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Enriching vermicompost by nitrogen fixing and phosphate solubilizing bacteria as organic waste and its impact on growth of canola

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Abstract

Canola is an important crop. Using vermin composts specially enriched ones could increase plants production and quality. This has been done in several ways. In order to investigate effects of applying enriched vermin composts on growth of canola this study was being prepared. In this study use of vermicompost enriched with nitrogen-fixing bacteria and phosphate solubilizing with vermicompost enriched with chemical elements on canola plant was conducted at 4 levels. Canola growth parameters such as plant height (PH), stem diameter (SD), number of leaves (NL), leaf area (LA), relative water content (RWC) and leaf chlorophyll (LC) as representations of growth scale of the plant were measured and promising results has been gained. According to the results, the highest growth indices observed in two of treatments - vermicompost enriched with nitrogen, sulfur and phosphorus. Vermicompost enriched with *Azotobacter* and *Pseudomonas* and the lowest indices in the treatment vermicompost enriched with nitrogen were observed. No significant differences in the values of leaf area, chlorophyll index and relative water content were found, but exception of relative water content with increasing use of vermicompost had was strange, somehow.

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Introduction

Canola (*Brassica napus* L.) is an important annual oil seed crop for both industrial and nutritional purposes and recently has served as a source to produce bio-diesel fuel (Ashraf and Mc Neilly, 2004). Like soybean, canola contains both high oil content as well as high protein content. It is Obvious that more healthy oil production needs more investigations in order to reach high quality and high yield of oil seeds kike canola.

Managing solid wastes in this intensive agriculture and industrialized world became important issues during last few years. Utilization of organic waste could be a promising solution to this problem. One way to use these kinds of waste is to convert them as low cost degradable environmental friendly matters. Here, Vermicomposting is being considered as a potential option in the hierarchy of integrated solid waste management that involves the stabilization of organic material by the action of different microorganisms. Furthermore enriched vermicomposts could help more crop production and development of sustainnable agriculture (Kumar and Singh, 2001).

Vermicomposts are finely divided peat-like materials with high porosity, aeration, drainage, water-holding capacity (Edwards, 1998). They have greatly increased surface areas, providing more *microsites* for microbial decomposing organisms, and strong adsorption and retention of nutrients (Shi-wei and Fu-Zhen, 1991). Albanell *et al.* (1988) reported that vermicomposts tended to have pH values near neutrality which may be due to the production of CO_2 and organic acids produced during microbial metabolism (Albanell *et al.*, 1998). They also reported that their moisture content was reduced progressively during vermicomposting giving final moisture contents between 45% and 60%, the ideal moisture contents for land-applied composts (Edwards, 1983).

Plant roots perform as a niche for some species of soil born bacteria in which some are beneficial for plant growth (Compant *et al.*, 210).

These bacteria, including Plant Growth Promoting *Rhizobacteria* (PGPR) could influence plant growth in different ways by reducing toxicity of heavy metals (Pandey *et al.*, 2013), controlling soil born plant pathogens (Simonetti *et al.*, 2012) and changing plant hormonal levels (Jalili *et al.*, 2009). *Pseudomonas fluoresense* is one of the PGPRs that could increase growth and yield of plants specially root length under environmental stress (Jalili *et al.*, 2009; Nadeem *et al.*, 2013).

The aim of this study is to apply enriched vermicompost by bacteria treatments in a crop production system in order to investigate its effect(s) on canola growth factors and yield. Results of this study could help farmers to produce high quality organic crops with a high yield in a sustainable agriculture base and could help environment to be healthy.

The change in growth is consequence of several physiological responses including modifications of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency, carbon allocation, and utilization (Prusinkiewicz, 2004). Photosynthesis.

The process of capturing light energy and converting it to sugar energy, in the presence of chlorophyll using carbon dioxide (CO_2) and water (H_2O) and stomatal conductance are two important factors determining plant growth. Photosynthesis is influenced by two categories of factors - external or environmental and internal or plant factors (Hohmann-Marriott and Blankenship, 2011). Therefore, evaluation of effect(s) of applying vermicompost in plant growth environment by measuring photosynthesis and stomatal conductance could be proper parameters.

Materials and methods

Vermicompost Preparation

Vermicompost (VC) was produced from *Eisenia fetida* grown in a mixture of cow manure and plant residues (ratio of 3:1 w/w) during a five-month period at the vermicompost research-educational station of College of Agriculture and Natural Resources of Tehran University (CANRTU), Iran.

Cow manure and plant residues were initially decomposed in pits (0.7 m W, 0.5 cm H, and 2 mL) in sunlight for one month. After excess irrigation and leachate removal *Eisenia fetida* were added to the pits at a density of about 500 per 100 kg of manure/plant residue material. Pit moisture was maintained at about 50-60% and moisture content with daily irrigation. Earthworms were removed from the VC after four months (Gupta 2003).

Bacterial Strain Inoculation

Bacerial isolates were obtained from the Soil Science Department at Tehran University. The Ps 59 strain of *Pseudomonas* genus was selected due to its P-solubilizing ability, and strain Az 21 was selected from the *Azotobacter* genus, for its N2-fixing ability, based on previous studies as MSc or PhD thesis. *Azotobacter* and *Pseudomonas* bacteria belonged to *Azotobacter chroococcum* and *Pseudomonas florescense* species, respectively. After renewing bacterial cultu-res, populations of fresh inoculant were adjusted to about 4×109 cfu./ml, based on dilution factor and then 25 mL of each inoculant were applied per kg of VC (wet base) (Busato *et al*., 2012).

All treatments were kept at 60% holding capacity with additions of distilled water. VC samples (one kg per each replication) were incubated in a chamber with optimum aeration at 28 ºC for 60 days and during incubation moisture of VC samples were maintained about 60 percent by distilled water.

Preparation and Cultivation of Seeds/Experimental Design

In order to present investigation to study effect(s) of enriched vermicompost, four treatments, each in four levels (0, 1, 2 and 3 %) with three replications as a factorial based on completely randomized experimental design were applied and data were analyses using SAS 9.2 software.

Main factor was the treatments and the sub factor was levels of treatments. Mean comparisons was done using Duncan's Multiple Range Test with 95 percent probability by MSTAT-C and Figs were prepared using Microsoft Excel.

Then the cultivar Canola RGS was planted into three-Kg plastic pots. In order to do this, canola seed, cultivar RGS were prepared from Karaj seed and plant improvement institute, then germinated and transplanted to plastic pots in which contained composition of the treatments (Table 1).

Five plants were transplanted in each plastic pot and green house condition applied. This condition was under control to maintain 20-28ºC minimum and maximum temperature respectively and 75-80 percent air moisture with a 14-houre day light using Tungsten/Fluorescent light bulbs for four months and irrigation was done to maintain 0.75-0.8 field capacity (FC).

Table 1. Composition of different treatments.

Treatment	Vermicompost	*Azotobacter chroococcum* (21Az)	*Pseudomonas fluoresense* (Ps59)	Nitrogen	Sulfur	Phosphorus
VCC	Yes	No	No	No	No	No
VC-AS	Yes	Yes	Yes	No	No	No
VC+N	Yes	No	No	Yes (1%)	No	No
VC+NSP	Yes	No	No	Yes (1%)	Yes (1%)	Yes (1%)

Measurements

Canola growth parameters such as plant height (PH), stem diameter (SD), number of leaves (NL), leaf area (LA), relative water content (RWC) and leaf chlorophyll (LC) as representations of growth scale of the plant were measured when plants have been flowering.

Plant height was measured accurately from crown to the end of flag leaf with a metric unit, centimeter (cm). Leaf Area was measured using Delta T-Devices UK (ΔT Area Meter MK_2), Leaf chlorophyll was measured by SPAD-502 plus chlorophyll meter (manufactured by Konika Minolta) without

tissue damage and in order to measure leaf relative water content, a sharp razor blade was used to cut the leaf base. Leaves were then immediately weighed (fresh mass, FM).

The FM obtained from each sample was above the minimum 0.5 g recommended by Clausen and Kozlowski (Clausen and Kozlowski, 1965). In order to obtain the turgid mass (TM), leaves were floated in distilled water inside a closed petridish. During the imbibition period, leaf samples were weighed periodically, after gently wiping the water from the leaf surface with tissue paper.

The petridishes were maintained under dim light (around 20 mmol/m²s) and under naturally fluctuating temperature conditions in the laboratory. At the end of the imbibition period, leaf samples were placed in a pre-heated oven at 80°C, for 48 h, in order to obtain the dry mass (DM). All mass measurements were made using an analytical scale, with precision of 0.0001 g. Values of FM, TM, and DM were used to calculate RWC, using the equation 1.

Equation 1: RWC (%)=[(FM - DM)/ (TM - DM)]* 100.

The rate of CO_2 uptake in plants is one way to measure photosynthesis. Uptake of CO_2 can be measured with the means of an IRGA (Infra-Red Gas Analyzer) which can compare the CO_2 concentration in gas passing into a chamber surrounding a leaf/plant and the CO_2 leaving the chamber.

An instrument (i.e. IRGA model LCA4-ADC) was utilized to have photosynthesis rate as micromoles of CO_2 m^{-2} S^{-1} of leaves. This instrument was used to detect stomatal conductance which is the measure of the rate of passage of carbon dioxide (CO_2) entering, or water vapor exiting through the stomata of a leaf.

Results and discussion

Analysis of variance for effects of different enriched vermicompost treatments on some morpho/physicological attributes of canola RGS are presented in Table 2. According to this statistical analysis effects of most of the treatments were significant.

The treatments of N fertilizer addition, compost type, and compost rate all significantly influenced shoot height (Table 2). There were statistically significant two-way interactions between the main factors of compost type, compost rate on shoot height (Table 2).

This indicates that each of the main factors did influence shoot length but to understand their impacts the individual treatment combinations must be examined. These results were same for all measured attributes except RWC.

Table 2. Analysis of variance for six morpho/physiological attributes of canola under four treatments of enriched vermicompost with four levels and three replications.

		Mean squared					
	df	RWC	SPAD	LA	L.N.	S.D.	P.H.
A	11	2.6 Ns	18.1**	2557.7**	24.9**	4.2**	309.1**
B	3	198.3**	396.5**	38397.7**	310.6**	52.4**	3435.7**
R	2	2.6 Ns	0.7 Ns	194.5*	2.3 Ns	0.01 Ns	19.6 Ns
A×B	33	1.3 Ns	4.3**	538.0**	5.2**	1.4**	98.9**
Error	94	2.16	1.4	56.5	2.0	0.15	12.5
C. V.	-	1.61	2.79	4.37	9.70	5.71	5.70

Abbreviations: A: Main Factor; B: Sub Factor; R: Replications (Blocks); A×B: Interaction Effect of Main Factor and Sub Factor; C.V.: Coefficient of variation.

Mean comparison shows that the VC-AS and VC-NPS treatments have the highest levels of attributes, while VC-N had the lowest level (Table 3). Fig. 1 to 6 indicates that increasing level of different treatments of enriched vermicompost because significant increase in all attributes.

The vermicompost which has been enriched by urea (N) causes more pH in VC-N so that plant would not able to absorb most macro and micro elements (Adamtey *et al.*, 2009); which would affect different growth parameters. Kumae *et al.* (2011) reported that

amount of vermicompost affects yield as the more vermicompost is available the more yield is reported (Kumar *et al.*, 2011). Probably this result is due to more available nitrogen for plant.

Table 3. Means of different attributes of canola under applied treatments.

Treatments	VC+AS	VC+NSP	VC+N	VCC
Shoot height	69.19a	70.08a	60.46c	64.38b
Stem diameter	7.82a	7.66a	7.05b	6.88b
Number of leaves	16.13a	16.25a	13.92c	14.21b
LAI	176.0a	177.7a	175.7ab	172.7b
SPAD	41.8ab	42.1a	41.3b	41.4b
RWC	92.5a	92.3a	91.7b	92.0ab

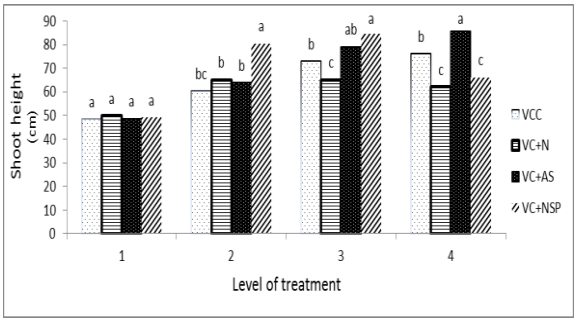

Fig. 1. Mean Comparison for P.H. in different levels of treatments.

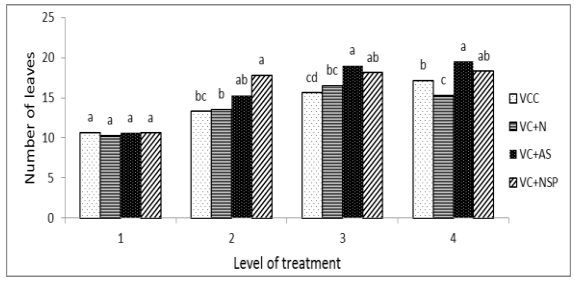

Fig. 2. Mean Comparison for L.N. in different levels of treatments.

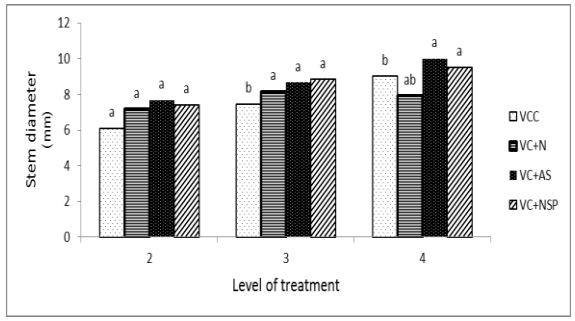

Fig. 3. Mean Comparison for S.D. in different levels of treatments.

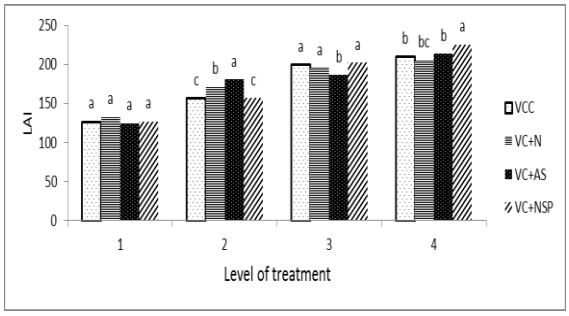

Fig. 4. Mean Comparison for L.A. in different levels of treatments.

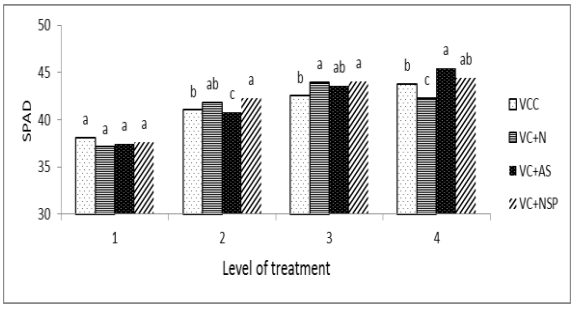

Fig. 5. Mean Comparison for Chl. (SPAD) in different levels of treatments.

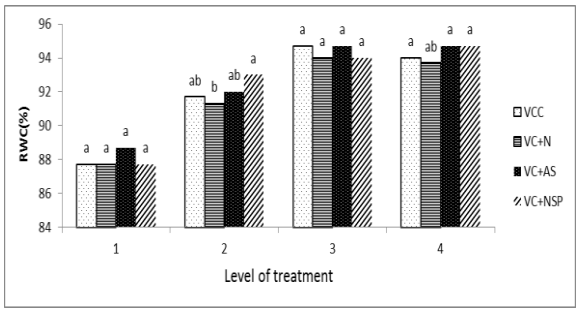

Fig. 6. Mean Comparison for RWC in different levels of treatments.

Our results show that LC and RWC did not change according to applied treatments but others indicated more growth parameters by increasing levels of enriched vermicompost.

This results are in same direction with Asciutto *et al.* (2006) who has reported that more levels of vermicompost causes more lea area (Asciutto *et al.* 2006). Same results have been reported for different crop/field plants.

When cabbage was grown in compressed blocks made from pig waste vermicompost, after transplanting to the field they were larger and more mature at harvest compared to those grown in commercial blocking material (Edwards and Neuhauser, 1998). More shoot biomass and increased seed yield of cowpeas was reported by (MBA, 1983).

The effects of different levels of introduced vermicompost treatments to canola on photosynthesis (PS) and stomatal conductance (SC) are shown in table 1 by analysis of variance and table 2 by comparison of means.

It is obvious that there are significant differences between different levels of vermicompost and by increase in vermicompost the PS and SC rate increase. On the other hand, applying VC+AS and VC+N were same as VCC in photosynthesis rate but higher than VC+NPS. This comparison for stomatal conductance was like the other treatment. These results are depicted in Fig. 7 and Fig. 8 respectively for PS and SC.

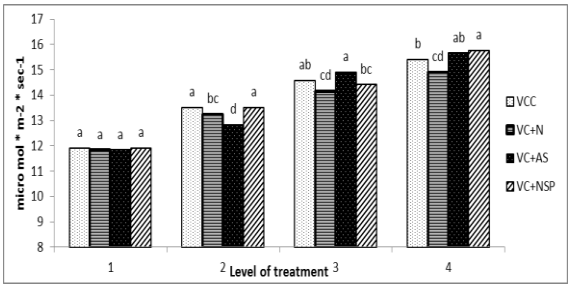

Fig. 7. Mean Comparison for stomatal conductance in different levels of treatments.

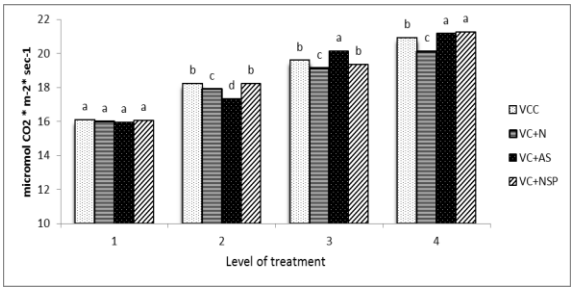

Fig. 8. Mean Comparison for photosynthesis in different levels of treatments.

There are several reasons how vermicompost could positively affect plant growth. Firstly vermicomposts contribute to improvements in physico-chemical and biological characteristics of the planting media or field soils that favored better plant growth (Atiyeh *et al.*, 2001). When vermicompost is used in plant root growth environment, nutrients such as N, P, K, Mn, Fe and Cu are available for plant, so that it could utilize them to increase chlorophyll content, photosynthesis and reach more growth (Theunissen *et al.*, 2010).

Reddy and Reddy (1999) reported significant increases in micronutrients in field soils after vermicompost applications compared to those in soils treated with animal manures (Reddy and Reddy, 1999). In other experiments, amounts of soil nitrogen increased significantly after incorporating vermicomposts into soils (Kale *et al.*, 1992; Nenthra *et al.*, 1999; Sreenivas *et al.*, 2000) and the amounts of P and K available also increased (Venkatesh *et al.*, 1998).

On the other hand there is a very substantial body of evidence demonstrating that microorganisms, including bacteria, fungi, yeasts, actinomycetes and algae, are capable of producing plant growth hormones and plant growth regulators (PGPRs) such as auxins, gibberellins, cytokinins, ethylene and abscisic acid in appreciable quantities (Arshad and Frankenberger, 1993; Frankenberger and Arshad, 1995).

Since, Normal irrigation was applied to the plants; it is expected to gain these results. Daneshmand (2006) claimed that drought stress could significantly

decrease morphological and physiological indices such as leaf area and relative water content (Daneshmand, 2006).

Inoculated compost was clearly superior to non-inoculated compost in promoting plant growth. Since the process of vermicomposting increases microbial diversity and activity dramatically, it is possible that vermicomposts could be a definitive source of plant growth regulators produced by interactions between microorganisms and earthworms, which could contribute significantly to enhancement of plant growth. On the other hand, enriching vermicomposts with main elements such as N, P and S could make these elements available for plants so that they could utilize them to increase macromolecule production and cell division. Finally, we hope that with enrich ing the vermicompost correctly individual problems of each chemical and biological fertilizers, bio-fertilizer would further improve performance.

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