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Vermicompost, plant growth promoting bacteria and humic acid can affect the growth and essence of basil (*Ocimumbasilicum*L.)

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ABSTRACT

To evaluate the effect of vermicompost, plant growth promoting bacteria and humic acid on growth and essence of basil an experiment was conducted in agriculture research field of Islamic Azad University, Karaj, Iran. The treatments were including; plant growth promoting bacteria (inoculation and non inoculation), vermicompost (application and non application), humic acid (non application, seed treated and foliar application) applied in the form of factorial experiment based on randomized complete block design. The results depicted that inoculation of PGPRs and application of vermicompost significantly affects almost the measured trait except for wet dry weight, leaf area and treatments were in the different statistical group. Whereas, seed treated of humic acid was superior to spraying it or to control. The highest wet and dry yield, essence yield and chlorophyll a, b and a+ b was observed in the interaction between PGPRs and vermicompost, indicating the synergistic effect. The interaction between PGPRs and humic acid led to the highest essence percentage, essence yield and plant height. Regarding to the replacement of chemical fertilizers with biologic fertilizers, it seems that applying biofertilizers, vermicompost and humic acid can decrease the use of chemical fertilizers and their negative effect. In addition they may play an important role to obtain the purposes of sustainable agriculture.

Key words: Basil, Vermicompost, PGPRs bacteria, Humic acid

INTRODUCTION

Although chemical fertilizers are one of the main factor to maintain soil fertility [10], excessive application of them has negative effect on physical, chemical and biological properties of soil and increase the possibility of soil erosion [15], meanwhile considering low input farming system and resource management in order to reach the sustainable agriculture purposes are of great interest. Thus biologic fertilizers application such as mycorrhizal fungi, phosphate-solubilizing microorganisms and vermicompost in a sustainable agriculture system not only can maintain the health of environment, but also increase the quality and stability of yield especially in medicinal plant production [18, 30]. Nowadays by revealing the side effects of chemical medicines, worldwide attention in medicinal plant production is turned the spotlight onto improving the quality, quantity and health of active ingredients of natural product via

ecologic farming. Therefore, it is obvious that using biologic fertilizers has the most conformity with the production purpose of these plants which leads to improve the qualitative and quantitative yield of them [25, 19, 18]. Offering organic matter in soil to eliminate the most important plant requirement is one of the outstanding advantages of the biofertilizers [24]. Furthermore, nutrient providing in the form of natural nutrition, developing biodiversity, increasing biological activity, improving quality and maintaining the health of environment are the other advantages of biologic fertilizers. Plant growth promoting bacteria (PGPRs) can be considered as biofertilizer. *Aztobacter*, *Azosperilliumand pseudomonas* are the most important of PGPRs. These species by biosynthesis of phytohormone such as different types of auxin, gibberellins and cytokine can influence the growth and development of plants [31]. On the other hand a species of earthworm that is called waste worm can digest organic waste like sewage sludge, manure and turn them to useful material for crop's growth[16, 17, 5]. This kind of materials is called vermicompost which have significant impact on water maintenance capacity of soil, nutrient supplying, phytohormone production [32]. Vermicompost as an organic fertilizer include an active biological mixture of bacteria, enzymes, plant residue, manure and cyst (capsule) of earthworm can cause the continuum analysis of organic matter and improve microbial activity in planting bed [9]. Humic material is a mixture of different organic compound which is obtained from plant and animal residue [21]. In other words humic acid is a natural organic polymer which is produced as a consequence of decomposing of soil organic matter, pit, lignin, etc. it can be applied to increase yield and its quality [4, 34]. As a whole humic acid has two function: 1) direct effect, as a pseudo hormonal compound [11, 12, 36].2) Indirect effect: by increasing mineral uptake such as Na, k, mg, Zn, Ca, Fe, Cu, etc. because it has the reduction, ability and can maintain membrane permeability [14, 26, 29], increase the metabolism of microorganisms, improve soil physical properties, increase the plant root and shoot growth [3, 6]. Basil (*Ocimumbasilicum*L.) as a medicinal crop belongs to *Lamiaceae*can cultivate almost in Mediterranean region. As Fertilizer management has been recognized as an important factor for successful medicinal crop cultivation, and also global attention is turned to sustainable agriculture and its management methods such as application of biologic and organic fertilizers in order to improving the quantitative and qualitative yield of medicinal crops, so that this study was investigated toevaluate the effect of vermicompost, PGPRs, humic acid on growth, chlorophyll content, wet and dry matter yield, essence percentage and essence yield of basil.

MATERIALS AND METHODS

Field plots were located at the research field of Islamic Azad University of Karaj branch in mahdasht (51° 6′ E, 35° 59′ N and 1,300 m above sea level). The soil was sampled for analysis of physico-chemical properties. Physical and chemical properties of the soil are given in under table.

Depth	Clay	Silt	Sand	Ec ds/m	pH	(O.C.) (%)	(T.N.V.) (%)	(N) (%)	P(ava)	K.(ava)
0-30	34	22	44	5.82	7.81	0.85	7.81	0.17	7.68	240

The experiment consisted of twelve treatments in factorial design with four replications based on randomized complete block design. The factors were PGPRs (inoculation, noninoculation), vermicompost (non application, 5 ton/ha) and humic acid (non application, soil drench application, foliar application). The used vermicompost was produced by manure and a species of earthworm (*Eiseniafoetida)*.

Humidity (%)	Oc (%)	Nt (%)	OM (%)	C/N ratio (%)	K (%)	P (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)	Ca (meq/lit)
25	37.7	4.92	65	7.66	3.19	0.16	36.50	27.40	15.25	15.5

The used bacterial inoculum was comprised of 3 PGPRs (*Aztobacter*, *Azosperilliumand pseudomonas*).

strain	*Pseudomonas putida*	*Azospirillumlipoferum*	*Azotobacterchroococcum*
Cell density (CFU g-1)	1×10^{9}	2×10^{17}	4×10^{8}

Treatment plots were 2×2 m which contained four rows spaced 25 Cm apart. Vermicompost were distributed and mixed with the soil on top of each rows. The Bacterial inoculum was applied as seed treated. Chlorophyll a, Chlorophyll b, Chlorophyll a+b, plant height and leaf area were measured at flowering stage. After harvesting shoot dry and wet weight were determined. Essence percentage was measured before flowering using Clevenger with 50 gr of dry matter. The content of Chlorophyll a, b, and a+ b in leaves was determined by spectrophotometer

according to Sestak&Catasky andChdolvadova et al. [28, 13]. Data were evaluated by analysis of variance using the MSTATC - SAS programs. Duncan's multiple range tests (P≤0.05) were used to compare means.

RESULTS AND DISCUSSION

The result depicted that vermicompost, PGPRs and humic acid had significant effect on measured traits. Shoot dry weight (28.28%), shoot wet weight (13.77%), essence yield (24.84%), plant height (13.2%), leaf area (17.17%), chlorophyll a (17.17%), chlorophyll b (17.37%), and chlorophyll a+b (17.35%) was enhanced by vermicompost relative to control, while it had no apparent effect on essence percentage (table 3). Relative to control inoculation of seed with PGPRs resulted in increase in shoot wet weight (34.9%), shoot dry weight (44.7%), essence yield (47.32%), plant height (15.85%), leaf area (22.04%), chlorophyll a (63.23%), chlorophyll b (61.86%), chlorophyll a+b (62.96%) (Table 3). However, humic acid application (either seed treated or foliar application) showed positive effect on mention traits. While seed treated was superior to foliar application of humic acid.

Table 1- Analysis of measured characters

S.O.V.	df	Shoot fresh weight	Shoot dry weight	Essential oil	Essential oil yield	Plant height	leaf surface	chl a	chl b	chla+b
Rep	3	1486875*	62500ns	0.001*	2.888*	19.464ns	1.291ns	2.689*	0.769**	4.421*
Factor A	1	13125208.3 **	1801875 **	0.002 ns	47.738 **	182.13 **	16.18 **	13.707 **	2.489 **	27.877 **
Factor B	1	69841875**	4200833.3 **	0.001 ns	142.69 **	254.38 **	236.368**	127.108 **	21.373 **	252.72 **
AB	1	4380208.3*	1110208.3 **	0.004 ns	43.463 **	45.047 **	1.277 ns	3.472 *	0.008 ns	3.819 ns
Factor C	2	849375 ns	239739.5 *	0.004 *	15.092 **	26.099 **	0.234 ns	2.445 *	1.017 **	6.503 *
AC	2	447708.3 ns	23593.7 ns	0.01 ns	0.732 ns	8.036 *	14.671 **	1.396 ns	0.101 ns	1.945 ns
BC	2	2030625 ns	45364.5 ns	0.011 **	7.8 *	18.786 **	15.435 **	1.071 ns	0.307 ns	2.517 ns
ABC	2	1678958.3 ns	16302.0 ns	0.002 ns	1.634 ns	15.766 **	0.753 ns	1.223 ns	0.242 ns	2.004 ns
Error	33	1107632.5	52159.0	0.002	2.352	2.6	0.947	0.616	0.312	1.521
C.V.(%)	-	12.96	14.13	6.99	17.01	15.14	14.35	11.60	19.85	12.87

***, * and ns show significant differences at 0.05, 0.01 probability level and non-significant, respectively.*

The interaction of vermicompost × PGPRs significantly affected plant height, chlorophyll a, shoot dry weight, wet weight and essence yield (table 2). Inoculation of bacteria together with vermicompost application was superior to individual application of these two factors which resulted in improving plant height, chlorophyll a, shoot dry weight, wet weight and essence yield (table 3). Based on suitable substrate that is being available by vermicompost, PGPRs could produce more. It seems vermicompost via stimulating bacterial activity in soil and the effect of bacteria themselves on root growth and development resulted in plant growth enhancement and plant dry matter. Regarding the influence of vermicompost and bacteria on nutrient availability and plant nutrient uptake (i.e. Mn, Fe), they resulted in chlorophyll enhancement in plant and increase plant height as well. Simultaneous application of these two factors showed synergistic effect which led to photosynthesis and plant dry weight increase. Essences are tripnoyid compound that their subunits, such as Isopantil pyrophosphate (IPP) and di-metylealil pyrophosphate (DMAPP), joinment need ATP and NADPH which in return need N and P [20]. So that, inoculation of microbial fertilizers (PGPRs) by influencing root growth can cause the N and P uptake by basils root and increase photosynthesis which finally resulted in essence increase and biological yield improvement. Stimulating effect of vermicompost on bacterial activity by providing suitable condition can increase solubility of inorganic phosphate in soil and organic phosphate in vermicompost that led to essence yield enhancement. The same effect of vermicompost on medicinal plant have been documented byPandey,Mcginnis et al.and Atiyeh et al.[23, 22, 6].

The combine effect of vermicompost × humic acid significantly affected plant height and leaf area (table 2). Treatment combination of A1C0, A1C1, A1C1 were superior to A0C0, A0C1, A0C2 respectively, which is because of vermicompost application in comparison with non application. Humic acid treatments (either seed treated or foliar application) with vermicompost were more likely effective than the other treatments, which resulted in plant height increase. Whereas humic acid (either seed treated or foliar application) increased leaf area in the absence of vermicompost. The announced increase in photosynthesis, shoot growth, plant height and leaf area can be referred to co-effect of vermicompost and humic acid, Due to vermicompost ability in increasing nutrient availability, water maintenance capacity, improving soil physical properties and its biological activity and also the role of humic acid in increasing nutrient uptake, improving physical properties of soil and synthesizing semi-hormonal substances. The positive effect of humicacid have been demonstrated by Turkmen et al. andAyas&Gulsar[33, 7]. The interaction between bacteria and humic acid had significant effect on leaf area, plant height, essence percentage and yield (table

2). Bacteria inoculation with humic acid application showed synergistic effect as their combination was more pronounced than their single application or in compared to control. The highest essence percentage, essence yield and plant height belonged to B1C2 and B1C1 respectively. This means that humic acid provided desirable condition for bacterial activity. Increasing leaf area was more demonstrated in treatment B1C0.Balakumbahan&Rajamani [8] have suggested that humic acid with the so called bacterium *Panckakavya*and the extract of Moringa have synergistic effect which led to improve the growth and yield of *Cassia angustifolia*. Humic acid include carbon which is the source of energy for N-fixation bacteria and can promote their biological activity [35]. Scientist have suggested that enzyme activity in plant stimulate when humic acid and biofertilizers especially *Azetobacter*+ mycorrhiza are co-inoculated [2]. They also have demonstrated that either co inoculation of *Azetobacter*and mycorrhiza or their single inoculation resulted in improved growth trait, increased photosynthesis pigment and nutrient uptake compared to other treatments without humic acid. In addition the highest grain yield and biological yield were produced by application of humic acid and dual inoculation of *A. chroococcum*and mycorrhizal fungi or single inoculation of *A. chroococcum*.Sellamuthu&Govindaswamy [27] have found out that rhizosphere microbial population and enzyme activity increased in the case of humic acid application which cause efficient use of nutrient. While, the maximum enzyme activity was observed when humic acid and biofretilizer were both applied that it could be because of the mechanisms of *Azetobacter*and mycorrhiza on soil properties. The effect of biofertilizers on medicinal plants have well documented by many researchers [1, 25].

Table 2- Mean comparison of characters

Tretment	Shoot fresh weight kg/ha	Shoot dry weight kg/ha	Essential oil gr/100gr	Essential oil yield kg/ha	Plant height cm	leaf surface cm2	chl a mg g-1 FW	chl b mg g-1 FW	chla+b mg g-1 FW
A0	7595.833 B	1422.917 B	0.561 A	8.018 B	29.438 B	21.782 B	6.233 B	2.588 B	8.822 B
A1	8641.667 A	1810.417 A	0.547 A	10.012 A	33.333 A	22.943 A	7.302 A	3.044 A	10.346 A
B0	6912.5 B	1320.833 B	0.55 A	7.291 B	29.083 B	20.143 B	5.14 B	2.149 B	7.289 B
B1	9325 A	1912.5 A	0.559 A	10.739 A	33.688 A	24.581 A	8.395 A	3.483 A	11.878 A
A0B0	6692. C	1279. C	0.5656 A	7.245 C	26.17 C	19.73 D	4.875 C	1.934 C	6.809 C
A0B1	8500. B	1567. B	0.5571 A	8.791 B	32.71 B	23.84 B	7.592 B	3.243 B	10.83 B
A1B0	7133. C	1363. C	0.5346 A	7.337 C	32.00 B	20.56 C	5.406 C	2.363 C	7.769 C
A1B1	10150. A	2258. A	0.5603 A	12.69 A	34.67 A	25.33 A	9.198 A	3.724 A	12.92 A
C0	7950. A	1509. B	0.5364 A	8.071 B	30.22 B	22.23 A	6.319 B	2.546 B	8.866 B
C1	8381. A	1750. A	0.5694 A	10.01 A	32.75 A	22.38 A	7.037 A	3.046 A	10.08 A
C2	8025. A	1591. AB	0.5573 A	8.963 AB	31.19 B	22.47 A	6.946 A	2.856 AB	9.802 A
A0C0	7613. BC	1331. C	0.5447 AB	7.241 C	28.81 D	20.55 C	5.493 C	2.234 B	7.726 C
A0C1	7813. ABC	1513. BC	0.5803 A	8.773 BC	30.00 CD	22.45 B	6.801 AB	2.830 AB	9.631 AB
A0C2	7363. C	1425. C	0.5590 AB	8.039 C	29.50 D	22.34 B	6.406 B	2.701 AB	9.108 B
A1C0	8288. ABC	1688. B	0.5281 B	8.901 BC	31.63 BC	23.91 A	7.146 AB	2.859 A	10.01 AB
A1C1	8950. A	1988. A	0.5586 AB	11.25 A	35.50 A	22.31 B	7.274 A	3.261 A	10.53 A
A1C2	8688. AB	1756. AB	0.5556 AB	9.887 AB	32.88 B	22.61 B	7.486 A	3.011 A	10.50 A
B0C0	7100. B	1275. C	0.5602 ABC	7.146 C	29.13 CD	18.88 D	4.757 C	1.895 C	6.653 C
B0C1	7175. B	1425. C	0.5590 ABC	7.979 BC	30.13 BC	20.77 C	5.125 C	2.233 C	7.358 C
B0C2	6463. B	1263. C	0.5310 BC	6.747 C	28.00 D	20.78 C	5.539 C	2.319 C	7.858 C
B1C0	8800. A	1744. B	0.5126 C	8.996 B	31.31 B	25.59 A	7.881 B	3.197 B	11.08 B
B1C1	9588. A	2075. A	0.5799 AB	12.04 A	35.38 A	23.99 B	8.950 A	3.859 A	12.81 A
B1C2	9588. A	1919. AB	0.5836 A	11.18 A	34.38 A	24.16 B	8.354 AB	3.394 AB	11.75 AB
A0B0C0	7350 CDEF	1263. E	0.5860 A	7.391 DE	27.00 E	17.11 D	3.890 E	1.558 E	5.448 G
A0B0C1	6975. DEF	1363. CDE	0.5828 A	7.932 DE	27.25 E	21.13 C	5.380 D	2.168 DE	7.547 F
A0B0C2	5750. F	1213. E	0.5280 AB	6.412 E	24.25 F	20.94 C	5.355 D	2.078 DE	7.432 F
A0B1C0	7875. CDE	1400. CDE	0.5035 B	7.091 E	30.63 D	23.99 B	7.095 C	2.910 BCD	10.00 DE
A0B1C1	8650. BCD	1663. C	0.5777 A	9.614 CD	32.75 BCD	23.78 B	8.222 BC	3.492 AB	11.72 BCD
A0B1C2	8975. ABC	1638. CD	0.5900 A	9.666 CD	34.75 B	23.74 B	7.457 C	3.325 BC	10.78 CD
A1B0C0	6850. EF	1288. DE	0.5345 AB	6.901 E	31.25 D	20.65 C	5.625 D	2.233 DE	7.858 F
A1B0C1	7375 CDEF	1488. CDE	0.5353 AB	8.027 DE	33.00 BCD	20.41C	4.870 DE	2.298 DE	7.168 FG
A1B0C2	7175. DEF	1313. CDE	0.5340 AB	7.082 E	31.75 CD	20.63 C	5.722 D	2.560 CD	8.283 EF
A1B1C0	9725. AB	2088. B	0.5218 AB	10.90 BC	32.00 CD	27.18 A	8.667 AB	3.485 AB	12.15 ABC
A1B1C1	10530. A	2488. A	0.5820 A	14.47 A	38.00 A	24.21 B	9.677 A	4.225 A	13.90 A
A1B1C2	10200. AB	2200. AB	0.5773 A	12.69 AB	34.00 BC	24.59 B	9.250 AB	3.463 AB	12.71 AB

Numbers followed by the same letter are not significantly different at P≤0.05.
Vermicompost: with 5 ton/ha (A1) and without (A0), Bacteria: with (B1) and without (B0) and humic acid : non application (C0), soil drench application (C1), foliar application (C2)

soil physical properties. On the other hand soil-born bacteria also increase nutrients uptake, regulate phytohormone synthesis and induce perfect condition for other microorganisms to produce so application of humic acid with PGPRs will show synergistic effect that result in increase nutrients uptake and affect growth index especially plant height and leaf area. Nutrients uptake enhancement especially phosphate led to increase the essence or hormonal precursor, hence better plant growth and essence. Tripartite interaction of vermicompost × bacteria × humic acid significantly affected plant height (table2). The highest plant height belonged to treatment A1B1C1 (table3) that is because of the co-effect of vermicompost, bacteria and seed treated of humic acid. Their tripartite interaction has also shown synergistic effect.

In summary, data presented in this paper indicated that inoculation of biofertilizers, humic acid and vermicompost together or individually via providing suitable substrate for plant establishment and growth can improve photosynthesis that result in basil's dry matter, essence percentage and essence yield increase. Regarding to the need of medicinal plant production and replacement of chemical fertilizers with biologic fertilizers, it seems that applying biofertilizers, vermicompost and humic acid can decrease the use of chemical fertilizers and their negative effect. In addition they may play an important role to obtain the purposes of sustainable agriculture.

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