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Influence of vermicompost and bacterium of Bacillus and Pseudomonas on growth, yield and morphological traits of saffron

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ABSTRACT

Saffron is a spice derived from the flower of *Crocus sativus* L. Biofertilizers and organic fertilizers are new alternatives to mineral fertilizers for increasing soil productivity and plant growth in sustainable agriculture. Therefore, it is necessary to determine the effects of biofertilizers and organic fertilizers on valuable medicinal plants such as saffron. To determine the effects of biofertilizers (*Bacillus* and *Pseudomonas*) and vermicompost application on growth and yield of saffron (*Crocus sativus* L.) an experiment was conducted in Faculty of Agriculture, Shahed University, Tehran, Iran during 2012-2013 growing season in a randomized complete block design with three replications and four fertilizer treatments. The treatments were control (without any fertilizer), 10 ton per hectare vermicompost, bacteria (mixture of *Bacillus* and *Pseudomonas*) and 10 ton per hectare vermicompost along with bacteria. The results showed that vermicompost and bacteria application had great impact on saffron growth and yield especially when these two fertilizers were mixed to each other, more positive results were observed. According to obtained results, combined application of vermicompost and bacteria can be useful in order to reduction in application of chemical fertilizer in agro-ecosystems which is towards minimizing environmental pollution and helping sustainable agriculture.

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INTRODUCTION

Saffron belongs to Iridaceae family and grows to 20–30 cm and bears up to four flowers, each with three vivid crimson stigmas (Kafi *et al*., 2006) which are used mainly in various cuisines as a seasoning and colouring agent. Saffron is native to Greece or Southwest of Asia (Hill, 2004) and was first cultivated in Greece (McGee, 2004). It is mostly distributed in Irano-Turanian region with low annual rainfall, cold winters and hot summers. At present, saffron is cultivated in Iran and a few countries. Iran is leading country in saffron production with 47200 ha cultivated area and 160 ton annual production (3.4 kg ha^{-1} yield) (Kafi *et al*., 2006). The main saffron production areas in Iran are located in Khorasan, Fars and Kerman provinces.

Saffron grows and develops in friable, low-density, well-watered, and well-drained clay-calcareous soils with high organic content (Deo, 2003) which promote good drainage. The soil organic content is a crucial soil property to guide agricultural applications and is historically boosted via application of organic substances such as cattle manure, crop residue and other type of composts also includes the remains of microorganisms and soil micro fauna (Koochaki and Gholami, 2006). Organic matters affect crop growth and yield directly by supplying nutrients and indirectly by modifying soil physical properties that can improve the root environment and stimulate plant growth (Bandyopadhyay *et al*., 2010).

Nowadays, low application of organic fertilizers such as composts and uncontrolled use of chemical fertilizers are the main reasons for soil degradation, decrease of soil organic matter and ultimately increased density of soils (Alidadi *et al*., 2013). So, organic substances like vermicompost can be a good substitute for chemical fertilizers to overcome their adverse effects (Joshi *et al*., 2013). Vermicompost is produced by biodegradation of organic material through interactions of earthworms and micro-organisms. It has higher levels of nutrition compared to the original materials of composts that have been created from other methods. It is a rich source of macronutrients, microelements, vitamins, enzymes and hormones that stimulating plant growth (Prabha *et al*., 2007). Essential elements such as nitrogen, phosphorus, potassium and calcium in the form of organic waste convert into available forms in the process of producing vermicompost (Ndegwa and Thompson,

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2001). It has been shown that vermicompost stimulates plant flowering, increases the number and biomass of the flowers (Arancon *et al*., 2008, Ramasamy and Suresh, 2011), as well as increases fruit yield (Singh *et al*., 2008).

Use of biofertilizers containing beneficial microorganisms instead of synthetic chemical are known to improve plant growth through supply of plant nutrients and may help to sustain environmental health and soil productivity (O'Connell, 1992). Nitrogen enters ecosystems via atmospheric deposition or biological nitrogen fixation. The latter pathway is mediated by prokaryotes, so called diazotrophs (Furnkranz *et al*., 2008). Various bacteria belonging to very different phylogenetic groups share the ability to reduce atmospheric N_2 to ammonium via the enzyme nitrogenase. So far considerable number of bacterial species mostly associated with the plant rhizosphere, have been tested and found to be beneficial for plant growth, yield and crop quality. They have been called 'plant growth promoting rhizobacteria including the strains in the genera *Acinetobacter Alcaligenes, Arthrobacter, Azospirillium, Azotobacter, Bacillus, Pseudomonas, Beijerinckia, Burkholderia, Enterobacter*, *Erwinia, Flavobacterium, Rhizobium* and *Serratia* (Sturz and Nowak, 2000; Sudhakar *et al*., 2000). Several species of rhizobacteria have also been found to synthesize plant hormones and have been suggested to play a key role in stimulating plant growth (Lindow *et al*., 1998; Beattie and Lindow, 1999). In previous studies, it was found that rhizobacteria could stimulate growth and increase yield (De Silva *et al*., 2000; Sudhakar *et al*., 2000; Esitken *et al*., 2002, 2003; Zahir *et al*., 2004). However, not much is known about promoting effects of *Bacillus* and *Pseudomonas* on growth and yield of saffron.

Organic farming of saffron is important due to medicinal and edible consumption of this plant. Although, there has been little discussion about organic farming of saffron, no comprehensive research has been done so far studying the effect of concurrent use of vermicompost and plant growth promoting bacteria in saffron cultivation. The key aims of the present study were to determine the result of vermicompost and bacteria treatments on growth, yield and morphological traits of saffron in comparison with conventional agriculture.

MATERIAL AND METHODS

A field experiment was conducted in Faculty of Agriculture, Shahed University, Tehran, Iran during 2013 growing season (latitude: 35° 559 N; longitude: 51° 389 E; 1062 m altitude; rainfall: 160 mm; mean temperature: 15.3°C).

Before planting, five soil samples were collected randomly at the depth of 30 cm and sent to laboratory to determine chemical properties. Soil properties are given in table 1. The experiment was laid out in a randomized complete block design with four treatments and three replications. The treatments included control (without any fertilizer), 10 ton per hectare vermicompost, bacteria (mixture of *Bacillus* and *Pseudomonas*) and 10 ton per hectare vermicompost along with bacteria. Samples of vermicompost used in this study were chemically analysed and the results of analysis were summarized in table 2. Nitrogen was measured by Kjeldhal method (Bremner and Mulvaney 1992), available phosphorus by John (1970) method, potassium, magnesium and sodium by flame photometer, Fe, Zn, Mn and Cu by atomic absorption and organic carbon base on Nelson and Sommers (1982) method.

The land was ploughed and then disked twice with the help of tractor. After land partitioning saffron corms were planted in the plots. Each experimental plot was 10 m long and consisted of 8 rows spaced 0.5 m apart. There were 2 m gaps between the blocks, to prevent lateral water movement and other interferences. The hill planting method was chosen and five saffron corms with the average weight 10-12 g were planted in each hole. Distance of holes was 25 cm. Irrigation was performed in a 10-day interval. Weeds were harvested manually and herbicide was not used. Plants were protected against possible pest and diseases by usual application methods.

Treatments were applied before flowering stage. Vermicompost was mixed into the soil at the certain amount and bacteria were added into the irrigation water (10^8 CFU). At harvest time, growth and yield related traits were studied and data were collected. All data were first analyzed by ANOVA to determine significant ($P \leq 0.05$) treatment effects. Significant differences between means were determined using Duncan's multiple range test (DMRT).

Table 1: Chemical properties of the soil

pH	$dS.m^{-1}$	Field capacity	T.N.V %	S.P %	O.C %	N %	P $mg.kg^{-1}$	K $mg.kg^{-1}$	Fe $mg.kg^{-1}$	Zn $mg.kg^{-1}$	Mn $mg.kg^{-1}$	Cu $mg.kg^{-1}$
8	3.5	25.4	19.3	40	1.05	0.1	87	896	4.9	2.14	9.2	1.3

Table 2: Chemical properties of the vermicompost

pH	$dS.m^{-1}$	O.C %	N %	P $mg.kg^{-1}$	K $mg.kg^{-1}$	Fe $mg.kg^{-1}$	Zn $mg.kg^{-1}$	Mn $mg.kg^{-1}$	Cu $mg.kg^{-1}$	Mg $Meq.l^{-1}$	Ca $Meq.l^{-1}$
8.5	6.21	17.5	2.24	1020	9340	75	37.85	30.5	6.12	11.8	5.2

RESULTS AND DISCUSSION

Analysis of variance showed that the effect of the fertilizer treatments was significant on all traits except for lateral corm fresh weight (Table 3). In addition, all leaf and flower related traits were affected by fertilizer treatments except for leaf length (Table 3). Comparison of means on corm weight indicated that vermicompost application and mixture of fertilizers had the highest effect on this trait (Figure 1). On the other hand, control treatment was found as ineffectual treatment (Figure 1). Mother corm diameter increased due to application of mixture and vermicompost treatments (Figure 2). Similarly, mother corm fresh and dry weight increased on account of vermicompost and mixture of fertilizers (Figure 1). This is the first study demonstrating that *Bacillus* and *Pseudomonas* and vermicompost can increase growth and yield of saffron under organic growing conditions. The plant growth and yield enhancement effects of bacteria and vermicompost used in this study on saffron could be explained by this fact that vermicompost, with high water-holding capacity and proper supply of macro- and micro-nutrients has a positive effect on biomass production and subsequently the enhanced plant growth. In addition, we found that the application of bacteria and vermicompost increased N, P and K content of saffron leaves which provide the additional evidence supporting our findings. Availability of nitrogen increases growth and leaf area index of plant which in turn increases absorption of light leading to more dry matter and yield (Taleshi *et al*. 2011). Bacteria application had the lowest effect on diameter of the corms (Figure 2). Moreover, control treatment had little effect on mother corm fresh and dry weight (Figure 1). Vermicompost application through the improvement of biological activities of soil and mineral element absorption (Arancon *et al*., 2004), caused more biomass production and flower number. It has been reported that vermicompost has high microbial activity due to presence of fungi, bacteria and actinomycetes (Tomati *et al*., 1988).Bacteria application significantly increased daughter corm number (Figure 3). Although daughter corm fresh and dry weight increased due to vermicompost application, mixture of fertilizers led to decrease in this trait (Figure 1).

Table 3: Analysis of variance on growth and yield related traits

S.O.V	d.f	Corm weight	Mother corm			Daughter corm			Lateral corm		
			Diameter	fresh weight	Dry weight	Number	Fresh weigh	Dry weight	Number	Fresh weight	Dry weight
Replication	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fertilizers	3	*	*	*	*	*	*	*	*	ns	*
Error	6	0.02	0.23	465837.42	662520.08	2413194444	1207537.22	1030327.14	18708854166	4242618.64	1786555.14
C.V (%)		16.37	19.42	22.64	37.81	49.90	34.92	75.35	62.46	31.43	68.41

S.O.V	d.f	Leaf						Flower		Stigma		
		Length	Width	area	LAI	Fresh yield	Dry yield	Number	Fresh yield	Fresh yield	Dry yield	Fresh weight/dry weight
Replication	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fertilizers	3	ns	*	*	**	*	*	*	*	*	*	**
Error	6	9.00	0.001	1.53	0.002	196242.08	120290.98	408663	654	2.60	0.12	0.22
C.V (%)		10.58	13.33	12.11	8.83	13.27	20.16	13.41	9.9	18.22	26.74	3.25

*, ** and ns significant at 0.01, 0.05 and no significant, respectively

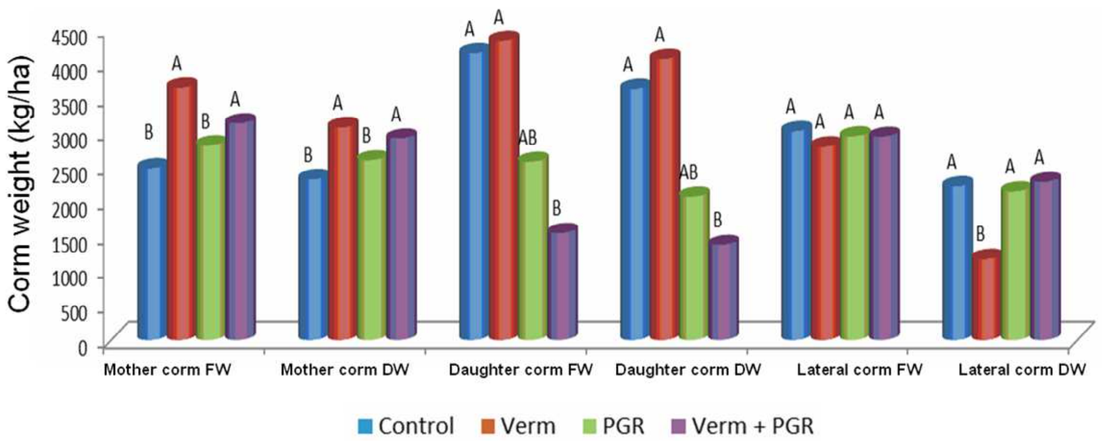

Fig. 1: Effect of biofertilizers and vermicompost on corm weight.

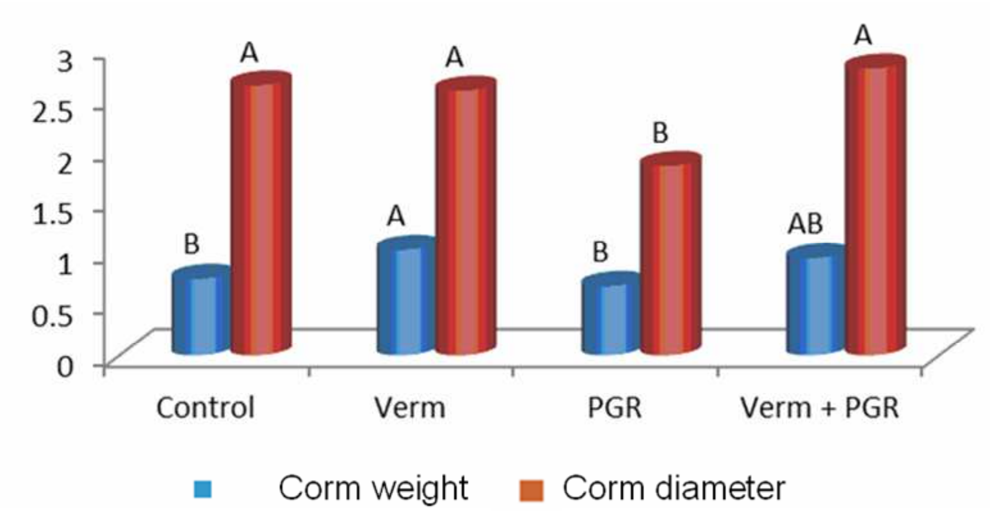

Fig. 2: Effect of biofertilizers and vermicompost on corm weight and corm diameter.

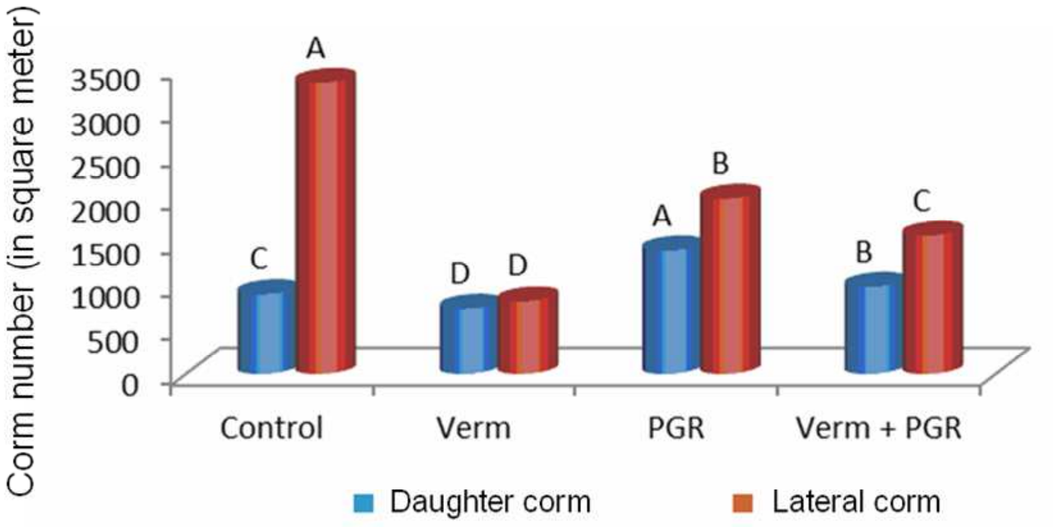

Fig. 3: Effect of biofertilizers and vermicompost on daughter and lateral corm number

Effect of control treatment on lateral corm number and effect of mixture of fertilizers on lateral corm dry weight were the most effective treatments (Figure 3 and 1). By contrast, vermicompost application demonstrated the lowest effect on this trait. In general, mixture of fertilizers and vermicompost application were known as the best treatments while control treatment and bacteria application had the lowest effect on these quantitative traits. Leaf width, leaf area and leaf area index increased due to vermicompost application (Figure 4 and 5). Earthworm casts have been shown to increase plant dry weight (Edwards 1995). The substitution of vermicompost in soil has always been associated with increasing germination, percentage and yield of vegetables even at low substitution rates and independent of nutrient supply in various experiments (Atiyeh *et al*., 2000). Growth and development of plants is due to the presence of humic acids (Arancon *et al*. 2005) and micro and macronutrients in vermicompost (Atiyeh *et al*., 2002; Fernández-Luqueño *et al*. 2010). The maximum increase in plant height of *Matricaria chamomomile* was observed by (Hadi *et al*. 2011) when vermicompost was applied at the rate of 20 ton per hectare.

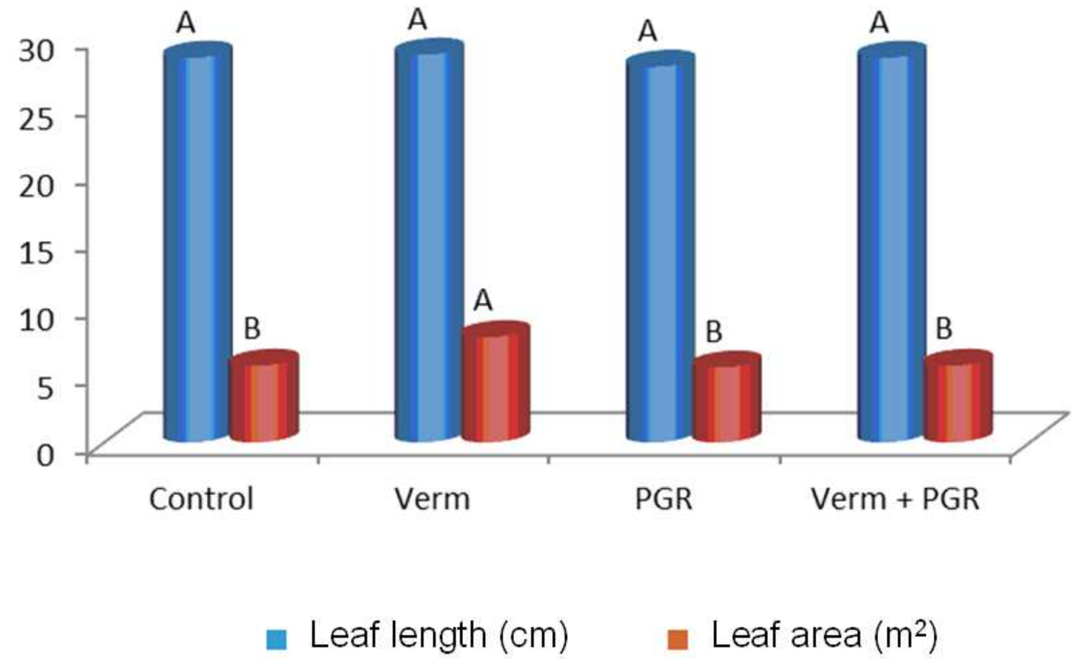

Fig. 4: Effect of biofertilizers and vermicompost on leaf length and leaf area

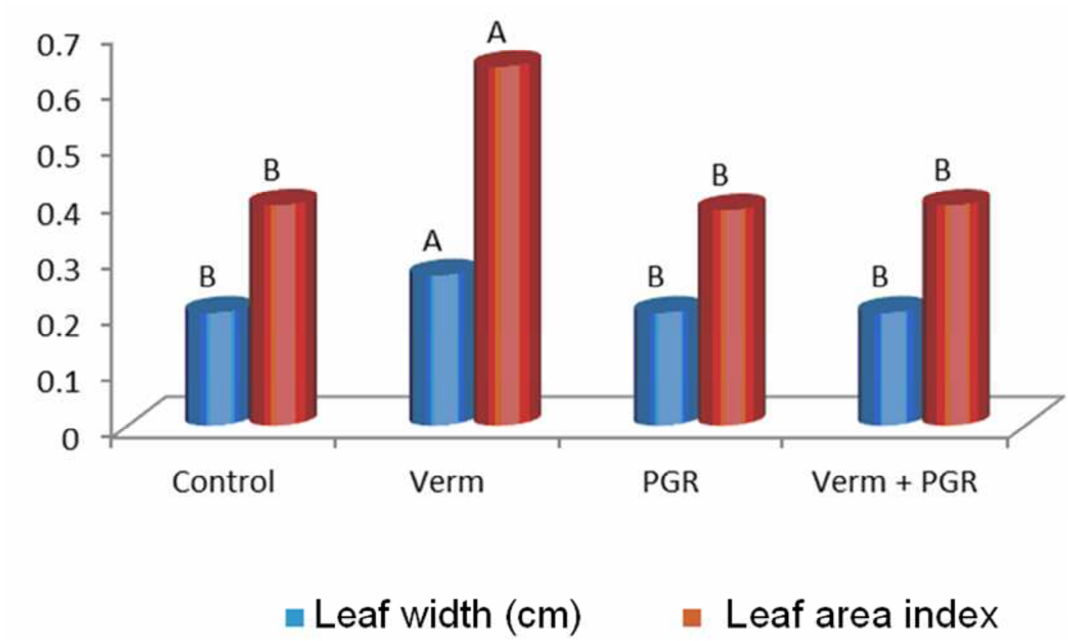

Fig. 5: Effect of biofertilizers and vermicompost on leaf width and lead area index

In similar studies, application of vermicompost increased stem diameter of *Lilium* plant (Moghadam *et al*. 2012) and okra (Ansari and Kumar Sukhraj 2010). Leaf fresh weight increased in control treatment while leaf dry weight increased in control, mixture and vermicompost treatments, however there was no significant difference between them (Figure 6). Bacteria application led to the lowest values regarding these traits but this reduction was not statistically significant (Figure 6). Generally, vermicompost and bacteria had the highest and the lowest effect on these traits, respectively. The results revealed that flower fresh yield, flower number and stigma dry yield increased due to mixture treatment while these traits decreased in control treatment (Figure 7, 8 and 9). Similar results were found when stigma fresh yield was measured, stigma fresh weight increased because of mixture treatment and decreased due to bacteria application (Figure 10). Overall, mixture treatment and vermicompost application had the most effect on quantitative traits related to the flowers. In a study, Azarmi *et al*.2009 reported that leaf dry weight and number of leaves of tomato increased on vermicompost applications. Vermicompost increased growth of various plants because of high porosity, aeration, drainage, and water-holding capacity (Edwards and Burrows 1988), presence of beneficial microflora (Tomati *et al*. 1987), nutrients such as nitrates, phosphates, and exchangeable calcium and soluble potassium (Orozco *et al*. 1996) and plant growth regulators (Tomati *et al*. 1988).

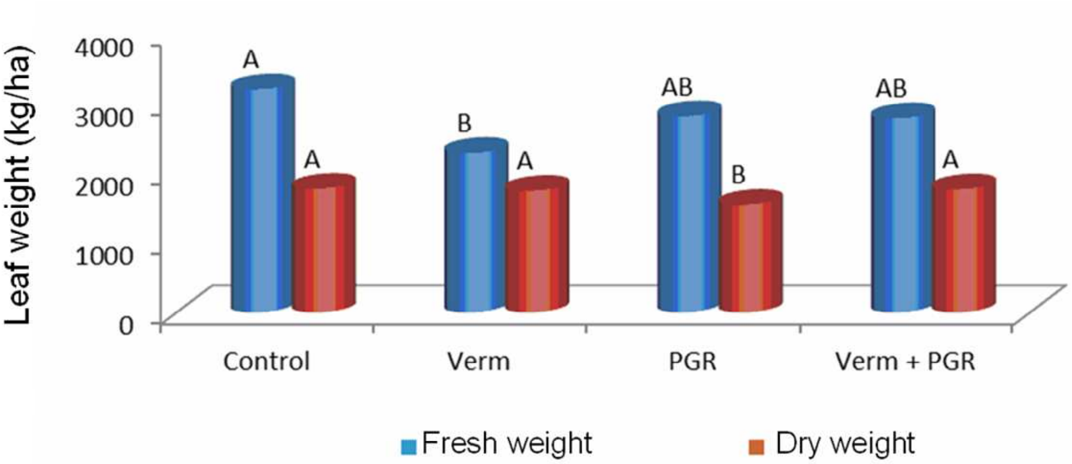

Fig. 6: Effect of biofertilizers and vermicompost on leaf fresh an dry weight

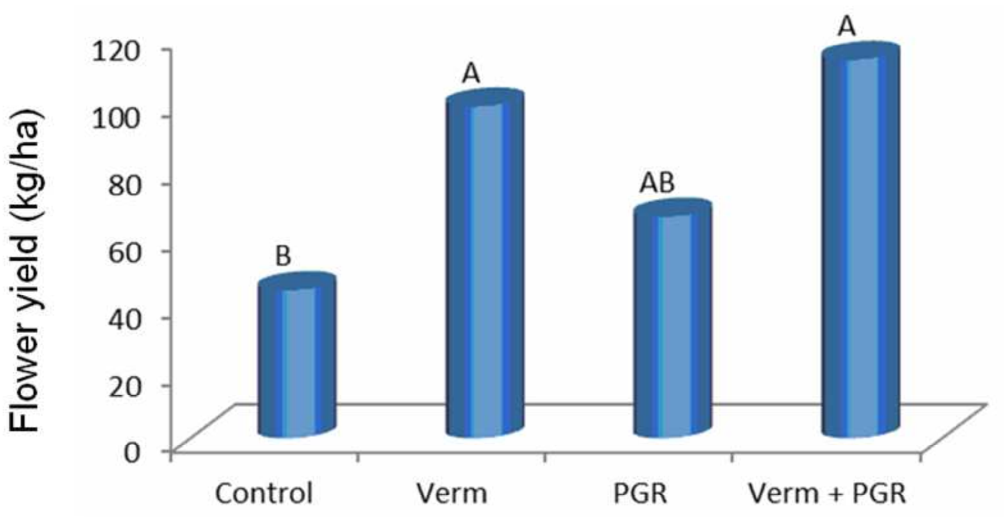

Fig. 7: Effect of biofertilizers and vermicompost on flower fresh yield

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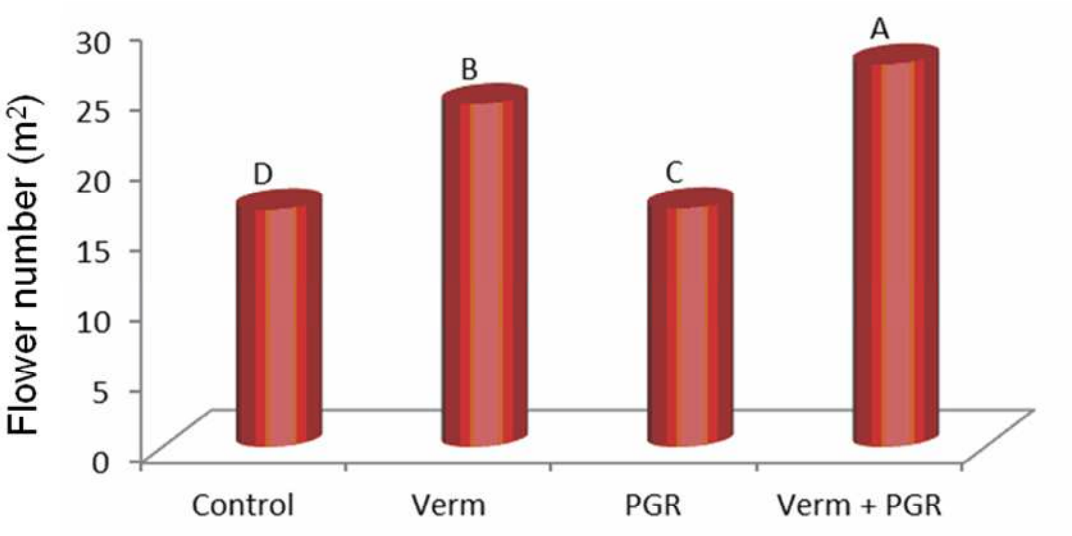

Fig. 8: Effect of biofertilizers and vermicompost on flower number

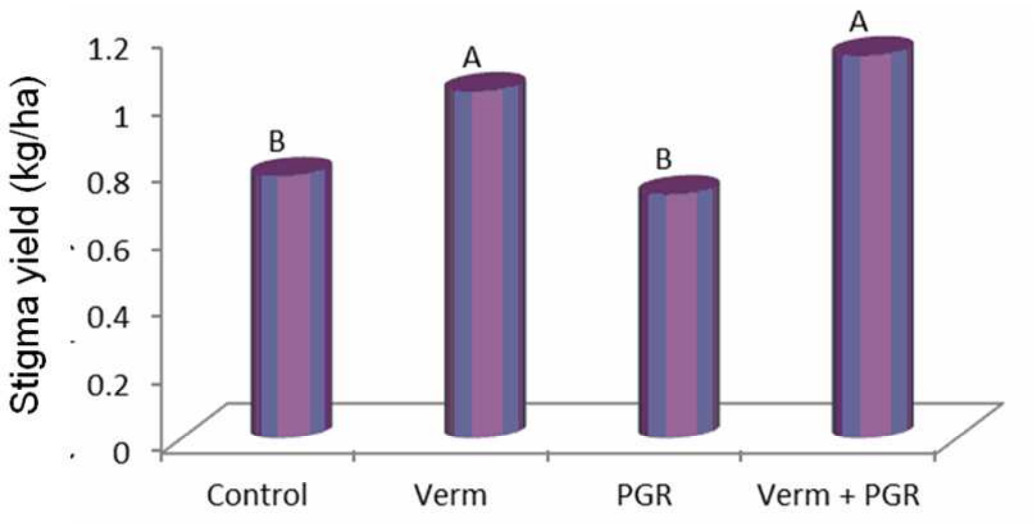

Fig. 9: Effect of biofertilizers and vermicompost on stigma dry yield

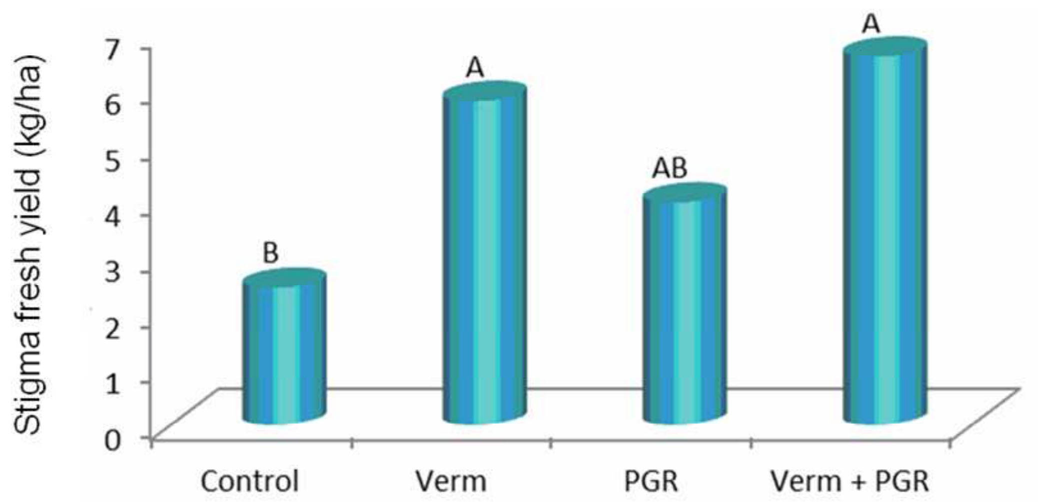

Fig. 10: Effect of biofertilizers and vermicompost on stigma fresh yield

Conclusion:

It is clear from the present study that biofertilizers and vermicompost successfully manipulate the growth of saffron, resulting in beneficial changes in yield. The highest biological and yield was obtained by using 10 ton vermicompost per hectare along with bacteria. Thus, combined application of vermicompost and bacteria can be helpful in developing of production and yield in saffron.

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