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Original Article

Effect of Chemicals and Bio-fertilizers on Yield, Growth Parameters and Essential Oil Contents of Funnel (*Foeniculum vulgare* Miller.)

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Abstract

In order to investigate the influence of bio- and chemical fertilizers on growth parameters and essential oil of fennel (*Foeniculum vulgare* Miller.), an experiment based on randomized complete blocks design with five treatments and three replications was carried out. Arbuscular mycorrhizal fungi (strain *Sebacina vermifera*) and phosphate dissolving bacteria (genera *Peseudomonas*) were applied as bio-fertilizers. Five treatments including T_1 as a control (100% recommended dosage of NP without inoculation), T_2 (50% recommended NP without inoculation), T_3 (*Sebacina vermifera* + 50% NP) T_4 (*Peseudomonas* + 50%), T_5 (*Sebacina vermifera* + *Peseudomonas* + 50% NP) were used. The results showed that inoculation of bio-fertilizers applied with 50% recommended dosage of NP, increased vegetative growth (plant height, number of umbel per plant, plant dry weight) of fennel plants compared to chemical fertilizer treatments only. Plants treated with combined of bio-fertilizers (arbuscular mycorrhizal strain *Sebacina vermifera* and phosphate dissolving bacteria genera *Peseudomonas*) had the highest plant height (72.6 cm), number of umbels per plant (45.5), dry weight (36.2 g) and fruit yield per plant (7.7 g). Application of bio-fertilizers also increased the amount of N, P and K in plant tissues compared to non-inoculated plants. Plants treated with combined bio-fertilizers had the highest amount of N (14.1 mg g^{-1}), P (8.2 mg g^{-1}) and K (9.2 mg g^{-1}) in their tissues, while the least amount of N (8.9 mg g^{-1}), P (4.7 mg g^{-1}) and K (5.9 mg g^{-1}) occurred in plant tissues treated with 50% NP fertilizers. The highest essential oil yield was also observed in combined bio-fertilizers + 50% NP. However, the least amount of essential oil yield was obtained in plants treated with half dose of chemical fertilizer.

Key words: Bio-fertilizer, Essential oil, *Foeniculum vulgare* Miller., Funnel, Plant growth.

Introduction

Medicinal and aromatic plants have been used by 80% of global population for their medicinal therapeutic effects as reported by world health organization (WHO) [1]. Many of these plants synthesize substances (secondary metabolites) that are useful to the maintenance of health in human and other animals [2]. Crop production and consequently secondary metabolites depend on nutritional management which is an important factor in success of agriculture. For optimum plant growth, nutrients must be balanced and should be sufficient for plants. In other words, the soil must have nutrients that are needed for plants [3]. However, some of soil-nutritional elements are mostly in an un-accessible form to plants and need long time period to release part of them through biological activity and chemical processes [4]. Hence, in order to increase crop yield per unit area, large amount of chemical fertilizers are used. Application of mineral fertilizers during large period of time has been leaded to the environmental pollution particularly in water and soil that threaten human society. Sustainable agriculture based on using biological fertilizers is an effective solution for overcoming these problems [5]. Using bio-fertilizers that contain different microbial strains has lead to a decrease in the use of chemical fertilizers and has provided high quality products free of harmful agrochemicals for human safety. The beneficial effects induced by the inoculated fungi such as arbuscular mycorrhizal and bacteria on plant growth

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are attributed to improvement of water and nutrients uptake, especially those of limited availability in soil such as nitrogen and phosphorus and micronutrients [6]. It was reported that phosphate solubilising bacteria, improved biological function and absorption of nutrient elements in crops [7]. Yazdani *et al*. [8] declared that inoculation of corn with phosphate solubilising bacteria reduced application of chemical phosphorus fertilizer by 50% without affecting corn yield. Shaharoona *et al*. [9] also reported that phosphate solubilising bacteria increased wheat yield. Jat and Ahlawat [10] reported that combined inoculation of phosphate solubilising bacteria and one strain of rhizobial bacteria on pea plants significantly increased biological yield, grain yield and grain protein level compared to control plants. Ratti *et al*. [11] found that the combination of the arbuscular mycorrhizal fungus, and *Bacillus polymyxa* and *Azospirillum brasilense* maximized biomass and phosphorus content of the aromatic grass palmarosa (*Cymbopogon martini*) when grown with an insoluble source of inorganic phoaphate. Likewise Toro *et al*. [12] reported that *Enterobacter sp*. and *Bacillus subtilis* promoted the establishment of the AM.

Fennel (*Foeniculum vulgare* Miller.) which belongs to Apiaceae family is one of the most important medicinal and aromatic plants due to its estrogenic activities and uses as a carminative, diuretic, anti-inflammatory and antimicrobial. Considering the importance of biological fertilizer for sustainable agriculture and the necessity to minimize chemical fertilizers application in agricultural ecosystems in Iran, this study was conducted to evaluate the effect of bio-fertilizers on growth, yield and essential oil of fennel plants to minimize usage of inorganic fertilizer.

Materials and Methods

An experiment based on randomized complete blocks design with three replications was carried out in farm conditions at Department of Medicinal Plants, Shirvan Faculty of Agriculture, Ferdowsi University of Mashhad, to investigate the effect of bio- and chemical fertilizers on growth traits and essential oil of fennel plants (*Foeniculum vulgare* Miller.). The bio-fertilizers used for inoculation of fennel seeds were: arbuscular mycorrhizal fungi (strain *Sebacina vermifera*) and phosphate dissolving bacteria (genera *Peseudomonas*). Five treatments including T_1 (100% recommended dosage of NP without inoculation as control), T_2 (50% recommended NP without inoculation), T_3 (*Sebacina vermifera* + 50% NP) T_4 (*Peseudomonas* + 50%), T_5 (*Sebacina vermifera* + *Peseudomonas* + 50% NP) were used. The full amount recommended of chemical fertilizers (NP) were ammonium nitrate (33% N) at the rate of 200 kg ha^{-1} and calcium super phosphate (15.5% P_2O_5) at the rate of 100 kg ha^{-1}. The inoculums of bio-fertilizers were kindly provided from Institute of Bio-Green Researches.

Plant density was kept uniform for all treatments (18 plants m^2). The distance between the plants within rows was 20 cm. Hand weeding was done when needed. Ten plants were randomly harvested from each plot and growth parameters such as plant height (cm), number of branches per plant, plant dry weight (g), number of umbels per plant, fruit yield per plant (g) were recorded. Concentrations of nitrogen, phosphorus and potassium in dried leaves were determined using the method of Cottenie *et al*. [13]. The volatile oil from fennel fruits was isolated by hydro distillation in order to extract the essential oils using the method of Guenther [14]. The isolated volatile oil was dehydrated over anhydrous sodium sulphate.

The data for all characters were analyzed using the analysis of variance procedure of Statistical Analysis System (SAS) software, version 6.12. Means were compared by Duncan's multiple range tests at the 0.05 probability level for all comparisons.

Results and Discussion

The results showed that inoculation of seeds with bio-fertilizers had significant effect on fennel growth parameters. Plants treated with *Sebacina vermifera* + 50% NP (T_3) and *Pesedomonas* + 50% NP (T_4) had higher growth parameters compared to plants treated with 50% NP only (T_2) (Table 1). Plants treated with T_5 (combination of bio-fertilizers + 50% NP) significantly had the highest plant height (72.3 cm) compared to other treatments except T_1 (full recommended of NP). There were no significant differences in number of branches per plant among different treatments.

However, fennel plants treated with T5 significantly had the highest number of umbels per plant (45.5) and consequently highest fruit yield per plant (7.7 g), while fennel plants treated with 50% NP (T_2) only had lowest number of umbel per plant (29.5) and fruit yield (4.6 g) (Table 1). Fennel plants dry weight also affected by fertilizer treatments. The highest (36.2 g) and lowest (23.3 g) dry weight were observed in plants treated with T_5 and T_2, respectively.

Table 1 Effect of different fertilizers treatments on growth traits and yield of fennel plants. Each number represents the means ± S.D of four measurements. Means followed by the same letter are not significantly ($p \leq 0.05$) different by Duncan's multiple range test within columns.

Treatment	Plant height (cm)	No. of branches per plant	No. of umbel per plant	Plant dry weight (g)	Fruit yield per plant (g)
100% NP (T_1)	69.1 ± 1.7^a	7.1 ± 0.5a	38.5 ± 2.3^b	30.1 ± 2^b	5.7 ± 0.8^b
50% NP (T_2)	55.3 ± 3.0^c	6.2 ± 1.1^a	29.5 ± 1.5^c	23.3 ± 1^c	4.6 ± 0.5^c
S. vermifera + 50% NP (T_3)	64.1 ± 1.5^b	7.1 ± 0.8^a	39.1 ± 1.7^b	32.3 ± 2.1^{ab}	6.2 ± 0.9^b
Pesedomonas + 50% NP (T_4)	63.5 ± 2^b	6.3 ± 1^a	42.3 ± 2^{ab}	33.6 ± 1.3^{ab}	6.4 ± 0.4^b
Combination of bio-fertilizer+ 50% NP (T_5)	72.6 ± 3^a	8.5 ± 1.5^a	45.5 ± 1.4^a	36.2 ± 1.9^a	7.7 ± 0.7^a

The next maximum growth parameters were observed in plants treated with T_4 (dissolving phosphorus bacteria + 50% NP) but was not significant compared to plants treated with T_3 and T_1 (Table 1). The highest (1.97%) and lowest (1.1%) essential oil were obtained in plants treated with T_5 and T_2, respectively (Fig 1). Results also showed that bio-fertilizers increased the amount of nitrogen, phosphorus and potassium in plant tissue (Fig. 2). The highest and lowest percentage of NPK in plant tissues were observed in plants treated with T_5 and T_2, respectively (Fig 2).

Fennel plants treated with T_5 had significantly the highest amount of nitrogen (14.1 mg g^{-1} dry weight), phosphorus (8.2 mg g^{-1}) and potassium (9.2 mg g^{-1}) in their tissues while plants treated with T_2 had the lowest nitrogen (8.9 mg g^{-1}), phosphorus (4.7 mg g^{-1}) and potassium (5.9 mg g^{-1}). These results are in agreement with those of Gad [15] for fennel (*Foeniculum vulgar* Miller.) and dill (*Anethum graveolens*), who reported that bio-fertilizers on these plants increased growth and yield. Increasing of growth parameters can be attributed to the positive effect of bio-fertilizers on nutrient uptake by plants. O'Keefe and Sylvia [16] reported that mychorrhizal fungi improve nutrient uptake of sweet potato, especially phosphorous uptake by improving physical exploration of the soil pore. In addition, phosphate dissolving bacteria secrete organic acids, which lead to transfer of fixed phosphate to available phosphate. This may increase growth of roots in the soil that can take up phosphorus. Some investigators [8,12] have explained the role of *Bacillus megatherium*, which increases the availability of phosphorus in the soil. Consequently there is an increase in phosphorus absorption as well as phosphorus accumulation in plant tissues. Although, there was no significant differences in some growth parameters of plants treated with full dose of recommended NP fertilizers (T_1) and T_5, bio-fertilizers appear to decrease the need of chemical fertilizers by contributing to the demand of optimum nutrient elements especially macro elements such as nitrogen, phosphorus and potassium for the growth of plant.

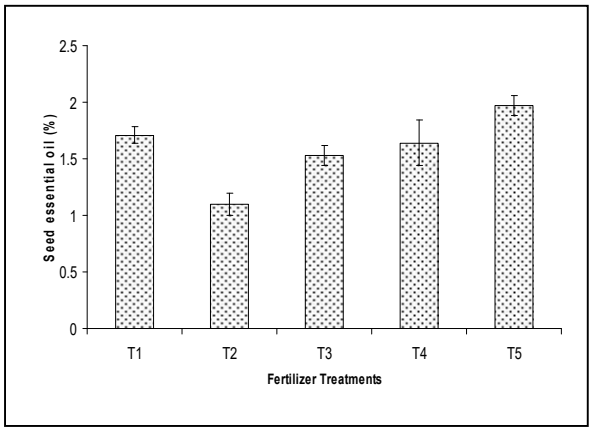

Fig 1 Effect of chemical and bio-fertilizers on seeds essential oil (%) of fennel plant. T_1 (full recommended chemical NP fertilizers), T_2 (50% recommended NP), T_3 (*S. vermifera* + 50% NP), T_4 (*Pesedomonas* + 50% NP) and T_5 (Combination of bio-fertilizer + 50% NP).

Nitrogen is one of the main constituent of protein and nucleic acid, which greatly influenced the cell division, cell enlargement and thereby it could increase the shoot length. Some researches [17,18] reported that bio-fertilizers increased the plant growth hormones like Indole Acetic Acid (IAA), Gibberelic acid (GA) and cytokinins. These phytohormones might have caused proliferation of roots and increased the uptake of nutrients.

The increase in activity of plant growth substances in plants inoculated with bio-fertilizers might have been responsible for increasing vegetative growth [19]. The reason for increasing essential oil might be due to the influence of nutrients (such as nitrogen and phosphorus) on synthesis of essential oil.

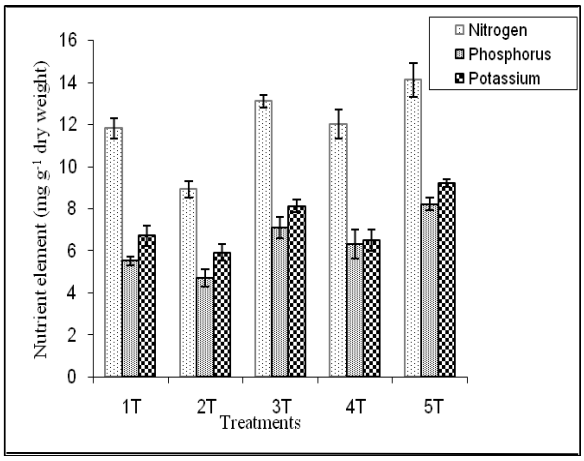

Fig 2 Effect of chemical and bio-fertilizers on amount of nitrogen, phosphorus and potassium of fennel plant tissues. T_1 (full recommended chemical NP fertilizers), T_2 (50% recommended NP), T_3 (*S. vermifera* + 50% NP), T_4 (*Pesedomonas* + 50% NP) and T_5 (Combination of bio-fertilizer + 50% NP).

It can be concluded that bio-fertilizers that are considered as an important part of environment friendly and sustainable agricultural practice, reduce the application of chemical fertilizers by 50% without any reduction in growth and yield of fennel plants.

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