Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 2 (6) May 2013: 112- 117

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com

ORIGINAL ARTICLE

Biochemical Change of Seeds and Yield of Isabgol (*Plantago ovata*) under Bio-fertilizer, Organic Manure and Chemical Fertilizer

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ABSTRACT
Biofertilizers and organic manure has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. The objective of this Field study was to evaluate the effects of organic manure, Biofertilizers and chemical fertilizer on yield, mucilage percentage, swelling factor and seed chemical components of isabgol. Experiment was conducted at Zabol University Iran, in randomized complete block design (RCBD) with four replicates. The treatments were managed with manure (20 ton ha-1) (T1); T2: vermi-compost (10 ton ha-1) (T2); phosphate bio-fertilizer (E-barvar 2) (100 g ha-1) (T3); phosphate bio-fertilizer (E- barvar 3) (100 g ha-1) (T4); phosphate chemical fertilizer (Triple Superphosphate) (50 kg ha-1) (T5); and control (T6). Results showed that the effect of fertilizers application had significant effect on any of the evaluated traits in this experiment. The effect of vermin-compost on yield (992.50 kg ha^{-1}) and mucilage (19.65%) was more than other treatments. Maximum swelling factor (22.63 mmM^3), N (0.33) and K (0.39%) concentration, Protein (1.75%) and content of total carbohydrate (4.40 mg/g DW) were obtained at the animal manure. The effect of the phosphate bio-fertilizer (E- barvar 3) on P (0.24%) uptake of seeds was more than other treatments.
Key words: *Chemical change, Fertilizer, Isabgol*

Received 17.04.2013 Accepted 15.05.2013

INTRODUCTION

Nowadays, given the side effects of chemical medications and growing tendency to herbal medicine, mass cultivation of various medicinal herbs has been interested. Use of chemical inputs, some of the bottlenecks in the production of agricultural products is eliminated. Although this technology has had problems with other ecological, Such chemical fertilizers dose a health hazard and microbial population problem in soil besides beings quite expensive and making the cost of production high [26]. Extensive use of chemicals as fertilizers to improve plant health and productivity and for control of pathogens has disturbed the ecological balance of soil and has led to the depletion of nutrients. Hence there is a need to search for alternative strategies to improve soil health without causing damage to environment as well as soil [40]. cultivation conditions can be optimized to maximize synthesis of pharmacologically active constituents [19]. Application of fertilizers and manures in the soil are aimed to supply nutrients to the plant [5]. In such situation the bio-fertilizers play a major role. In the present agricultural practices there are number of microbial inoculants used as bio-fertilizers. A group of bacteria are now referred to plant growth-promoting rhizobacteria T (PGPR), which participate in many key ecosystem processes such as those involved in the biological control of plant pathogens, nutrient cycling and seedling establishment, and therefore deserve particular attention for agricultural or forestry purposes [16]. Some strains have multiple functions for plant growth. For example, strains of *Pseudomonas putida* and *Pseudomonas fluorescens* could increase root and shoot elongation in canola, lettuce and tomato [20,17].

There are intensive efforts worldwide to use organic manures to provide the same amount of food with less fossil fuel-based inorganic fertilizer. Organic manure is a suitable source of macro- and micronutrient, so using it as fertilizer can be an important disposal method [29]. Brown [7] showed that earthworms can modify the diversity and function of the rhizosphere microbial community (i)

by altering the physical and chemical environment through their burrowing and casting activities, (ii) by grazing leading to selection in favour of faster growing organisms, (iii) by dispersal of microorganisms either on their body surface or after surviving passage through the gut. Application of organic manure such as compost has a positive effect on crop production [45] wherein the nutrient deficiencies are correlated to the compost addition. The potential of the vermicompost to supply nutrient and support beneficial microbes is being recognized recently.

Animal manure is a good source of macro and micro nutrient, so using it as fertilizer is an important disposal method [29]. This contributes to diminishing the environmental pollution from manure disposal [11]. Vermin-compost and animal manure are good sources of organic matter and play a vital role in soil fertility improvement as well as supplying primary, secondary and micronutrients for crop production.

Isabgol (*Plantago ovata* Forsk.) 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 is a major contributor to the export earnings. The seeds of isabgol are composed of many different types of chemicals that are used as medicine. it contains mucilage, fatty oil, Proteins, carbohydrates, mineral element, etc. Seed oils are used to make soap, paint, printing inks, and other industrial supplies. Proteins are made up of long chains of amino acids, and are the main substrates for odour production. Proteins are the precursors for sulphurous, indolic and phenolic compounds and volatile fatty acids [28]. Carbohydrate increases neural activity in a number of regions of the brain and in the mouth has been shown to improve performance during prolonged physical activity [8]. Isabgol husk traditionally used against constipation, diarrhoea, intestinal irritation, etc., is a very good source of dietary fiber and has hypo cholaesterolemic activity [23]. Recently, it is increasingly being used as a food additive in several processed materials like cookies, ice-cream, bread, etc. [34,43].

Plantain cultivation without application of fertilization produced significantly lower yields of leaves, seeds [21], and some active substances content. With regards to importance of fertilizers on the Isabgol feeding the present research was done in order to evaluate the effect of bio-phosphate, chemical fertilizers and organic manure application to improve the seeds chemical contain and increasing of yield.

MATERIAL AND METHOD

Field trials of the present study were carried out at the experimental field of Zabol University (61° 29 N, 31° 23E, 450 m above sea level), in Southeast Iran, during the 2011 and 2012 growing season. The pH of soil field experiment was 8.4 and soil texture was sandy loam, (physical and chemical properties of soil in experimental field were presented in table 1). The experimental site is located in warm and arid region with mean annual precipitation of 63 mm and annual mean long–term average temperature of 23°C. The experiment was laid out in randomized complete block design with four replications. The treatments used in the experiment were manure (20 ton ha-1) (T1); T2: vermi-compost (10 ton ha-1) (T2); phosphate bio-fertilizer (E-barvar 2) (100 g ha-1) (T3); phosphate bio-fertilizer (E- barvar 3) (100 g ha-1) (T4); phosphate chemical fertilizer (Triple Superphosphate) (50 kg ha-1) (T5); and control (T6). The biofertilizers (BPB) used for this experiment was supplied by Green Bio-tech Co.; which Phosphate-E- Barvar2 consisted of two kinds of phosphate solubilizing bacteria such as Pseudomonas putida (strain p13) and Bacillus lentus (strain p5). Pseudomonas putida by producing of organic acids cause abandon the phosphate from inorganic compounds and Bacillus lentus by secretion and producing of phosphatase enzyme couse abandon the phosphate from organic compounds. the Phosphate-E- Barvar3 more than this strains is including of a new strain namely MC1. This biofertilizer is in initial stages of experiments. 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. Each experimental plot was 3 m long and 2 m wide with total area of 6 m2. During the growth period all plots were weeded manually. No serious incidence of insect or disease was observed and no pesticide or fungicide was applied to either crop. At the end of growth period 10 isabgol plants were sampled and yield per hectare were recorded. Mucilage percentage and swelling factor according to Ebrahimzadeh *et al*. [13] method were determined. Total N was determined by micro-kjeldahl method by titration method with sulphuric acid after the ammonium gases produced during distillation under alkaline condition had been absorbed by dilute boric acid [22], P by SnCl2 method

using spectrophotometer (Olsen et al., 1954) [33] and K content were determined directly by using flame emission spectrophotometer. Protein was calculated by % total N × 6.25 [3]. Total carbohydrates content was determined colorimetrically according to the method described by Michel *et al* [30].

Table 1. Soil, vermincompost and manure analysis result for physical and chemical characteristics

	pH	C:N ratio	N (%)	P	K	Mn	Cu	Zn	Fe
				mg kg^{-1}					
Soil	8.4	25.8	0.05	31.7	115	8.3	10.2	7.6	21.2
Vermicompost	7.8	11.3	0.600	1732.9	5451.4	331.8	87.7	66.0	6543.7
manure	7.6	27.4	1.71	322.0	2595.6	397.5	35.0	99.0	1677.2

Statistics
Data collected were subjected to the analysis of variance (ANOVA). The Duncan Multiple Range Test between mean values was calculated at the 5% probability levels.

RESULTS AND DISCUSSION
Seed yield
Analysis of variance showed that all properties that measurement in this study were significantly affected by various treatments (Table 2). Application of all kind of fertilizers significantly increased seed yield, seed chemical components and mucilage yield compared to control (Table 3). However maximum seed yield (992.50 kg ha-1) observed in vermin-compost application. The increase with vermi-compost may be due to more number of siliqua and bolder seeds as a result of availability of major and minor nutrients at all the essential stages of growth and development. According to Edwards *et al*. [14] Vermi-compost can have dramatic effects upon germination, growth, flowering, fruiting and yields of most of the crops. A good supply of organic manure also results more productive tillers, more number of spikes per unit area, number of grains per spike, grains yield. Findings of Mahmoud and Amara [31] confirmed these results. Vanaja and Raju [44] reported that highest seed and stalk yield of sunflower obtained with the application of organic manure.
Mucilage and swelling factor
The highest mucilage yield (19.65 %) and swelling factor (21.75mm M^3) was obtained in Vermi-compost and animal manure respectively (Table 3). Yadav *et al*. [46] also reported that biological, seed and mucilage yields of Isabgol significantly increased by using organic fertilizers, which can increase the available nutrition for plant roots and improve photosynthesis process. Singh *et al*. [42] reported that biological, seed and mucilage yield of Isabgol could be increased with application of organic and integrated systems due to improving of physical and chemical soil properties. 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. Ebrahim Zadeh *et al*. [13] also reported the high correlation between mucilage percentage and swelling factor in Isabgol. Khalil [24] recorded that mucilage content in Plantago afra herb was affected by organic manures with biofertilizers and this fertilizers producing the largest content of mucilage.
Seed mineral elements concentration
The nutrients absorbed by isabgol plant were influenced by the fertilizers. That would be propitious to meet the nutrient requirement for isabgol growth [42]. Therefore different fertilizers should select to study on the nutrients requirement of isabgol according to the character of nutrient absorbed and rule of isabgol growth. There was significant difference in seed nitrogen (N) concentration between treatments compared to the control (Table 3). However, the animal manure significantly increased seed N concentration than other treatments. Nitrogen concentration is related to chlorophyll content, and therefore indirectly to one of the basic plant physiological processes: photosynthesis [36,6]. Organic manure plays a very vital role in the process of nitrogen absorption by increase leaf area of the crop and by intercepting more sun light. Sengar *et al* [38]

reported that N, P, K uptake by rice was significantly increased by the application of N fertilizer and manure.

The minimum value of phosphorous percentage was observed in seeds that were obtained from control plots, while the maximum phosphorous percentage was found in seeds obtained from phosphate bio fertilizer plots (Table 3). Colonization of plant roots by micro organism greatly enhances the uptake of phosphorus and other nutrients [10]. There is increasing evidence that phosphor bacteria improve plant growth due to biosynthesis of plant growth substances rather than their action in releasing available phosphorus. Chaykovskaya *et al* [9] reported that treatment with phosphate solubilizing bacteria resulted in increased yield of pea and barley. Such obtained results are in agreement with those mentioned by Sarg and Hassan [37], Abo El-Salehein *et al* [1] on pea.

From the data presented (Table 3), it seems that animal manure have highest effect on the K concentration of isabgol seeds. Much research shows that incorporation of organic manure into soils, significantly stimulates soil microbial activity, microbial biomass and enzyme activity [27,41]. The enhanced soil enzyme and biological activities are believed to be direct indicators of the enhancement of soil fertility resulting from the incorporating organic manure [27], which helps increase N, P and K uptake by plants. Naguib and Aziz [32] indicated that application of organic manure either alone or in combination with NPK, were positively affected on N, P, K content of *Hyoscyamus muticus* plants. Similar results were obtained also with Ashorabadi *et al* [4] on fennel plant, and Anwar *et al* [2] on French basil. The obtained results are in harmony with those of Kotb [25] who stated that application of organic manure increased the total uptake of N, P and K by pea plants than control treatment.

Table 2: Analysis of variance for the mean squares of yield and seed chemical components of Isabgol

	df	Mucilage (%)	swelling factor (mmM³)	N (%)	P (%)	K (%)	Protein (%)	Carbohydrate (mg/g DW)	Yield (Kg ha⁻¹)
Block	3	1.06 ns	0.17 ns	0.02 ns	0.009ns	0.89 ns	0.01ns	0.01ns	8788.67 ns
Fertilizer	5	69.81**	6.83**	0.02**	0.009**	256.82**	0.76**	1.61*	292232.71**
Error	15	0.39	0.15	0.0002	0.0005	1.19	0.007	0.08	7094.82
CV(%)		4.69	1.88	10.11	3.97	3.88	9.79	8.61	12.42

ns= Non significant, ** = $p < 0.01$ and * = $p < 0.05$

Table 3: Effect of different fertilizers on yield and seed chemical components of Isabgol

	Mucilage (%)	swelling factor (mmM³)	N (%)	P (%)	K (%)	Protein (%)	Carbohydrate (mg/g DW)	Yield (Kg ha⁻¹)
T_1	16.17b	22.63a	0.33a	0.17c	0.39a	1.75a	4.40a	941.00a
T_2	19.65a	21.75b	0.23b	0.16c	0.35b	1.20b	3.82a	992.50a
T_3	14.43c	19.75d	0.14d	0.23a	0.24d	0.73cd	3.65b	443.30cd
T_4	11.48d	19.75d	0.16c	0.24a	0.28c	0.84c	3.75a	551.50c
T_5	9.80e	21.13c	0.13d	0.19b	0.21e	0.70cd	3.27c	717.30b
T_6	8.25f	19.38d	0.11e	0.11d	0.18f	0.59d	2.50d	322.80d

Columns means followed by the same letter are not significantly different at 0.05 or 0.01 probability level

Protein content of the seed

All treatments significantly increased the protein content of isabgol seeds (Table 2); the range varied from 0.59% to 1.75% according to treatment. The highest protein content was obtained with animal manure treatment (Table 3). The significant effect of animal manure may be due to the fact that this manure consists of different nutritive elements, which is why it is considered to be a balanced fertilizer that encourages the photosynthetic process and other physiological factors that increase protein synthesis. El Tilib *et al*. [15] reported that protein content increases with improved plant

nutrition and that the application of manure results in a high exchangeable capacity, hence a considerable quantity of phosphorus is diverted to available form and thus increased protein. Nitrogen is one of the essential nutrients involved as a constituent of bio-molecules such as nucleic acids, coenzymes and proteins [39], any deviation in these constituents would inhibit the growth and yield of plants. Protein concentrations in plants tend to increase with fertility level of the growth medium [18]. Dixit and Gupta [12] reported that farm yard manure or biofertilizer either alone or in combination showed an increasing tendency of protein content in rice grain.

The seeds content of total carbohydrates

As shown in Table (2), seeds content of total carbohydrates was significantly increased due to using the fertilizer treatments compared with control plants. Animal manure recorded the highest values in this respect. The positive effect of organic manure on chemical contents (total carbohydrates) of isabgol seeds may be due to their involvement in one or more of important biological functions such as synthesis of chlorophyll, electron transport system, oxidation-reduction reactions and protein synthesis [35].

CONCLUSION

Results of this study indicated that organic systems could produce the most yield, mucilage percentage, swelling factor and seed chemical components. Barvar Phosphate Biofertilizers (BPB) increased seeds measurement parameters compared with control. This biofertilizer could be used as a suitable fertilizer along with organic and chemical fertilizers to achieve the maximum benefits.

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How to Cite this Article
Abdolshakoor R., M. Galavi, M. Zafaraneieh, M. Soluki, S. R. Mousavi. (2013). Biochemical Change of Seeds and Yield of Isabgol (*Plantago ovata*) under Bio-fertilizer, Organic Manure and Chemical Fertilizer.*Bull. Env. Pharmacol. Life Sci.,* Vol 2 (6): 112-117.