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Studying the Effect of Seed Priming on the Growth and Yield of Marigold (*Calendula officinalis* L.) Under Different Levels of Vermicompost

Farzin Saeedzadeh

Department of Agriculture, Astara Branch, Islamic Azad University, Astara, Iran

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***Corresponding author's email:** shokriziba6@gmail.com

Abstract

The present study aims to investigate the effect of different levels of vermicompost on the growth and yield of the essential oil of marigold plant under seed priming with *Azospirillum* and humic acid under different levels of vermicompost factorial experiment carried out based on a randomized complete block design (RCBD) in the research farm of Islamic Azad University, Astara Branch. The test treatments included seed priming including *Azospirillum brazilense* bacteria, humic acid, *Azospirillum* + humic acid and control and different levels of vermicompost (0, 5 and 10 t ha^{-1}). The results showed that some traits such as chlorophyll a, anthocyanin, essential oil yield and biological yield were affected by vermicompost interaction and seed priming. But plant height, number of seeds and flowers, flower weight, seed weight, essential oil amount and total chlorophyll were affected by the main effect of vermicompost, but seed priming was not significant on some of these traits. The highest amounts of chlorophyll a and total, biological yield, essential oil yield and dry weight of flowers were obtained from humic acid + *Azospirillum* treatment under the application of 10 t vermicompost ha^{-1}.

Keywords: Anthocyanin, *Azospirillum*, Essential oil, Marigold, Vermicompost.

INTRODUCTION

The marigold belongs to the Asteraceae family, which usually flowers in spring. Today, marigold cultivation has been developed all over the world as an ornamental-medicinal plant. Marigolds are edible and are widely used for coloring food and salad, making tea and extract of marigolds, in addition to cosmetic and health industries, in the production of ointments, creams, eye drops, and oils. One of the most famous marigold products is calendula ointment, which is used to heal insect bites or superficial wounds (Catalano *et al*., 2022).

Optimum soil quality plays an important role not only in the production and growth of plants, but also in the quality and quantity of effective substances of medicinal plants (Ghadimi *et al*., 2021). Organic farming has gained a special position in most countries. In Europe, from 2015 to 2020, it has increased from 100,000 ha to 2.8 million ha. Its basis is the increase of organic matter in the soil and as a result maintaining soil fertility in the long term by applying organic management (Mandal *et al*., 2023).

The application of compost in the soil is generally in order to maintain and increase the soil stability, the fertility of agricultural and garden soils, which has been of special importance in the past decades (Liu *et al*., 2017). On the other hand, the use of urban waste compost in the soil has caused some concerns due to the aspects of phytotoxicity, the uncertainty of the nutritional value of the available nutrients for the plant and the environmental consequences (Tejada and González, 2009). Vermicompost contains nutrients such as phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in a form that can be easily absorbed and accessed by plants (Amanullah, 2016). Some researchers also reported that organic matter increases the P available to plants and indirectly prevents the precipitation of P in alkaline pHs, which is unabsorbable for plants (Mosavi-Azandehi *et al*., 2023). In addition, significant amounts of micronutrient elements (Amanullah, 2016), humic acid (Maji *et al*., 2017) and plant growth promoting substances such as auxins, gibberellins and cytokinins Mosavi-Azandehi *et al*., 2023) and dissolving bacteria P, enzymes and vitamins (Bakhshi *et al*., 2023), are present in vermicompost. The existence of these compounds leads to the implementation of vegetative growth and economic yield of the plant (Joshi *et al*., 2015). The results of various studies on the application of vermicompost showed that the highest plant weight, essential oil content and yield of Moldavian dragonhead (*Dracocephalum moldavica* L.) oil were obtained in the treatment of using 10 t ha^{-1} of vermicompost (Darzi *et al*., 2016). Dry matter yield in chamomile (*Matricaria recutita* L.) (Fallahi *et al*., 2013), basil (*Ocimum basilicum* L.) (Makkizadeh *et al*., 2011) and thyme (*Thymus daenensis* Celak L.) (Safaei *et al*., 2014) using vermicompost in increased compared to the control.

Humic acid as an organic growth stimulant has been commercially available to farmers for several decades in order to increase crop growth and economic efficiency (Olk *et al*., 2018). Humic acid is produced by the decomposition of organic materials, especially those of plant origin, and is found in soil, coal, and peat. Humic acid forms a stable and insoluble complex with microelements. Humic acid, a mixture of very large molecules with the ability to chelate nutrients, increase their absorption and soil fertility, along with folic acid, are the most important components of soil humus (Mackowiak *et al*., 2001). The results of the application of humic acid organic fertilizer on marigold showed that the application of humic acid increased plant height, fresh flower yield, and number of flowers, seed yield, petal yield and thousand seed weight compared to the control (Abedini *et al*., 2015).

Biological fertilizers also include sufficient amounts of one or more types of beneficial soil microorganisms, which are supplied with suitable preservatives and play a positive role in meeting the nutritional needs of plants and improve their growth conditions. The most important nitrogen-fixing bacteria are symbiotic Rhizobium and non-symbiotic *Azotobacter* and *Azospirillum*, which are present in the soil rhizosphere environment (Ahmadian *et al*., 2011). These microorganisms, when used on the seed, root surface or in the soil, stimulate root growth and increase the plant's growth by increasing the plant's accessibility to minerals (Vessey, 2003). It has been reported that *Azospirillum* increased stem weight and capitol diameter (Shokrani *et al*., 2012) and essential oil yield (Hosseini Mazinani and Hadipour, 2014) in marigold. The positive effect of biological fertilizers on the growth of garden thyme (*Thymus vulgaris* L.), savory (*Satureja hortensis* L.) and rosemary (*Salvia rosmarinus* L.) has also been reported by some researchers (Leithy *et al*., 2006; Nasiri-Dehsorkhi *et al*., 2018).

Considering the importance of producing medicinal plants in a sustainable production system, protecting natural resources and producing healthy medicinal products, the purpose of this research is to investigate the effect of using vermicompost, *Azospirillum* and humic acid and their interaction on some of the ecophysiological properties and essential oil content of marigold.

MATERIALS AND METHODS

The field experiments was conducted at an experimental farm in Astara, Guilan (Long. 48°52' E., Lat. 38°22' N., the elevation of 10 meters from sea level) in 2022. The study site has been reported to have a temperate and humid climate based on the climatic classification. Its annual precipitation and mean annual temperature have been reported at about 1326 mm and 19.8 °C, respectively. Table 1 depicts the climatic conditions of Astara including temperature and precipitation. Before the experiments, the farm soil was sampled to determine its properties. The results are presented in table 2.

Table 1. Average, maximum and minimum temperature and monthly rainfall in the Astara region during the plant growth period.

Months	**Monthly sunny hours**	**Precipitation (mm)**	**Temperature (°C)**		**Mean**
			Max	**Min**	
May	179.3	45.4	24.9	19.4	22.1
June	237.7	76.5	27.1	20.3	23.7
July	264.2	69.1	28.6	21.8	25.2
August	292.2	72.2	27.4	20.2	23.85
September	218.4	89.3	26.6	18.1	22.35

The study carried out as factorial experiment based on a randomized complete block design with three replications. The first factor was the vermicompost of cow manrure (VM) at a rate of 0 (VM0), 5(VM1), 10 (VM2) t ha^{-1}; seed priming treatments at four levels including *Azospirillum* brasilense (PA), humic acid 15 g l^{-1} (Ph), A. brasilense + humic acid (PA+Ph) and control (P0).

Table 2. Physical and chemical characteristics of the experimental field soil.

pH	**Electrical conductivity**	**Organic carbon (OC)**	**Nitrogen (N)**	**Phosphorus (P)**	**Potassium (K)**	**Sand**	**Silt**	**Clay**
	(dS m-1)	**(%)**		**(mg kg^{-1})**			**(%)**	
7.18	0.67	1.04	0.218	19.9	234	17.3	37.5	45.2

Agronomic practices

The seeds of the marigold of the Gitanana F1 cultivar with yellow petals, which were obtained from Pakan Seed Co., Isfahan. The *Azospirillum* brasilense was provided by the Soil Biology Research Department of the Soil and Water Research Institute. Each experimental plot was composed of six 5–m–long sowing rows with an inter–row spacing of 40 cm and on–row spacing of 10 cm. After the plots were prepared, the vermicompost was distributed according to the planting plan and was completely mixed with the soil. To inoculate the seeds, they were first impregnated with gum arabic, and the bacteria and humic acid were added to them. After the seeds were inoculated and dried, they were sown manually at a depth of 2 cm on May 10 and were immediately irrigated by the sprinkler method.

Plant sampling

In order to evaluate the traits related to a single plant (plant height, number of sub-stems per plant, diameter of the main stem, number of flowers per plant, leaf greenness index), seven plants from each experimental plot, taking into account the margin effect, were randomly selected and traits were measured. A caliper was used to measure the diameter of the main stem.

To estimate chlorophyll, the leaves were cut before the flowering stage, their chlorophyll was measured by Mazumdar and Majumdar›s (2003) method. Finally, leaf chlorophyll a, b and total was calculated by the following equation:

$$\text{Chlorophyll a (mg g}^{-1}\text{ FW)} = 9.93\ (\text{A660}) - 0.777\ (\text{A642}) \qquad (1)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ FW)} = 17.6\ (\text{A642}) - 2.81\ (\text{A660}) \qquad (2)$$

$$\text{Total chlorophyll (mg g}^{-1}\text{ FW)} = 7.12\ (\text{A660}) - 16.8\ (\text{A642}) \qquad (3)$$

Petal anthocyanin was estimated by spectrophotometry and the following formula:

$$\text{Petal anthocyanin (mg 100 g}^{-1}\text{ DW)} = (e\times b\times c)/(d\times a)\times 100$$

Where, a, b, c, d, and e were the reading, sample size, whole solution, taken sample size, and sample weight, respectively.

After the flowers opened, harvesting was done manually from the plants of the middle two rows of each plot and once every seven days. The harvesting operation started on July 20 and continued until the end of September. After each harvest, in order to maintain the quality, the harvested flowers were immediately dried in a suitable place away from direct sunlight, and after drying, they were kept in suitable paper envelopes until essential oil extraction. The total dry weight of harvested flowers in all harvests was considered as flower yield per unit area. After the end of the flower harvesting period, the bushes whose flowers were already harvested were cut from the top of the crown and weighed after drying. The total weight of harvested plants and flowers was considered as biological yield.

To determine the seed yield in each plot, one row was selected and the seeds were harvested and weighed at the ripening stage and recorded as seed yield, and then the weight of 1,000 seeds harvested was also measured. To determine the percentage of essential oil, a mixture of harvested dry flowers (sum of harvests) was used. In order to extract the essential oil, the water distillation method was used based on the method suggested by the European Pharmacopoeia and the Clevenger device, so that from each sample, 100 g of crushed flowers were extracted for four hours using the Clevenger device (Farjami and Nabavi, 2014). The following relationships were also used to determine the percentage and yield of the essential oil:

Equ.1: Concentration of essential oil = (Weight of extracted essential oil (g))/(Dry weight of flowers (100 g))×100

Equ. 2: Weight of flowers (g m^2) × Percentage of essential oil (%) = Yield of essential oil (g/m^2)

Statistical analysis

Data were subjected to the analysis of variance (ANOVA) for statistical analysis. The significance of the treatment effect was determined by the magnitude of F–value ($P < 0.05$). When the F–test revealed the significance of the treatments, the means were separately compared using the LSMEANS method with LSD adjustment at $P = 0.05$. The statistical analysis of the results was performed by the general linear model (GLM) in the SAS (Ver. 9.2) software suite.

RESULTS

Leaf chlorophyll

The results of analysis of variance showed that the content of chlorophyll a, b and total chlorophyll were affected by the interaction of vermicompost × seed priming (Table 3). The chlorophyll b increased with the increase of vermicompost application, so that the chlorophyll b at 5 and 10 t/ha levels increased by 24.6 and 39.3%, respectively, compared to the without vermicompost application treatment (VM0) (Table 6). The mean comparison showed that the highest chlorophyll a and total were obtained from the combined treatment of *Azospirillum* + humic acid (PA + Ph) under the application of 10 t/ha of vermicompost (VM2) (Table 7).

Petal anthocyanin

The results of analysis of variance showed that the anthocyanin was affected by the interaction of vermicompost × seed priming (Table 3). The mean comparison showed that treated plants increased the amount of anthocyanin by 13.3 to 42.8% compared to control plants. In all levels of vermicompost, the highest content of anthocyanin was obtained from PA (*Azospirillum*) and PA + Ph (*Azospirillum* + humic acid) (Table 7).

Plant height and number of branches

The results of analysis of variance showed that the main effect of seed priming and vermicompost on the plant height was significant (Table 4). The mean comparison showed that there was no significant difference between 5 (VM1) and 10 (VM2) t ha^{-1}, but compared to the VM0, they increased the plant height by 27.5 and 25.3% (Table 5). Seed priming treatments also increased plant height by 24.3 to 27.4% compared to control (Table 6). The number of branches was only affected by vermicompost and the application levels of vermicompost (MV_1 and MV2) increased the number of branches by 35.7 and 64.4% compared to the MV0 (Table 5).

Table 3. Analysis of variance in the effect of different levels of vermicompost and seed priming on physiological traits.

S.o.V	df	MS: Chlorophyll a	Chlorophyll b	Total chlorophyll	Anthocyanin	Concentration of essential oil	Yield of essential oil
Block	2	0.553^{ns}	0.671^{**}	5.02^{**}	481^{ns}	0.0025^{ns}	0.036^{ns}
Vermicompost (V)	2	11.93^{**}	0.8109^{**}	17.41^{**}	9274^{**}	0.0074^{ns}	0.513^{**}
Priming (P)	3	12.63^{**}	2.374^{**}	31.42^{**}	26004^{**}	0.023^{**}	0.773^{**}
V × P	6	22.18^{**}	0.336^{*}	23.69^{**}	49619^{**}	0.019^{**}	0.276^{*}
Error	22	0.378	0.089	0.412	562.1	0.0042	0.051
CV (%)		14.64	25.12	11.28	7.94	8.03	16.83

*, ** and ns: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test, respectively.

Leaf greenness index

The results of analysis of variance showed that the leaf greenness index was only affected by vermicompost (Table 4). The mean comparison showed that with the increase in vermicompost application, the leaf greenness index also increased (Table 5).

Number of flowers

Based on the results of analysis of variance, the main effect of vermicompost and seed priming on the number of flowers was significant at the 5 and 1% probability level, respectively (Table 4). The results of mean comparison of vermicompost levels showed that with the increase of vermicompost application, the number of flowers plant^{-1} increased so that the most flowers were obtained from VM2 level (Table 5). The results of the comparison of seed priming also showed that seed priming had a significant advantage over the non-prime treatment (P0) and the highest number of flowers was obtained from the *Azospirillum* + humic treatment (34.3 flowers plant^{-1}) (Table 6).

Table 4. Analysis of variance in the effect of different levels of vermicompost and seed priming on morphological traits.

S.o.V	df	MS: Plant height	Number of stems	Number of flower plant^{-1}	Leaf greenness index	Dry weight of flower	Seed number capitol^{-1}	Seed weight	Biological yield
Block	2	112.0^{*}	29.4^{ns}	25.03^{ns}	5.12^{**}	1437^{ns}	0.487^{ns}	97.4^{ns}	835^{ns}
Vermicompost (V)	2	268.7^{**}	146^{**}	118.9^{*}	18.84^{**}	9610^{**}	51.7^{**}	1987^{**}	7848^{**}
Priming (P)	3	837^{**}	32.7^{ns}	315^{**}	1.17^{ns}	4425^{*}	95.63^{**}	2210^{**}	16848^{**}
V × P	6	39.8^{ns}	58.2^{ns}	18.9^{ns}	0.39^{ns}	2147^{ns}	2.87^{ns}	879^{*}	14823^{**}
Error	22	26.07	33.16	49.7	0.863	1870	10.56	263.4	1725
CV (%)		3.92	13.44	9.35	22.10	39.55	29.40	33.25	9.23

*, ** and ns: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test, respectively.

Table 5. Means comparison for the main effect of different levels of vermicompost on the measured traits.

VM	Plant height (cm)	Number of stems	Number of flower plant^{-1}	SPAD	Weight of dry flower (g m^{-2})	Seed number capitol^{-1}	Biological yield (g m^{-2})	Chlorophyll b (mg ml^{-1})
VM0	37.21d	9.44c	16.44c	36.73c	163b	26.4	641.0c	0.846c
VM1	40.78b	12.72b	22.3b	38.05b	269ab	33.2	707.5b	1.124b
VM2	41.97a	14.96a	29.96a	49.83a	282a	35.6	744.9a	1.406a

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test. Vermicompost (VM) levels: 0 (VM0), 5(VM1), 10 (VM2) t ha^{-1}.

Dry weight of flowers

Based on the results of analysis of variance, the main effect of vermicompost and seed priming on the dry weight of flowers was significant at the 5 and 1% probability level, respectively (Table 4). The highest dry weight of flowers (282.3 g m^{-2}) was obtained from the application of 10 t ha^{-1} vermicompost (VM2), which showed an increase of 57.8% compared to the VM0, and the application of 5 t ha^{-1} of vermicompost (VM2) was ranked next with the production of 269.2 g dry flowers m^{-2} (Table 5). Also, the results of the mean comparison of seed priming showed that the Azospirillum + humic acid (PA + Ph) treatment had the highest dry weight of flowers (247.6 g m^{-2}), which caused a 55.4% increase in the dry weight of flowers compared to the control (Table 6).

Table 6. Means comparison for the main effect of seed priming treatments on the measured traits.

Priming treatments	Plant height (cm)	Number of flower plant^{-1}	Yield of dry flower (g m^{-2})	Seed number capitol^{-1}	Biological yield (g m^{-2})	Chlorophyll b (mg mL^{-1})
P0	37.82d	15.24c	137.6b	23.98d	57.28a	0.7083d
Ph	39.99c	18.38b	191.9ab	28.86c	54.279b	1.0058c
PA	41.08b	23.92b	217.8ab	34.44b	51.470c	1.2817b
PA+Ph	43.15a	34.9a	247.2a	39.86a	48.664d	1.7550a

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.Priming treatment levels: P0: Control, Ph: Humic acid seed priming, PA: Bio priming by *Azospirillum*, PA+Ph: Bio priming by *Azospirillum* + Humic acid seed priming.

Number of seeds per capitol

The results of the analysis of variance showed that the main effect of vermicompost and seed priming on the number of seeds per capitol was significant at the 1% probability level (Table 4). Based on the results of mean comparison, the application of 10 t ha^{-1} of vermicompost (VM2), with 35.6 seeds per capitol, increased the value of this trait by 24.5% compared to the VM0 (Table 5). *Azospirillum* + humic acid (39.86 seeds per capitol) also increased its amount by 74.1% compared to the control (Table 6).

Seed weight

The results of analysis of variance showed that the interaction effect of vermicompost × seed priming on seed weight was significant at the 5% probability level (Table 4). Based on the mean comparison results, the highest seed weight was obtained in *Azospirillum* (271 g m^{-2}) and PA + Ph (260 g m^{-2}) treatments under the application of 10 t/ha of vermicompost and these two treatments compared to the control increased the seed weight by 68.6 and 67.1%, respectively (Table 7).

Biological yield

Based on ANOVA results in table 4, it was observed that the interaction of vermicompost × seed priming was significant on biological yield (Table 4). The mean comparison of the treatments showed that at all levels of vermicompost, the priming treatments had higher biological yield than the control. All priming treatments showed the highest biological yield at the MV2 level, and PA + Ph (576 g m^{-2}) and Azospirillum (551 g m^{-2}) treatments were significantly superior to other treatments (Fig. 1).

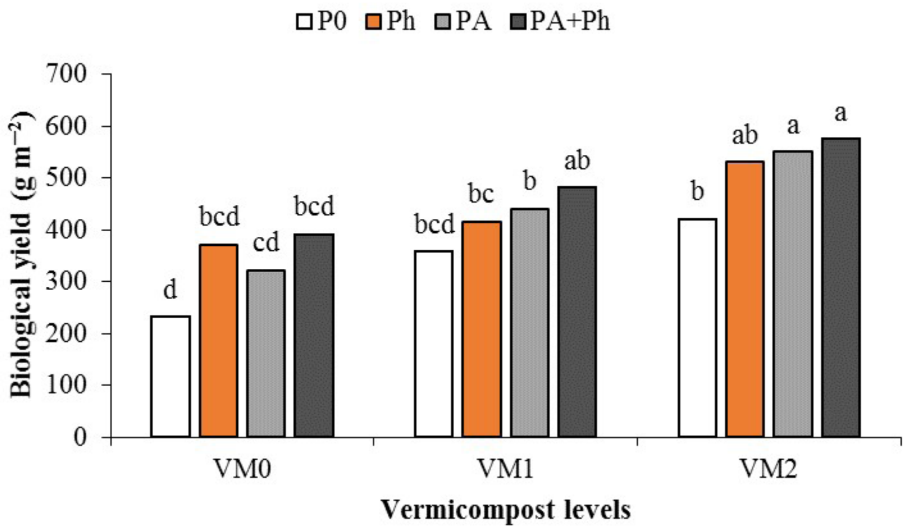

Fig. 1. Mean comparison of interaction effect of vermicompost × seed priming on biological yield. Priming treatment levels: P0: Control, Ph: Humic acid seed priming, PA: Bio priming by *Azospirillum*, PA+Ph: Bio priming by *Azospirillum* + Humic acid seed priming. Vermicompost (VM) levels: 0 (VM0), 5(VM1), 10 (VM2) t ha^{-1}. In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Concentration of essential oil

The results of analysis of variance showed that the interaction effect of vermicompost × seed priming was significant on the concentration of essential oil (Table 4). The PA+Ph treatment under VM0 level (0.132%) and Ph treatment under VM1 (0.168%) and VM2 (0.185%) levels showed the highest concentration of essential oil. Among all treatments, the control plants (P0 + MV0) showed the lowest concentration of essential oil (Table 7).

Yield of essential oil

The results of analysis of variance showed that the interaction effect of vermicompost

× seed priming was significant on the yield of essential oil (Table 4). Based on the means comparison results, the highest yield of essential oil was obtained in of PA + Ph (0.943 g m^{-2}) and *Azospirillum* (0.775 g m^{-2}) treatments, under the application of 10 t ha^{-1} of vermicompost, and these two treatments were 79.6 and 68.6%, respectively, compared to the control (Fig. 2).

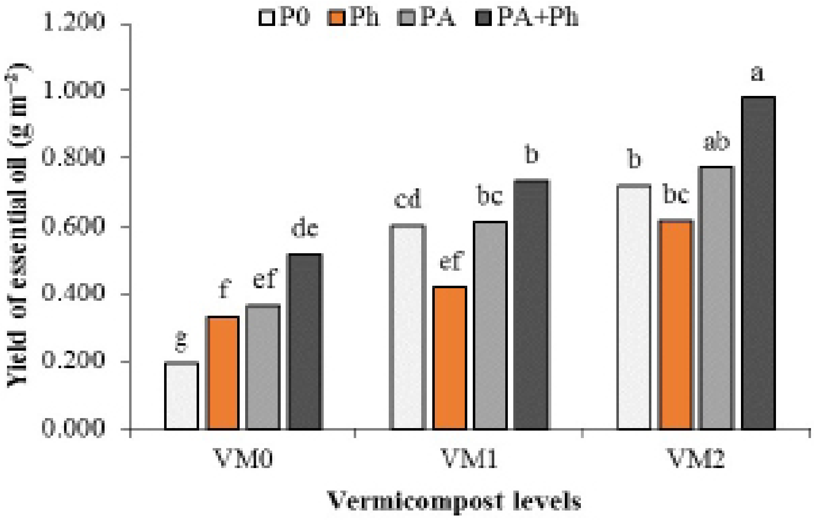

Fig. 2. Mean comparison of interaction effect of vermicompost × seed priming on yield of essential oil. Priming treatment levels: P0: Control, Ph: Humic acid seed priming, PA: Bio priming by *Azospirillum*, PA+Ph: Bio priming by *Azospirillum* + Humic acid seed priming. Vermicompost (VM) levels: 0 (VM0), 5(VM1), 10 (VM2) t ha^{-1}. In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Table 7. Means comparison for the interaction effects vermicompost × seed priming on the measured traits.

VM	PT	Chlorophyll a (mg ml^{-1})	Chlorophyll b (mg ml^{-1})	Chlorophyll total (mg ml^{-1})	Anthocyanin (mg 100 g^{-1})	Concentration of essential oil (%)	Seeds weight (g m^{-2})
VM0	P0	1.910h	0.446f	2.826h	210.1h	0.062i	85.00i
	Ph	2.820fgh	0.970de	3.830fgh	283.2ef	0.095h	129.3defg
	PA	3.670defg	0.706ef	4.770def	314.0cde	0.113fg	124.0efg
	PA+Ph	2.656gh	0.993de	5.206cde	241.6gh	0.132e	96.67hi
VM1	P0	3.556e-g	1.153c-e	3.443gh	300.8de	0.101gh	106.0ghi
	Ph	4.080c-e	1.643bc	5.816cd	333.1bcd	0.168b	119.6fgh
	PA	4.926bc	1.123de	4.623ef	248.0f-h	0.139cde	174.0c
	PA+Ph	4.486bcde	1.640bc	5.886c	323.0cd	0.152c	147.6de
VM2	P0	5.480ab	2.016ab	7.523b	372.9a	0.116f	154.3cd
	Ph	4.600bcd	1.056de	8.913a	250.8fg	0.185a	214.3b
	PA	5.340b	1.363cd	4.493efg	343.0abc	0.137de	271.3a
	PA+Ph	6.466a	2.240a	8.703a	369.1ab	0.170b	260.3a

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test. Priming treatment levels: P0: Control, Ph: Humic acid seed priming, PA: Bio priming by *Azospirillum*, PA+Ph: Bio priming by *Azospirillum* + Humic acid seed priming. Vermicompost (VM) levels: 0 (VM0), 5(VM1), 10 (VM2) t ha^{-1}.

DISCUSSION

Soil organic matter is a key factor in soil fertility and has been included in the UN Environmental Program as a fundamental environmental challenge at a global scale (Jhariya *et al*., 2022). In addition to soil health and quality, the presence of organic matter in soil is a proper indicator of soil fertility, which is the result of the interaction of physical, chemical, and biological processes (Ghadimi *et al*., 2021). Organic fertilizers, especially composted animal manure, have a large amount of organic substances, which are a rich source of N, P and K and over time, these elements are provided to the plant and increase the vegetative growth. These increases may be due to the fact that the application of animal manure facilitates the plant's access to water and sufficient nutrients, especially nitrogen, and as a result, it is effective in increasing the height of the plant and the number of branches through the effect on the division and enlargement of cells (Adamipour *et al*., 2019). Mahboub Khomami *et al.* (2021) reported in their research that the application of organic fertilizers caused a significant increase in the height of the ornamental foliage plants. In relation to the increase in plant height due to the use of humic acid and its combination with *Azotobacter chroococcum,* it seems that humic acid, through hormonal effects, influences the metabolism of plant cells, and by having the power of chelating and increasing the absorption of nutrients, causes it increases the growth and height of the plant (Sharafabad *et al*., 2022). The increase in the height of the vitex (*Vitex trifolia* L.) with the use of humic acid has been reported in another study (Ashour *et al*., 2020). In a research on the peppermint (*Mentha piperita* L.), the results indicated that the use of humic acid by creating better nutritional conditions for the vegetative growth of the plant caused a significant increase in traits such as plant height, shoot dry weight and dry matter yield (Askari *et al*., 2012).

The number of flowers is considered as one of the most important components of yield in the marigold, and with its increase, the weight of flowers also increases. The increase in the number of flowers and the weight of flowers with the use of vermicompost can be related to the effect of this fertilizer in increasing soil nutrients and providing the ability to absorb them in the plant (Mashayekhi *et al*., 2019), as a result of which the absorption efficiency of nutrients also increases. The reason for the increase in the number of flowers and flowers weight with the use of humic acid and *Azospirillum* can be because humic acid has increased fertility and production in the plant through chelating essential elements and increasing the absorption of nutrients (Nasiri Dehsorkhi *et al*., 2018). In addition to fixing N, *Azospirillum* causes the release of plant hormones such as gibberellic acid and auxin, and under these conditions, root growth and the availability and absorption of nutrients such as N and P increase, which ultimately increases the height of the plant, the number of secondary stems, and the number of flowers. An increase in the number of flowers and the dry yield of flowers has been reported in coneflower (*Echinaceae purpurea* L.) with the use of *Azotobacter chroococcum* biofertilizer (Agha Alikhani *et al.*, 2013) and in chamomile (*Matricaria chamomilla* L.) with the use of humic acid (Mashayekhi *et al*., 2019).

The use of larger amounts of vermicompost through increasing the water absorption power and the optimal provision of high-use and low-use nutrients has a positive effect on the amount of chlorophyll in the leaves and causes an increase in chlorophyll, and in addition, by providing the nutritional needs of soil microorganisms, it increases the number of and their activity increases, and as a result, the amount of absorption of micro elements such as iron, manganese, and magnesium, which play an important role in the production of chlorophyll, is increased by the plant, and finally they increase the synthesis of chlorophyll (Khosravi Shakib *et al*., 2019). It seems that the application of 10 t ha^{-1} of vermicompost improves and increases the content of organic matter in the soil, maintenance and proper provision of moisture and nutrients such as N, P and K, on the increase in the biological yield of marigold. It has an effect on the shape and weight of the seeds and has improved the yield of the seeds. In relation to the use of *Azospirillum*, it seems that the inoculation treatment increased the biological yield through the obvious effect that increased the number of branches plant^{-1}. On the other hand, probably, humic acid also increases the absorption of nitrate (NO^-_3) and the activity of the ATPase enzyme in the plasma membrane of root cells, leading to an increase in the absorption of nutrients from the soil, and as a result, by increasing the photo-center in the plant, it has led to an increase in yield (Manivannan and Nanthakumar, 2021).

It seems that adding vermicompost to the soil improves the activity of soil microorganisms and provides the necessary conditions for P solubility and more access to soil N, and as a result, P and N needed for the biosynthesis of ATP and NADPH to make terpenoid compounds (essential oils) in placed near the plant and essential oil production increases (Nasiri Dehsorkhi et al., 2018; Upadhya *et al*., 2022).

Since the use of vermicompost, *Azospirillum* and humic acid has led to an increase in the plant weight and percentage of essential oil of marigold, therefore, the yield of essential oil, which is a result of the last two parameters, also increases. In accordance with the results of this research, Massey *et al.* (2021) observed that the use of appropriate amounts of vermicompost in the cultivation of lemongrass (*Cymbopogon flexuosus* L.) significantly increased the yield of essential oil. They stated that the addition of organic matter to the soil has increased the growth of aerial organs and the production of dry matter and finally improved the yield of essential oil. Kumar *et al.* (2019) also reported that the use of organic fertilizers in marigold increases the yield of essential oil. Regarding the positive effect of the use of humic acid on the percentage and yield of essential oil, it can be argued that humic acid, by increasing the activity of the Rubisco enzyme, increases the photosynthetic activity of the plant and, as a result, the production of photosynthetic products, and because essential oils belong to the chemical group of terpenes, and because glucose is considered as a suitable precursor in the synthesis of essential oil and especially monoterpenes, photosynthesis and the production of photosynthetic products are directly related to the production of essential oil (Chaupoo and Kumar, 2022). Sajjadi *et al.* (2020) reported that the percentage of essential oil increased as a result of seed inoculation with *Azotobacter chroococcum* and *A. lipoferum* bacteria due to the increase of N absorption by the plant.

CONCLUSION

In general, the use of vermicompost can help improve plant growth conditions by increasing organic matter and storing soil nutrients. The use of *Azospirillum* also increases the plant's access to nitrogen and other nutrients and stimulates plant growth, and the use of humic acid increases the concentration of chlorophyll, anthocyanin, growth characteristics, flowers weight, concentration and the yield of marigold essential oil.

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