maize (*Zea mays* L.) [10]. the effects of Parthenium and Lantena on germination and seedling vigor of hybrid rice and noticed stimulation of germination at lower concentration (i.e., 5%) of leaf extract[11] .

Abul *et al*.,[12] reported the presence of the auxin IAA, the cytokinins tZR, DHZR and iP, and the gibberellins GA4, GA9 and GA20 in the rhizome and aerial sporophytic tissue of the ancestral fern *Pisoltum nudum*. Occurrence of nutrients and plant hormones (cytokinins and IAA) in the water fern *Salvinia molesta* during growth and composting have been reported by Arthur *et al*., [13]. The induction of protein and RNA synthesis in response to auxins and Gibberellins is evident[14]. While the study of Rijvan and Prakash [15] also indicates significant increase in RNA synthesis followed by enhanced protein synthesis of fenugreek seedlings. They further observed that the enhanced RNA synthetic activity is more significant when NAA and GA (5 fold) are used in combination than they used separately. In the present investigation the HPLC analysis of the leaf extracts also indicates the presence of NAA and GA in combination which may induces the protein synthesis followed by enhanced seed germination and seedling growth at lower concentration of these leaf extracts.

Our findings correlated with above results who found growth promoting activity of various aqueous leaf extract on economic plants. Thus, the bioprospecting of *M. pubescens* leaves for their biotonic potentials results in the development of several natural agrochemicals which will be useful in the development of chemical free organic farming in future.

This study interestingly indicated that *M. pubescens* plant extracts have strong biological activity in the field of agriculture. From this small scale study we may conclude that:

1.Aqueous extract of *M. pubescens* significantly stimulates the germination of economic crops.

2.Further pot experiment as well as field experiment with this extract during cultivation of vegetable crops for final conclusion.

3.Leaves of *M. pubescens* contain growth hormones and other bio-active substances.

Owing to high energy costs, increased costs of chemical growth regulators and stimulants and increasing concern over the adverse effects of long term use of the agro-chemicals on plants and animals through residual effects, there is now considerable interest in the use of renewable organic growth substances from plant sources. Thus, bioprospecting of useful natural sources for the enhancement of agricultural yield and productivity is the prime necessity of modern agriculture.

Conflict of interest statement

We declare that we have no conflict of interest.

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