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Effect of Different Soil Fertilizing Systems on Seed and Mucilage Yield and Seed P Content of Isabgol (*Plantago ovata* Forsk)

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Abstract: A greenhouse experiment was carried out to investigate the effects of chemical fertilizer include N and P (chemical fertilizing system), animal manure (organic fertilizing system), combined use of manure and chemical fertilizer (integrated fertilizing system) and Barvar Phosphate Biofertilizer (BPB) on seed yield, mucilage and seed P content of Isabgol at the Agriculture Research Center, University of Tehran, Karaj, Iran. The results showed that BPB inoculation significantly increased seed yield, mucilage and seed P content. The results also showed that seed and mucilage yields were significantly affected by fertilizing systems. Animal manure and combined use of manure and chemical fertilizer resulted in a greater seed yield, mucilage yield, mucilage percentage, swelling factor and P content than sole application of chemical fertilizer. The most seed (4.58 g pot^{-1}) and mucilage yield (0.876 g pot^{-1}) were observed in integrated use of animal manure and chemical fertilizers with BPB inoculation.

Key words: Fertilizing systems, biofertilizer, isabgol, mucilage and phosphorous

INTRODUCTION

Plants known as medicinal are rich in secondary metabolites and are potentially useful to produce natural drugs. The biosynthesis of the secondary metabolites, although controlled genetically, is affected strongly by environmental factors (Singh *et al*., 2003). Plantago ovata is an important herb that has been used in health care for many centuries in South Asia, whereas it is now widely used for its medicinal properties all over the world. Isabgol an Ayurvedic herb is found in Iran, India and Afghanistan. It is also native to the surrounding of Mediterranean region including Northern Africa, Europe and Pakistan. This plant can be grown under a wide range of agro-climatic conditions, but it is mostly confined to the arid areas of the world due to its low water requirements (Zahoor *et al*., 2004). Isabgol has been used as medicine since ancient times, but it has been cultivated as a medicinal plant only in recent decades (Yadav *et al*., 2002). The seed of Isabgol contains mucilage, fatty oil; large quantities of albuminous compound, a pharmacological inactive glycoside, namely Aucubin ($C_{13} H_{19} O_8 H_2O$) and a pentose sugar (Zahoor *et al*., 2004). The seed husk has the property of absorbing and retaining water which accounts for its application in controlling diarrhea. It is diuretic, alleviates kidney and bladder complaints, gonorrhea, arthritis and hemorrhoids (Zargari, 1994).

To provide economically sustainable yields, nutritional elements must be present in sufficient quantities in soil and they must be available for root uptake. Nitrogen and phosphorous are essential nutrients for plant growth and development (Blaise *et al*., 2005). The extensive uses of chemical fertilizers in Iran have increased crops productivity but did not promote the quality of medicinal and aromatic plants which are the most important characteristics for export. To increase the crops quality especially medicinal and aromatic plants, organic fertilization is more acceptable than chemical fertilizers (Karla, 2003). Organic farming can guarantee the quality of medicinal plants and that is why the medicinal plants consumers prefer to use the organic plant components (Griffe *et al*., 2003). Organic fertilizers are excellent sources of nutrients for crop production and improving physical and chemical properties of soil (Ewulo, 2005). It has been shown that addition of organic matter

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improved soil properties such as aggregation, water-holding capacity, hydraulic conductivity, bulk density, the degree of compaction, fertility and its resistance to water and wind erosion (Franzluebbers, 2002). Application of organic matter positively affects the growth and development of plant roots and shoots (Ghosh *et al*., 2004). Yadav *et al*. (2002) reported that integrated use of chemical fertilizer and animal manure increased the seed and mucilage yields of Isabgol. Singh *et al*. (2003) reported that the total dry matter, seed and mucilage yields of Isabgol can be increased with application of chemical and organic fertilizers. Also Parihar and Singh (1995) and Patel and Sadaria (1996) reported that chemical fertilizers can increase seed and mucilage yields, spike length and spike number per plant of Isabgol.

Microorganisms are important attributes in agriculture to promote the circulation of plant nutrients and reduce the need for chemical fertilizers (Rodríguez *et al*., 1999). Biofertilizers are organic products containing living cells of different types of microorganisms, which have the ability to convert nutritionally important elements from unavailable to available form through biological processes (Vessey, 2003). In recent years, biofertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yields through environmentally better nutrient supplies. P-solubilizing bacteria may be important for plant nutrition by increasing P uptake and play a significant role as PGPR in the biofertilization system. Trials with rhizosphere-associated plant growth-promoting P-solubilizing Bacillus species indicated that yield increases in sugar beet (Akmakc *et al*., 2006), wheat (De Freitas, 2000), sugar beet and barley (Ahin *et al*., 2004). Accordingly, great attention should be paid to studies and application of new combinations of phosphate-solubilizing bacteria. Barvar Phosphate Biofertilizer (BPB) is a new biofertilizer including P-solubilizing bacterial strains which has been produced by Green Bio-tech Co. in University of Tehran.

The primary objective of this study was to evaluate the efficiency of novel P-solubilizing bacterial strains isolated from Iran's soils (Barvar Phosphate Biofertilizer) and different soil fertilizing systems (chemical, organic and integrated) on the promotion of plant growth, mucilage percentage, seed swelling factor and seed P content of Isabgol. The secondary goal was to find out whether sufficient and high quality yields of the Isabgol crop could be produced by solely biologic fertilizer application in organic farming system in Iran conditions.

MATERIALS AND METHODS

This experiment was conducted in glasshouse of College of Agronomy and Animal Sciences, University of Tehran, Karaj, Iran, during Autumn, 2006. Seeds used in this experiment were obtained from the Jahad Medicinal Plant Research Center, Ministry of Jahad of Agriculture in Iran. The original seed were collected from the Khorasan province in northeastern part of the country. The Effects of different fertilizing treatments were investigated in pots. The treatments were arranged in factorial based on a completely randomized block design with three replications. The effect of Barvar phosphate biofertilizer (inoculated and without inoculation) was tested with two levels of chemical fertilizer including N and P (chemical fertilizing systems), two levels of animal manure (organic fertilizing systems), two levels of combined use of manure and chemical fertilizers (integrated fertilizing systems) (Table 1). Intact soil volume for this experiment was collected from Dolat-Abad farm in Karaj (Table 2). The soil was air-dried and grounded to pass through a 4 mm sieve. About 2.5 kg of soil was allocated to each pot and thoroughly mixed with different fertilizing treatments (Table 2). Fifteen viable seeds were sown in each plastic pot (20 cm diameter and 30 cm high) at a constant temperature (20±4°C). Ten days after seed germination, the emerged seedlings were thinned to 5 plants in each pot. The pots were watered on a daily basis to maintain the moisture at approximately 60% water holding capacity of the soil. The crop was harvested after 101 days. The sources of N, P and animal manure were urea, super phosphate and dairy cow manure (Table 3), respectively. The first half of the nitrogen fertilizer was applied before sowing and the remainder at spike formation stage. The total P and dairy cow manure were applied before sowing. The biofertilizer (BPB) used for this experiment was

Table 1: Soil fertilizing system treatments details

Soil fertilizing systems	Treatment code	Chemical fertilizer (kg ha^{-1}) P	N	Cow dairy manure (t ha^{-1})
Control	Co	-	-	-
Chemical	C1	30	50	-
	C2	15	25	-
Organic	M1	-	-	20
	M2	-	-	10
Integrated	IN1	10	20	20
	IN2	5	10	10

Table 2: Some of soil characteristics

K	P	N	Sand	Silt	Clay	pH	OM
(Mg kg^{-1})			(%)				(%)
166	5.21	0.07	42	27	31	8.17	1.21

Table 3: Percentage of some cow dairy manure characteristics

Ca	Mg	K	P	N	OC
3.6	1.34	4.23	0.8	2.65	27.1

supplied by Green Bio-tech Co.; which consisted of two kinds of phosphate solubilizing bacteria (*Pseudomonas putida* (strain p13) and *Bacillus lentus* (strain p5). The bacteria strains were originally isolated from farm soils in Iran. For seed inoculation according to instructions, 50 g of Isabgol seed was inoculated with one gr BPB before being immediately planted.

Measurements: Biological yield was determined by cutting and harvesting the whole plants per each pot. Samples were oven-dried at 75°C for 72 h for Dry Matter (DM) determination. Mucilage percentage, swelling factor according to Ebrahimzadeh *et al.* (1998) method, seed phosphorous content according to Olsen and Sommers (1990) method, seed and mucilage yield per pot, were measured after harvesting.

Data analysis: Two-way General Liner Model (GLM) ANOVA was used (SAS Institute Inc., 1989) for soil fertilizing systems and Barvar phosphate biofertilizer effects on yield, yield components and seed P content of Isabgol. Duncan at $p<0.05$ tested the significance of differences among treatment means.

RESULTS AND DISCUSSION

Results showed that all traits investigated in this study were significantly affected by fertilizing systems (Table 4). Barvar Phosphate Biofertilizer (BPB) inoculation significantly affected all traits except mucilage percentage and swelling factor. The interaction of these treatments significantly affected seed yield and seed phosphorous content (Table 5). Application of all kind of fertilizers significantly increased biological, seed and mucilage yield compared to control (Table 4). Among fertilizing systems, chemical system had less effect on above mentioned traits compared to other fertilizing systems. Integrated system could increase more seed and mucilage yield compared to organic system, so the highest seed yield (4.58 g pot^{-1}) was observed in $N_{20}P_{10}$ + 20 t ha^{-1} manure which were inoculated with BPB (Table 5). This could be attributed to the fact that animal manure can improve physical and chemical properties of soil (Blaise *et al.*, 2005).

Many studies have shown that the fertilizing power of animal manure is due to its content of stabilized organic matter and due to the amount of nutritive elements contained therein (Ewulo, 2005; Ghosh *et al.*, 2004). Yadav *et al.* (2002) also reported that biological, seed and mucilage yields of Isabgol significantly increased by using integrated application of chemical and organic fertilizers, which can increase the available nutrition for plant roots and improve photosynthesis process. Singh *et al.* (2003) reported that biological, seed and mucilage yield of Isabgol could be increased with application of animal manure and chemical fertilizers in organic and integrated systems due to improving of physical and chemical soil properties. The highest mucilage yield (0.876 g pot^{-1}) was obtained in $N_{20}P_{10}$ + 20 t ha^{-1} manure treatment, which was not significantly different from 20 t ha^{-1} manure (Table 4). Sing *et al.* (1996) reported total dry matter, seed and mucilage yields of Isabgol could be increased with application of chemical and organic fertilizers. Also Parihar *et al.* (1995) and Patel *et al.* (1996) reported that chemical fertilizers can increase seed and mucilage yields, spike length and spike number per each plant of Isabgol. Inoculation of BPB along with all fertilizing systems increased seed yield and this effect was more identical in low levels of fertilizer application. This result could be attributed less activity of phosphate solubilizing bacteria in rich phosphorous soils (Table 5). Phosphate solubilizing bacteria can increase yield of spinach (Urashima *et al.*, 2003), sugar beet and barley (Ahin *et al.*, 2004). Inoculation with BPB had no significant effect on swelling factor and mucilage percentage (Table 4). On the other hand all of the fertilizing systems increased seed mucilage percentage and the highest mucilage percentage (19.67%) were obtained in 20 t ha^{-1} manure which did not have a significant difference with $N_{20}P_{10}$ + 20 t ha^{-1} manure treatment (Table 4). Chemical fertilizing system compared to organic and integrated systems did significantly affect

Table 4: Mean comparison effects of Barvar Phosphate Biofertilizer (BPB) and fertilizing systems on yield, mucilage, swelling factor and seed P concentration. With Duncan test at the 5% level of probability

Treatments	Total dry matter (g pot^{-1})	Seed yield (g pot^{-1})	Mucilage (%)	Mucilage yield (g pot^{-1})	Seed swelling factor (mL)	Seed P concentration (%)
With BPB	14.48a	4.09a	18.81a	0.760a	9.52a	0.528a
Without BPB	13.58b	3.79b	18.68a	0.711b	9.45a	0.506b
Fertilizers						
Control	11.38e	3.18f	17.99e	0.573e	8.91c	0.492d
$N_{50}P_{30}$	13.89c	3.92d	18.38cd	0.721c	8.99c	0.528b
$N_{25}P_{15}$	12.16d	3.39e	18.14de	0.616d	8.94c	0.504c
20 t ha^{-1} M	15.53a	4.38b	19.67a	0.861a	10.10a	0.531b
10 t ha^{-1} M	14.34bc	3.94cd	18.71bc	0.736bc	9.69b	0.507c
$N_{20}P_{10}$+20 t ha^{-1} M	16.09a	4.51a	19.44a	0.876a	10.06a	0.549a
$N_{10}P_5$+10 t ha^{-1} M	14.82b	4.05c	18.86b	0.765b	9.81b	0.510c

Means with the same letter(s) in columns are not significantly different at $p<0.05$; BPB = Barvar Phosphate Biofertilizer $N_{50}P_{30}$ = 50 kg ha^{-1} nitrogen and 30 kg ha^{-1} phosphorous, M = Dairy cow manure

Table 5: Mean comparison of interaction effects of Barvar Phosphate Biofertilizer (BPB) and fertilizing systems on seed yield and seed P concentration. With Duncan test at the 5% level of probability

Treatments	Seed yield (g pot^{-1})	Seed P concentration (%)
Without BPB		
Control	2.95i	0.478g
$N_{50}P_{30}$	3.88f	0.520c
$N_{25}P_{15}$	3.26h	0.488f
20 t ha^{-1} M	4.30bc	0.522c
10 t ha^{-1} M	3.81f	0.494ef
$N_{20}P_{10}$+20 t ha^{-1} M	4.44ab	0.543b
$N_{10}P_5$+10 t ha^{-1} M	3.91ef	0.500de
With BPB		
Control	3.41gh	0.506d
$N_{50}P_{30}$	3.97ef	0.537b
$N_{25}P_{15}$	3.53g	0.519c
20 t ha^{-1} M	4.49ab	0.540b
10 t ha^{-1} M	4.07de	0.520c
$N_{20}P_{10}$+20 t ha^{-1} M	4.58a	0.555a
$N_{10}P_5$+10 t ha^{-1} M	4.20cd	0.521c

Means with the same letter(s) in columns are not significantly different at $p<0.05$; BPB = Barvar phosphate biofertilizer $N_{50}P_{30}$ = 50 kg ha^{-1} nitrogen and 30 kg ha^{-1} phosphorous, M = Dairy cow manure

swelling factor, however, the most swelling (10.10 mm) appeared in 20 t ha^{-1} manure which did not have a significant difference with $N_{20}P_{10}$ + 20 t ha^{-1} manure (Table 4). Correlation analysis showed that there is high and significant relation between mucilage percentage and swelling factor. Swelling factor will increase as mucilage percent increases. As a result, higher seed mucilage percentage can make higher swelling factor (data not shown). Ebrahim Zadeh *et al.* (1998) also reported the high correlation between mucilage percentage and swelling factor in Isabgol.

The results of this experiment showed that seed P content increased by BPB inoculation compared to non-inoculated plants (Table 4). This could be caused by more phosphorous availability resulted from phosphate solubilizing bacteria activities. These results support the findings of Urashima *et al.* (2003) on spinach and Ahin *et al.* (2004) on sugar beet and barley and Poberejskaya and Egamberdiyeva (2003) on cotton. The highest seed phosphorous content was observed in $N_{20}P_{10}$ + 20 t ha^{-1} manure treatment along with BPB (Table 5). This could be due to improving soil available P and physical and chemical properties of soil. Application of animal manure on farmlands, recycle nutrients from manure to soil for plant growth and add organic matter to improve soil structure and water holding capacity. On the other hand, phosphate solubilizing bacteria can increase soil available P by hydrolysis of P from inorganic and organic compounds due to acidification of soil or secretion of phosphatase enzymes (Akmakc *et al.*, 2006).

CONCLUSIONS

Results of this study indicated that organic and integrated fertilizing systems could produce the most mucilage percentage, swelling factor, seed phosphorous content and biological, seed and mucilage yields and provide with more profits compared to chemical fertilizing system. Barvar Phosphate Biofertilizer (BPB) increased seed and mucilage yields of Isabgol. This biofertilizer could be used as a suitable fertilizer along with organic and chemical fertilizers to achieve the maximum benefits. To prevent the environmental pollution from extensive application of chemical fertilizers, the biological fertilizers could be recommended to insure the public health and a sustainable agriculture.

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