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Response of Spring Safflower to Co-Inoculation with *Azotobacter chroococum* and *Glomus intraradices* Under Different Levels of Nitrogen and Phosphorus

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Abstract: Problem statement: In order to evaluate the effects of co-inoculation of Azotobacter and Mycorrhiza with nitrogen and phosphorus levels on yield and yield components of spring safflower, this study was carried out in the experimental field of Farahan University in Markazi province- Iran in 2006. **Approach:** A factorial experiment in the form of complete randomized block design with three replications has been used. Inoculation of Azotobacter (without and with inoculation by *Azotobacter chroococum*) and Mycorrhiza (without and with inoculation by *Glomus intraradices*) under different levels of nitrogen and phosphorus levels [$F_0 = N_0+P_0$(kg ha^{-1}), $F_1 = N_{50}+P_{25}$(kg ha^{-1}), $F_2 = N_{100}+P_{50}$(kg ha^{-1}) and $F_3 = N_{150}+P_{75}$(kg ha^{-1})] on spring safflower (*Carthamus tinctorius* L.-cv. IL-111) have been studied. **Results:** In this study some characteristics such as: Harvest index, hectolitre weight, root dry weight, seed yield, mycorrhizal root colonization, number of days to maturity were assessed. Results indicated that treatment ($A_1M_1F_2$) with average grain yield 1239 (kg ha^{-1}) and treatment ($A_0M_1F_0$) with average grain yield 723.7 (kg ha^{-1}) were significantly higher than other treatments. Seed inoculation at the planting date with Azotobacter and Mycorrhiza caused increasing grain yield about 6.13% in compare with control treatment. **Conclusion:** Seed yield and yield components of safflower have been affected significantly by the inoculation with Azotobacter and Mycorrhiza, because these biofertilizers can fix atmospheric nitrogen and increase phosphorus availability in soil and enhanced absorb elements by safflower.

Key words: Spring safflower, Azotobacter and Mycorrhiza

INTRODUCTION

The excessive use of chemical fertilizers have generated several environmental problems. Some of these problems can be tackled by use of biofertilizers, which are natural, beneficial and ecologically friendly. The biofertilizers provide nutrients to the plants and maintain soil structure. In Iran, the main oil seed crops are canola, sunflower, soybean and cotton, nevertheless safflower (*Carthamus tinctorius* L.) is one of the native plants and farmers don't produce safflower in a large scale because it does not have high grain yield and with a low oil content. However, safflower can be a potential oilseed crops for low-rainfall areas such as Iran.

Safflower has been grown for centuries, primarily for its colorful petals to use as a food coloring and flavoring agent, for vegetable oils and also for preparing textile dye in the Far East, central and northern Asia and European Caucasian[13]. It has also received considerable interest recently as forage plant[18]. Particularly, consumers have demanded healthier oils, naturally low in saturated fat such as olive, safflower, canola and sunflower oils. The seeds contain 35-50% oil, 15-20% protein and 35-45% hull fraction[28]. Most of the experiments have indicated that biofertilizers can play a major role on a soil with poor fertility that safflower could be grown on it[17]. Although biofertilizers and other alternatives are considered with suspicion by grown-promoting rhizobacteria and arbuscular mycorrhiza are known to be essential symbiosis without which the vast majority of plants could not survive in soils with normal levels of available phosphorus and nitrogen.

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Nitrogen is a major limiting nutrient for crop production. It can be applied through chemicals or biological resources, but chemical nitrogen fertilizers are expensive. Nitrogen is a fundamentally important element in biologically mediated production and nutrient cycling processes. N_2 containing constituents of organic molecules often confer bioactivity to these molecules. Major cellular, structural and functional constituents have essential and often highly specific requirements for N_2. Free living prokaryotes with the ability to fix atmospheric di-nitrogen (diazotrophs) are ubiquitous in the soil. But our knowledge of their ecological importance and their diversity remains incomplete. In natural ecosystems, biological N_2 fixation is most important source of nitrogen. The capacity for nitrogen fixation is widespread among bacteria. The estimated contribution of free-living N-fixing prokaryotes to the N input of soil ranges from 0-60 kg ha^{-1} $year^{-1}$[8]. Azotobacter is used as a biofertilizer in the cultivation of most crops. Azotobacter is an obligate aerobic diazotrophic soil-dwelling organism with a wide variety of metabolic capabilities, which include the ability to fix atmospheric nitrogen by converting it to ammonia. Azotobacter naturally, fixes atmospheric nitrogen in the plant rhizosphere. There are different strains of Azotobacter each has varied chemical, biological and other characters. However, some strains have higher nitrogen fixing ability than others[8]. *Azotobacter* sp. is a gram negative bacteria, polymorphic i.e., they are of different sizes and shapes. Old population of bacteria includes encapsulated forms and have enhanced resistant to heat, desiccation and adverse conditions. The cyst germinates under favorable conditions to give vegetative cells. They also produce polysachharides. These are free living bacteria which grow well on a nitrogen free medium. These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis[14]. Besides, nitrogen fixation, Azotobacter also produces, thiamin, riboflavin, indole acetic acid and gibberellins. When Azotobacter is applied to seeds, seed germination is improved to a considerable extent, so also it controls plant diseases due to above substances produced by Azotobacter[15].

Arbuscular Mycorrhizal Fungi (AMF) is one of the most important microbes of soil that form symbiotic associations with most of the terrestrial plants on the earth. These fungi are chiefly responsible for Phosphorus (P) uptake. Vesicular-Arbuscular Mycorrhiza (VAM) was able to alter water relation of its host plants and effects of VAM on morphology, metabolism and protective adaptation of host plants in the drought stress condition. The symbiosis of Arbuscular Mycorrhiza (AM) with host plant and hence, the production of a very extensive network of hypha, improves plant nutrient uptake and photosynthesis in the host plant[2]. Mycorrhizal symbiosis is actually a specialized network of hypha, enhancing the uptake and translocation of nutrients to the plant, compared with plant roots[20] especially under stress condition[11,21,22,29]. The mechanisms of VAM effect to enhance resistance of drought stress in host plant may include many possible aspects: (1) VAM improves the properties of soil in rhizosphere (2) VAM enlarges root areas of host plants and improves its efficiency of water absorption (3) VAM enhances the absorption of P and other nutritional elements and then improves nutritional status of host plant (4) VAM activates defense system of host plant (5) VAM protects against oxidative damage generated by drought and (6) VAM affects the expression of genetic material[30]. Many experiments have indicated that VAM were able to alter water relations and played a great role in the growth of host plant in the drought stress condition[5]. There is a great correlation between nutritional status of plant and its drought resistance, while VAM changed the nutritional status of its host plant. P concentrations themselves may affect host water balance, but it is often fixed in soil and not available to plant. Phosphatase produced by VAM fungi play an important role in changing fixed or insoluble into soluble P, which can be used by plant freely. At the same time, hyphae are also important ways of P transported in the soil. Other elements such as Zn and Cu can also not flow freely in soil[19]. This experiment designed to evaluate the effects of co-inoculation of Azotobacter and Mycorrhiza with different nitrogen and phosphorus levels on yield and yield components of spring safflower.

MATERIALS AND METHODS

This experiment was conducted in experimental field of Islamic Azad University- Farahan Branch (34°30`N, 40°41`E Long., 1779 m, height from sea level) in Markazi province- Iran in spring of 2006. Before sowing, combined soil samples from 0-30 and 30-60 cm depth were collected and their physical and chemical properties were analyzed. Specifically, our test included determination of soil texture using the hygrometry method[14], total N[25] and the concentration of available P (sodium bicarbonate extraction method[26]), available K(flame photometer method, emission spectrophotometry[16]), were determined (Table 1).

Table 1: Physical and chemical characteristics of soil

Soil texture	Loam	Loam
Clay (%)	25.00	-
Silt (%)	32.00	-
Sand (%)	43.00	-
Available K (ppm)	390.00	200-300
Available P (ppm)	9.30	10-15
Total N (%)	0.05	0.1>
Organic carbon (%)	0.39	>1.0
TNV	10.10	10.0<
pH	7.60	6.5-7.5
EC Mmos.cm^{-1}	0.90	2.0<
Depth (cm)	30-0	Optimum

The experimental design was a factorial arrangement in the form of randomized complete block design with three replications. Each plot consisted 4 rows, 5 m long with 50 cm spaced between rows and 5 cm distance between plants on the rows. Plant density was 40 seed m^{-2}. Treatments were included three agent: Azotobacter (without and with inoculation by *Azotobacter chorococum* with population 10^8 number per each ml, Mycorrhiza (without and with inoculation by *Glomus intraradices* with population 250-300 active spores for each planted seed and used combination of different rate of nitrogen and phosphorus in 4 levels: [F_0 = N_0+P_0(kg ha^{-1}), F_1 = N_{50}+P_{25} (kg ha^{-1}), F_2 = N_{100}+P_{50} (kg ha^{-1}) and F_3 = N_{150}+P_{75}(kg ha^{-1})] on spring safflower (*Carthamus tinctorius* L.-cv. IL-111). Urea (0, 50, 100 and 150 kgN ha^{-1}) was used; It was broadcasted to the plots meanwhile. Triple superphosphate (0, 25, 50 and 75 kgP_2O_5 ha^{-1}) was spread at sowing time. The plants were thinned after complete emergence in the 6 leaf stage as keeping on rows about 5 cm. Final harvest was performed at physiological maturity stage when a black layer was formed at seed base. Ten plants from the middle of each plot were harvested. In harvest stage, the two middle rows were used for sampling and measured parameters such as: hectoliter weight, mycorrhizal root colonization percent, roots length, harvest index, root dry weight, number of days to maturity, oil percent, oil content and grain yield were assessed. Grain yield in each plot measured with 14% humidity. Mean comparisons of treatments were conducted using Duncan's Multiple Range Test[31].

RESULTS

Results from the present study indicate that grain yield have been affected significantly by the inoculation with Azotobacter. In other word, Azotobacter could proper part of nitrogen for feed plants in the rhizosphere. But mycorrhiza could affect significantly on characters such as; harvest index, hectolitre weight, root dry weight and mycorrhizal root colonization. Combined application of nitrogen and phosphorus levels had significant effect on grain yield, oil content, root dry weight, mycorrhizal root colonization and number of days to maturity at 1 and 5% probability level on hectoliter weight.

The data (Table 2) indicated Azotobacter inoculation significantly increased grain yield of safflower (6.53%) in compared to treatments without inoculation. N-fertilization also significantly influenced the seed safflower yield, but Mycorrhiza inoculation had not significant influence. Maximum grain yield (1183 kg ha^{-1}) obtained when fertilizer was applied 100 and 50 (kg ha^{-1}) N and P respectively in compared with control treatment (762.4 kg ha^{-1}). Similar results have been observed by Anjum[3]. The interaction effect of inoculum, N and P was significant, highest and lowest grain yield obtained in $A_1M_1F_2$ with average 1239 kg ha^{-1} and $A_0M_1F_0$ with average 723.7 kg ha^{-1}, respectively (Table 3). Mycorrhiza inoculum had significant effect (10.79%) on mycorrhizal colonization percent, but chemical fertilizers decreased mycorrhizal colonization percent, significantly. Maximum mycorrhizal colonization percent (27.00%) was recorded from N and P applied 0-0 (kg ha^{-1}) which was comparable with N and P applied 150-75 (kg ha^{-1}) treatment (12.58%). Interaction effect of inoculums and mineral fertilizer was also statistically significant. The data (Table 4) further indicated that $A_0M_1F_2$ significantly increased mycorrhizal colonization (28.33%) as compared to control plants ($A_0M_0F_0$). Also, the results of correlation coefficients between traits show that grain yield has a positive and significant correlation with root dry weight and mycorrhizal colonization at 1 and 5% probability levels, respectively and a negative signification with days to maturity at 1% probability level (Table 5). Highest and lowest harvest index obtained in $A_0M_0F_3$ with average 28.73% and $A_1M_0F_1$ with average 22.77%, respectively. Therefore, $A_0M_0F_3$ was more successful than other treatments to transport of assimilate from sources to plant sinks and had highest harvest index. One of benefit effects of mycorrhiza is on plants photosynthesis, VAM plants often display higher rate of photosynthesis which is consistent with VAM effects on stomatal conductance. Most of the researchers suggested that VAM symbiosis increased the photosynthesis and increase the rates of photosynthetic storage and export at the same time[5]. It has been proved that concentration of chlorophyll in VAM plants was higher than their control plants. Therefore it can produce larger grains and enhance economical yield.

Table 2: Mean comparison of main effects of co-inoculation by *Azotobacter* and Mycorrhiza under nitrogen and phosphorus levels on harvest index, hectoliter weight, root dry weight, grain yield, mycorrhizal colonization and days to maturity

Treatment	Harvest index(%)	Hectoliter weight (kg.100 L^{-1})	Root dry weight (g)	Grain yield (kg ha^{-1})	Mycorrhizal colonization (%)	Days to maturity
A_0	26.10^{a}	52.14^{a}	3.57^{a}	989.3^{b}	21.88^{a}	109.0^{a}
A_1	25.10^{a}	52.73^{a}	3.65^{a}	1054.0^{a}	21.08^{a}	109.1^{a}
M_0	24.90^{b}	52.32^{a}	3.51^{b}	1028.0^{a}	20.38^{b}	108.9^{a}
M_1	26.73^{a}	52.55^{a}	3.69^{a}	1015.0^{a}	22.58^{a}	109.2^{a}
F_0	25.69^{a}	54.13^{a}	3.04^{b}	762.4^{b}	26.08^{a}	110.6^{a}
F_1	25.38^{a}	50.85^{b}	3.68^{a}	1023.0^{c}	27.00^{a}	108.8^{b}
F_2	25.76^{a}	51.56^{b}	3.80^{a}	1183.0^{a}	20.25^{b}	108.5^{b}
F_3	26.58^{a}	53.20^{a}	3.86^{a}	1117.0^{b}	12.58^{c}	108.3^{b}

Means which have at least one common letter are not significantly different at the 5% level using (DMRT)

Table 3: Mean comparison twofold interaction effects of co-inoculation of *Azotobacter* and Mycorrhiza under nitrogen and phosphorus levels on harvest index, hectoliter weight, root dry weight, grain yield, mycorrhizal colonization and days to maturity

Treatment	Harvest index(%)	Hectoliter weight (kg.100 L^{-1})	Root dry weight (g)	Grain yield (kg ha^{-1})	Mycorrhizal colonization (%)	Days to maturity
A_0M_0	25.22^{bc}	51.92^{a}	3.42^{b}	999.5^{b}	16.83^{c}	109.0^{a}
A_0M_1	26.98^{a}	52.36^{a}	3.66^{ab}	979.0^{b}	26.92^{a}	109.0^{a}
A_1M_0	24.73^{c}	52.73^{a}	3.60^{ab}	1057.0^{a}	23.92^{b}	108.8^{a}
A_1M_1	26.48^{ab}	52.73^{a}	3.71^{a}	1051.0^{a}	18.25^{c}	109.4^{bc}
A_0F_0	25.92^{b}	53.63^{ab}	3.06^{c}	743.5^{c}	26.33^{b}	110.7^{a}
A_0F_1	25.17^{b}	50.62^{c}	3.54^{b}	993.0^{b}	24.33^{b}	109.0^{b}
A_0F_2	25.23^{b}	51.13^{bc}	3.64^{ab}	1157.0^{a}	25.17^{b}	108.3^{b}
A_0F_3	28.07^{a}	53.17^{ab}	3.94^{a}	1063.0^{b}	11.67^{d}	108.0^{b}
A_1F_0	25.47^{b}	54.62^{a}	3.03^{c}	781.3^{c}	25.83^{b}	110.5^{a}
A_1F^{1}	25.58^{b}	51.08^{bc}	3.82^{ab}	1054.0^{b}	29.67^{a}	108.7^{b}
A_1F_2	26.28^{ab}	51.98^{bc}	3.97^{a}	1208.0^{a}	15.33^{c}	108.7^{b}
A_1F_3	25.08^{b}	53.23^{ab}	3.78^{ab}	1171.0^{a}	13.50^{cd}	108.7^{b}
M_0F_0	24.03^{c}	53.37^{ab}	3.07^{d}	766.5^{e}	25.00^{ab}	110.0^{b}
M_0F_1	24.15^{c}	51.73^{b-d}	3.64^{bc}	1057.0^{cd}	25.83^{ab}	108.7^{c}
M_0F_2	25.05bc	50.35^{cd}	3.56^{c}	1146.0^{b}	16.00^{c}	108.7^{c}
M_0F_3	26.67^{ab}	53.83^{ab}	3.77^{a-c}	1143.0^{b}	14.67^{c}	108.7^{c}
M_1F_0	27.35^{a}	54.88^{a}	3.02^{d}	758.3^{e}	27.17^{ab}	111.2^{a}
M_1F_1	26.60^{ab}	49.97^{d}	3.73^{a-c}	989.3^{d}	28.17^{a}	109.0^{bc}
M_1F_2	26.47^{ab}	52.77^{a-c}	4.03^{a}	1220.0^{a}	24.50^{b}	108.3^{c}
M_1F_3	26.48^{ab}	52.57^{a-c}	3.95^{ab}	1092.0^{bc}	10.50^{d}	108.3^{c}

Means which have at least one common letter are not significantly different at the 5% level using (DMRT)

Table 4: Mean comparison threefold interaction effects of co-inoculation of Azotobacter and Mycorrhiza under nitrogen and phosphorus levels on harvest index, hectoliter weight, root dry weight, grain yield, mycorrhizal colonization and days to maturity

Treatment	Harvest index(%)	Hectoliter weight (kg.100 L^{-1})	Root dry weight (g)	Grain yield (kg ha^{-1})	Mycorrhizal colonization (%)	Days to maturity
$A_0M_0F_0$	23.53^{de}	52.73^{ab}	3.16^{c-e}	763.3^{f}	18.33^{g}	110.0^{ab}
$A_0M_0F_1$	23.07^{e}	52.47^{a-c}	3.30^{b-e}	1043.0^{de}	23.33^{ef}	109.3^{bc}
$A_0M_0F_2$	23.07^{e}	48.90^{c}	3.28^{b-e}	1115.0^{b-d}	12.00^{hi}	108.7^{bc}
$A_0M_0F_3$	28.73^{a}	53.27^{ab}	3.95^{a}	1077.0^{cd}	13.67^{hi}	108.0^{c}
$A_0M_1F_0$	28.30^{ab}	54.53^{ab}	2.95^{e}	723.7^{f}	34.33^{ab}	111.3^{a}
$A_0M_1F_1$	24.80^{c-e}	48.77^{c}	3.78^{ab}	943.3^{e}	25.33^{de}	108.7^{bc}
$A_0M_1F_2$	27.40^{a-c}	53.37^{ab}	3.99^{a}	1200.0^{ab}	38.33^{a}	108.0^{c}
$A_0M_1F_3$	27.40^{a-c}	52.77^{ab}	3.94^{a}	1049.0^{de}	9.66^{i}	108.0^{c}
$A_1M_0F_0$	24.53^{c-e}	54.00^{ab}	2.99^{e}	769.7^{f}	31.67^{bc}	110.0^{ab}
$A_1M_0F_1$	22.77^{e}	51.00^{bc}	3.98^{a}	1072.0^{cd}	28.33^{cd}	108.0^{c}
$A_1M_0F_2$	27.03^{a-c}	51.80^{a-c}	3.83^{a}	1177.0^{a-c}	20.00^{fg}	108.7^{bc}
$A_1M_0F_3$	24.60^{c-e}	54.10^{ab}	3.60^{a-d}	1208.0^{ab}	15.67^{gh}	108.7^{bc}
$A_1M_1F_0$	26.40^{ab}	55.23^{a}	3.08^{de}	793.0^{f}	20.00^{fg}	111.0^{a}
$A_1M_1F_1$	28.40^{ab}	51.17^{bc}	3.67^{a-c}	1035.0^{de}	31.00^{bc}	109.3^{bc}
$A_1M_1F_2$	25.53^{b-e}	52.17^{a-c}	4.11^{a}	1239.0^{a}	10.67^{i}	108.7^{bc}
$A_1M_1F_3$	25.57^{b-e}	52.37^{a-c}	3.96^{a}	1135.0^{a-d}	11.33^{hi}	108.7^{bc}

Means which have at least one common letter are not significantly different at the 5% level using (DMRT)

Table 5: Correlation coefficients between traits

Trait	1	2	3	4	5	6
GY	1.00					
HI	-0.05^{ns}	1.00				
RDW	0.63**	0.10^{ns}	1.00			
MC	0.34*	-0.14^{ns}	-0.21^{ns}	1.00		
HW	-0.16^{ns}	0.33*	-0.27^{ns}	0.07^{ns}	1.00	
DTM	-0.63**	0.06^{ns}	-0.62**	0.27^{ns}	0.15^{ns}	1

1: Grain Yield (GY); 2: Harvest Index (HI); 3: Root Dry Weight (RDW); 4: Mycorrhizal Colonization (MC); 5: Hectolitre Weight (HW); 6: Days To Maturity (DTM)

Harvest index of safflower cultivars under water stress condition ranges from 23.4-28.4%[24]. Also Ashkani *et al*.[4] reported that harvest index of safflower cultivars ranges from 18.5-23.5%.

Inoculation with mycorrhiza and chemical fertilizers application were significantly effect on hectoliter weight. Therefore, if enough available nutrients existing in around of plants root, plants can absorb higher amount of macro and micro elements and produce more grain with higher hectoliter weight. Usually grains which have higher 1000 grain weight, have higher in hectoliter weight in compare with grains which have lower 1000 grain weight. Treatment $A_1M_1F_0$ with average 55.23 kg.100 L^{-1} has higher and $A_0M_1F_1$ with average 48.77 kg.100 L^{-1} has lower hectoliter weight among treatments. Camas *et al*.[9] showed fluctuates of 1000 grain weight from 30-49 g and it was correlated with grain yield (r = 0.45**), head diameter (r = 0.47**) and (r = 0.53**) or its components.

The main effects of inoculation with mycorrhiza was significant at 5% and use of different nitrogen and phosphorus levels was significant at 1% probability level on root dry weight. The interaction effect of Azotobacter and different levels of nitrogen and phosphorus was significant at 1% probability level. The highest and lowest root dry weight obtained in $A_1M_1F_2$ with average 4.11 g plant^{-1} and $A_0M_1F_0$ with average 2.95 g plant^{-1}, respectively. Thus, mycorrhiza fungi can causes higher growth in roots and increase root dry weight in plants which were inoculated with mycorrhiza. Bryla and Duniway[7] reported that root dry weight in inoculation with mycorrhiza was 0.49 g plant^{-1} and without mycorrhiza in average 0.46 g plant^{-1} in safflower cultivars.

All of main, twofold and threefold interactions effect of treatments had significant effect on grain yield, except main effect of mycorrhiza and twofold interactions effect of inoculation with Azotobacter and mycorrhiza. Results showed that treatment $A_1M_1F_3$ with average grain yield 1239 kg ha^{-1} and treatment $A_0M_1F_0$ with average grain yield 723.7 kg ha^{-1} were higher than other treatments, significantly.

In other word, mycorrhizal symbiosis could increase P uptake by plants. Threefold interactions effect of inoculation with Azotobacter and mycorrhiza with combination of nitrogen and phosphorus levels were significant at 1% probability level. The study of evaluated parameters varied greatly among the cultivars. Previous literature reports cited that grain yield of safflower ranging from 1168- 3325 (kg ha^{-1})[6,9,10,23,27] Thus, the lowest and highest yields observed in the current study are somewhat similar those found in the preceding works.

Many studies suggest that water extraction by plant roots can be enhanced when they are infected By Arbuscular Mycorrhiza (AM) fungi. In this study mycorrhizal colonization fluctuated from 9.66 in treatment $A_0M_1F_3$ to 38.33% in treatment $A_0M_1F_2$ and it was not correlated with grain yield, but it was correlated with 1000 grain weight trait. All of main, twofold and threefold interactions effect of treatments were significant on mycorrhizal colonization, except main effect of Azotobacter. Association of Arbuscular Mycorrhizal (AM) with crops was assessed at four Agro-Ecological Zones [AEZ-28(Joydebpur), AEZ-9 (Jamalpur), AEZ-11 (Ishurdi) and AEZ-23 (Hathazari)] of Bangladesh during 1999-2000. Mainly cereals, pulses, oilseeds, vegetables and spices crops were selected for assessment. The average AM root colonization in all crops differed among the locations during both years. Average colonization (in two years) was maximum (43.3%) at AEZ-9 (Jamalpur) and minimum (38.8%) at AEZ-28 (Joydebpur). A considerable variation was also observed in average spore population among different AEZs. Higher average spore number (157.4/100 g soil) was recorded at AEZ-23 (Hathazari) and minimum (98.8/100 g soil) at AEZ-28 (Joydebpur). The spore number varied within and between the zones[12]. Inoculation with *Azotobacter* and mycorrhiza could have not any significant effect on day to maturity. But different levels of nitrogen and phosphorus were significant on day to maturity at 1% probability. Among all of treatments, $A_0M_1F_0$ with average 111.3 days and $A_0M_1F_3$ with average 108 days had highest and lowest number of days to maturity. Number of days to maturity of safflower cultivars under water stress and non water stress condition reported ranges from 106-114 and 114-118 days, respectively[4].

CONCLUSION

The success of safflower for introduction in a new areas will largely depend on the extent of improvement made in grain yield and oil content[1]. Result from the present study indicated that grain yield and yield components of safflower have been affected significantly by the inoculation with Azotobacter and Mycorrhiza, because these biofertilizers can fix the atmospheric nitrogen and increase phosphorus availability in soil and enhance absorb elements by plant. Seed inoculation at sowing date with Azotobacter and Mycorrhiza increased grain yield about 6.13%.

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