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ORIGINAL ARTICLE **OPEN ACCESS**

Response of Wheat (*Triticum aestivum* L) to AM Fungal inoculum application on plant growth parameters and harvesting parameters in the agricultural field of Waghodia taluka, Vadodara

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ABSTRACT
The present study clearly states the effect and synergistic interaction between an efficient AMF i.e. Glomus fasciculatum, Glomus macrocarpum and Azotobacter on Triticum aestivum L was studied in unsterile soil in Parul University agricultural instructional farm, Patiyapura, Waghodia taluka, Vadodara.When compared to uninoculated plants, growth and harvesting parameters and chlorophyll content were maximum in plants treated with the combination of Glomus macrocarpum , Glomus fasciculatum and Azotobacter. Root colonization level was high (94.7%), there was remarkable increase in Nitrogen (2.849g/plant) and Phosphorus (0.783%) content. Outcome of the study showed that Arbuscular Mycorrhizal fungal species when used in combination with Azotobacter were more beneficial when compared to individual inoculum for better nutrition and much improved growth of wheat (Triticum aestivum L.).
Key words; AM Fungus, Azotobacter, Glomus fasciculatum, Glomus macrocarpum, Triticum aestivum. L.

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INTRODUCTION

In India, the cost of fertilizer has already escalated far behind the reach of poor farmers. Therefore, to estimate the cost of fertilizer use, application of bio fertilizer like mycorrhiza is restored for crops and is helping to sustain the production system for long term. Gaunt [9] stated that AM usually prefers some of the host exhibiting symbiotic response and increases the growth and yield of crop through nutrient uptake. Sreenivasa [23] observed that different species of AM fungi have lot of variation among and within its species for the ability to promote the growth of plants. This resulted in AM fungal host preference concept [11]. So, an efficient AM fungi should be selected for a specific soil-climate-host combination to procure maximum benefits. However, the beneficial effect and use of inoculants such as AM fungi, on a wide scale, as a source of plant nutrition for agriculture crop plants is dependent on development of crop growth promoting strains of AM fungi which are superior to native soil population of AM fungi [12-15]. Therefore, field study is requisite to recognize the copious and kind of native AM fungi present in the rhizosphere of the crop and high yield is dependent on depth of sowing. *Glomus fasciculatum* and *Glomus macrocarpum*spp were abundantly found in Waghodia taluka, Vadodara. In this view, the aim of study is to find the response of *Triticum aestivum* L., when inoculated with indigenous *Glomus fasciculatum* (Gerd. Koske & Walker),*Glomus macrocarpum* (Tul. &Tul,) and *Azotobacter* in unsterile soil either singly or in combination.

MATERIAL AND METHODS

Present study was undertaken and investigation was done in unsterile soil of field during Rabi season i.e. December to April to determine the effect of different AM fungi with other microorganisms on different growth parameters, yield parameters and nutrient availability of wheat crop (*Triticum aestivum.* L) at Instructional farm of Parul Institute of Agriculture, Parul University, Patiyapura, Waghodia taluk, Vadodara district, Gujarat which is situated 10 kms from Parul University. The farm is situated on the bank of river Devanadi at an altitude of 33 meters above sea level. The geographical location of Waghodia taluka, Vadodara district is 22.30^0 N latitude and 73.38^0 E latitude of east southern part of Vadodara

district, Gujarat state, India Patiyapura.The average temperature is 8^0 C to 44^0 C. The temperature and rainfall were in adequate quantity for wheat crop. Sandy loamy soil is the soil on which the experiment is conducted. Available Potash and organic content of the soil is sufficient for the wheat crop. 125kg per hectare grains of wheat variety GW496 is sown in Rabi season. Field area is 360 sq. meters with 6 treatments allocated randomly in split-plot design. Each treatment plot is 5x4 sq. meters replicated 3 times in 18 blocks with a spacing of 12 cms to 15cms between each row, depth of sowing is about 4-6cms. VAM fungal inoculum is applied near root zone of the plants as per recommended dose of bio inoculants. To evaluate the interaction study of microbial inoculants in improving the performance of wheat plants the following treatments were set. Treatments were designed as T-0 = control, T-1= VAM 1 (*Glomus fasciculatum*), T-2 = VAM 2 (*Glomus macrocarpum*), T-3= VAM 1 + VAM 2, T-4 = 50% RDF, T-5 = 50% RDF + VAM 1 + VAM 2 + *Azotobacter* (where, RDF = Recommended Dose of Fertilizers, T = Treatments.)

Soil pH was 6.65 which did not vary throughout the cultivation. Completely randomised beds were maintained for the crop in the open field wherein each bed (area) is treated with different VAM inoculums, 50% of recommended dose of fertilizers (RDF) and irrigated 4 times. Soil used for experiment contained organic carbon 0.57% , pH – 6.65%, available N 16 - kg per acre, available P_2O_5 – 9kg per acre, available K is 104kg per acre, electrical conductivity (EC) is 1:2 was 0.49 oh/m of soil. Soil root culture of *Glomus fasciculatum* and *Glomus macrocarpum* were cultivated in instructional farm, Patiyapura, Vadodara which was maintained on Rice (*Oryza sativum*) and Onion (*Allium cepa*) roots using mixture of sand:FYM:soil (1:1:1). The cultures containing chlamydospores and root segments of rice and onion colonized by particular AM fungus were used as mycorrhizal inoculum. Spores and fragments of VAM fungi filaments and infected root bits were applied at the time of sowing over 8kg per acre. In the experiment, treatments included uninoculated control, *Glomus fasciculatum, Glomus macrocarpum* and *Azotobacter* and their combination. Observations were recorded at 60DAS for chlorophyll content, growth parameters and harvesting parameters of wheat.

1. Growth parameters (table no. 1)

Shoot length: The Shoot length was measured from ground level to the tip of the plant using measuring tape and expressed in centimetres. Shoot length was observed at 20, 40, 60 days after sowing.

Number of leaves per plant: Number of leaves was observed at 20,40, and 60days after sowing.

Number of tillers per plant: Number of tillers was observed at20, 40, and 60 days after sowing

Root samples of each treatment were collected and stained in 0.05% cotton blue with Lactophenol [17]. Percent root colonization was calculated using the method of Giovannetti and Mosse [10].0020The AM fungal spores were recovered after extraction of Rhizospheric soil by wet sieving and decanting technique [10]. Nitrogen and Phosphorus from shoot of wheat were determined following Jackson method.

2. Harvesting parameters (table 2 & 3)

Spike length: Spikes from each plot were randomly selected and its length was measured with scale and recoded in centimeters. Average of spike length was calculated (fig no 2).

Spike weight (g): Spikes from each plot was selected randomly and it was measured by using weighing machine in grams and its average weight was calculated.

Number of grains per spike: Spikes were selected randomly from each plot and spikelet's threshing was done and average of number of grains and grains per spike was calculated.

Test (Grain) weight (g): Spikes were threshed after harvesting the plots and the weight of 1000 grains was measured in grams by weighing machine.

Grain yield (t / ha): After harvesting, each plot crop was threshed and weight of grains was checked and recorded the yield into ton per hectare.

Biological yield (t / ha): Crop weight was calculated from each plot after harvesting and converted into ton per hectare.

Straw yield (t/ha): After the harvesting of each plot, threshing of the crop was done and left behind straw yield was weighed and was calculated into ton per hectare.

Harvesting index (%): It is the rate of grain yield to the biological yield per plot. It was calculated by the formula of Donald and Hamblin , (fig no 3b).

$$\text{Harvest index} = \frac{\text{Grain yield (t ha}^{-1})}{\text{Biological yield (t ha}^{-1})} \times 100$$

Harvesting and Threshing: Crop were harvested from net plot area individually, tagged and weight was recorded and expressed in tons per hectare. Harvesting parameters were taken for the each plot and analysis of quality parameters was done. Harvesting was done when the leaves and stems turned yellow and became fairly dry. When there was 20% to 30% of moisture in grains, it was the right stage for harvesting. Threshing was done by power driven stationary thresher.

Chlorophyll pigment determination

Chlorophyll estimation was done by the method of Arnon [1].The amount of chlorophyll present in the extract i.e.mg chlorophyll per gram tissue was calculated using the equations as described by S. Sadasivan and A. Manickam [19] biochemical methods.

RESULT AND DISCUSSION

Field study was conducted to assess the impact of different VAM inoculum alone or in combination. Effect of growth response on wheat inoculated with triple inoculates (*Glomus fasciculatum, Glomus macrocarpum* and *Azotobacter*). The results obtained have been shown in (table no1). The growth with respect to Shoot length,number of leaves and number of tillers (table 1), percentage of VAM colonization , no. of spores per 50grams of soil (table 1), Nitrogen in shoot(g) Phosphorus in shoot were recorded at an interval of 20 days, 40 days and 60 days. Percent of Mycorrhizal colonization of experimental plants were represented in (table 1).

1. Growth parameters (table 1)

Shoot length (cms): At 20 DAS, T5 (24cms) showed highest significant value of shoot length as compared to other values of treatments followed by T5, T3, T4 and T1 and and T2 (16.67cm). Minimum value was noticed in control i.e. T0 (15.33cms). These amended plots showed significant shoot length and at every 20 days interval. Prashanti Sandegopu and M, Mamatha [18] reported an increase in shoot development in field and plant growth parameters were appreciably increased in association with VAM.

Number of leaves per plant: Among different treatments, number of leaves/plant varied from 2.67 to 33.6 from 20 DAS to 60 DAS as shown in the table no 1. T5 showed best performance in leaves count (33.6) as compared to control (17.1). Gupta. M.L. et al., [15] reported that VAM inoculation also increased shoot tissues, plant height and fresh herbage as compared to non-inoculated Mint cultivars, nutrient accession for prevailing economic production under field conditions.

Number of Tillers: Number of tillers / plant were recorded at 20, 40, 60, DAS. At 20 DAS, T5 showed highest number of tillers (3.0) followed by T3 (2.0). Up to 40 DAS and 60 DAS, T5 exhibited the maximum number of tillers i.e., 4.67, 5.67 and 6.67 respectively. A similar significant growth in the number of tillers was followed by T3, T4, T1 and T2 respectively. The least number of tillers was observed in T0 i.e. control. Effect of AM fungi on *Dactylisg lomerata* l showed higher shoot weight, increased number of tillers, height of tillers, and increased number of leaves as reported by [3].

Maximum percentage of root colonization was recorded in T5, while moderate result was noted in T3 and T1 and lowest colonization in T2. Higher number of spores per 50g soil was recorded in T5. However, AM in triple inoculum manifested healthier growth and P content as compared to the uninoculated i.e., in control plants. Increase of P content was significantly higher in T5, followed by T3, T4 and statistically similar in the treatment T1 and T2 and the lowest content was seen in T0. Maximum significant Nitrogen content was found in T5 followed by T4. Least content was observed in T0 compared to T2 [24] which helps in more absorption of phosphorus in the soil surrounding mycorrhizae colonized roots. Soil microorganisms were known to influence the germination of Arbuscular Mycorrhizal fungal spores [12]. Chishimba, Katongo [6]. The study revealed that VAM and *Trichoderma spp* significantly increased phosphorus uptake and grain yield for all wheat varieties. Fungal treatment increased grain yield and P value uptake by 200% and 400% respectively.

Table no 1. Impact of different treatments on growth parameters, Nitrogen, Phosphorus, percentage of root colonization and VAM spores/50g of soil for 60 days of wheat (*Triticum aestivum* L.)

Treatments	DAI	Shoot length (cm)	No.of eaves	No.of tillers	N in Shoot (g/plant)	P in shoot (g/plant)	% root colonization	VAM spores/ 50g of soil
Control (T 0)	20	15.33	2.67	1.67	0.833	0.087	--	---
	40	24.1	8.67	2.33	0.857	0.09	--	---
	60	56	17.1	4.33	0.879	0.094	--	--
VAM 1 (T 1)	20	18	4.33	1.33	1.005	0.119	30.4	253
	40	28.33	15	3	1.015	0.144	43	462
	60	61.33	21	5.33	1.053	0.179	65.1	702
VAM 2 (T 2)	20	16.67	3.33	1.33	0.914	0.094	22	169
	40	25.67	12.33	2.67	0.923	0.095	39.53	454
	60	59.33	18.67	4.33	0.958	0.126	62.4	704
VAM 1+ VAM 2 (T 3)	20	21.90	4.67	2	1.219	0.471	30.5	425
	40	33.60	18.67	4.4	1.254	0.497	43.8	661

	60	69.67	31	5.67	1.38	0.507	77.4	852
50% RDF (T 4)	20	20	4	1.6	1.605	0.227	---	---
	40	30.3	17.33	3.7	1.624	0.236	---	---
	60	65.33	26.67	5.3	1.849	0.255	---	---
VAM 1 + VAM 2 + AZO + 50% RDF (T 5)	20	24	5.7	3	2.735	0.741	33.2	584
	40	37.33	21.67	4.67	2.79	0.77	57.5	736
	60	73.33	33.6	6.68	2.849	0.783	94.7	1045

T-0 = control, T-1 = VAM 1, T-2 = VAM 2, T-3 = VAM 1 + VAM 2,T-4 =50% RDF,
T-5 = 50% RDF + VAM 1 + VAM 2 + *Azotobacter*.
VAM 1 = *Glomus fasciculatum*, VAM 2 = *Glomus macrocarpum*
Note: P-value* was for interaction effect between Intervention and Days.
** denotes significant difference between 20 (DAS) 40 and 60 (DAS).
Mixed ANOVA Models showed that there were significant effects of Intervention **X** Days AfterSowing on shoot length in cms, no.of leaves, no.of tillers, N & P content in shoot, percent root colonization, spores per 50g soil. P value was < 0.001 ***. There was significant increase in all treatments over time.

Harvesting Parameters (table 2 & 3) (fig no. 2 & 3).

Spike length (cm): Maximum significant value was found in T5 (16cm) followed by T3 (14cm), and values of the other treatments were T4, T1, T2 i.e. 12cm, 10.33cm and 10cm respectively. Minimum value was recorded in T0 i.e. 9cm. Within the treatments both T1 & T2 statistically had similar values (10.33cm & 10cm). Among all the treatments difference was found in spike length. The increase of spike length in T5 when compared to T0 was 7cm at the time of harvesting (table & fig no 2). Negi et al., [16] reported that bio fertilizer increases the spike length and number of tillers as compared to control plants. This statement was further reported by Abedi et al., [2].

Number of grains per spike: The increase in number of grains / spike in T5 as compared to Control Plot where no fertilizer was applied was by 40.67 grains. Highest number of grains was observed in T5 (76.67) followed by T3 (70.33), T4 (64.0), T1 (58.67), T2 (50.33) and least number of grains was recorded in T0 (36.0). However T5 also showed significantly improved values for number of grains per spike. The addition of inorganic fertilizers and bio inoculants leads to more uptake of nutrients at different physiological stages and thus more number of grains per spike was observed [14].

Spike weight (g): The maximum significant value was found in T5 (24.0g) followed by T3 (20.0), T4 (17.0g), T1 (12g) and T2 (10g) and the least value of spike weight was observed in T0 (8g). The increase in spike weight in T5 as compared to T0 was 16g (table & fig no 2). A significant improvement by AMF on plant height, number of tillers, spike length, number of spikelets and grains per spike, grain weight and test weight in AMF treated plants as reported by Babita Rani et al [4].

Test weight (g): Treatment T5 (58.33g) showed best performance with respect to test weight, followed by T3 i.e. dual inoculum (49g), T-4 with 50% RDF (44.67g), T-1 with VAM 1 (39.33g) and T2 with VAM 2 (36.67g). In T0 (control), 36 grams per 1000 grains was observed. Increase in 1000 grain weight percentage was found in T5 as compared to control at 22.33g (table & fig no 2). The combination use of Mycorrhiza and chemical fertilizers increases the test weight, no. of grains / spike and Number of tillers / spike as reported by Devi et al., (2011).

Biological yield (t/ha): Treatment T-5 and T-3 showed best performance and significantly similar statistical results (17.143 and 17.14 respectively) with respect to biological yield .Maximum significant value was found in T-5 and T-3 followed by T-4, T-1 and T-2 (table & fig no 3a). According to Singh and Agarwal (2001), observed the use of different and recommended dose increases grains per spike, biological & Straw yield of wheat.

Grain yield (t/ha):T-5 treatment showed the maximum significant value (9.163 t/ha) in grain yield followed by T-3, T-4, T-1, T-2 and minimum significant value was noticed in control i.e. T-0 (3.697 t/ha) plot. Plants inoculated with triple inoculum plots showed better result as compared to dual or single inoculum in combination or alone applications given to the plots (table & fig no 3a).

Straw yield (t/ha): Maximum significant value was noticed in T-3 followed by T-4, T-5 treatments showed best performance with respect to straw yield (t/ha).

Harvest index (%): Treatment T-5 (53.447%) showed better result followed by T-3, T-4, T-1 & T-2 and minimum significant value was found in T-0 (35.65 %) i.e. control (table & fig no 3a & 3b). Ajay Nair., Archana, S. Rao., L. Bhanu., Veena, S. More., K.S. Anantharaju and Sunil, S. More (2022) reported on Arbuscular Mycorrhizae, a treasured symbiont to agriculture. Their report stated that VAM helps the plant by enhancing the growth and yield, indirectly protects the crop from pathogens and enhancing the quality and quantity of production of agricultural commodities.

Estimation of chlorophyll: In my study it was found that the total chlorophyll content was high in plants inoculated with triple inoculum as compared to plants inoculated with dual inoculum of VAM fungi.T1 and T4 treatments have almost the same and sufficient amount of chlorophyll content. From the current study findings it can be concluded that in T5 treated plants showed higher chlorophyll content with approximately four fold increase in total chlorophyll content at 60 days after inoculation of plants with *Glomus fasciculatum, Glomus macrocarpum* and *Azotobacter* compared to uninoculated plants.

Table no. 2. Impact of different treatments on Harvesting Parameters like spike length, no. of grains, spike weight and test weight in field study

Treatments	T-0	T-1	T-2	T-3	T-4	T-5	P-value
Parameters	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	
Spike length	9±0.0	10.33±0.57	10±0.0	14±0.0	12±0.0	16±0.0	< 0.001*
No. of grains	36±0.0	58.67±.52	50.33±1.52	73.33±1.52	64±2.08	76.67±3.21	<0.001*
Spike weight	8±0.0	12.67±1.157	10.0±0.0	20.0±1.73	17.0±1.0	24±0.57	<0.001*
Test weight	36±0.57	39.33±0.57	36.67±0.57	49±1	44.67±0.57	58.33±0.57	<0.001*

T-0 = control, T-1 = VAM 1, T-2 = VAM 2, T-3 = VAM 1 + VAM 2, T-4 = 50% RDF, T-5 = 50% RDF + VAM 1 + VAM 2 + Azotobacter

VAM 1 = *Glomus fasciculatum*, VAM 2 = *Glomus macrocarpum*

ANOVA showed that there was effect of treatment on Spike length, no. of Grains, Spike weight and Test weight ($p<0.001^*$)

Table no 3. Impact of different treatments on harvesting parameters like biological yield, grain yield, straw yield and harvest index, Chl a, Chl b and total chlorophyll in field study.

Treatments	T-0	T-1	T-2	T-3	T-4	T-5	p value
Parameters	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	
Biological yield (t/ha)	10.367±0.086	15.177± 0.065	13.633± 0.122	17.14± 0.108	16.533 ± 0.325	17.143 ± 0.087	<0.001*
Grain yield(t/ha)	3.697 ±0.147	6.08 ± 0.036	5.22 ± 0.085	8.63 ± 0.145	7.237 ± 0.093	9.163 ± 0.07	<0.001*
Straw yield(t/ha)	7.353± 0.065	9.433 ± 0.09	9.11± 0.09	12.137 ± 0.112	11.173 ± 0.205	10.16± 0.07	<0.001*
Harvest index (%)	35.65± 1.173	40.056 ± 0.086	38.283 ± 0.285	50.343± 0.54	43.77 ± 0.349	53.447±0.17	<0.001*
Chlorophyll a	1.1897	2.9848	2.0187	3.031	2.391	3.3788	<0.001*
Chlorophyll b	0.6241	0.5414	0.5548	1.323	1.1452	2.2252	
Total chlorophyll Mg/g tissue	1.8133	3.5263	2.5735	4.354	3.536	5.6040	

T-0 = control, T-1 = VAM 1, T-2 = VAM 2, T-3 = VAM 1 + VAM 2, T-4 = 50% RDF, T-5 = 50% RDF + VAM 1 + VAM 2 + *Azotobacter* VAM 1 = *Glomus fasciculatum*, VAM 2 = *Glomus macrocarpum*

Since the normality assumption wasn't met, Krushkal Wallis test was performed to find significant difference in distribution of treatment. Post hoc analysis was carried to find the significant difference between groups. $p < 0.05$ was significant.

T-0 = control, T-1 = VAM 1, T-2 = VAM 2, T-3 = VAM 1 + VAM 2, T-4 = 50% RDF, T-5 = 50% RDF + VAM 1 + VAM 2 + Azotobacter ,VAM 1 = Glomus fasciculatum, VAM 2 = Glomus macrocarpum

In the field study, it has been concluded that in sandy loamy soil of Gujarat, after 60 DAS, the result showed that interaction between *Triticum aestivum* L. and plants raised with bio-inoculants like mycorrhiza & *Azotobacter* i.e. plots treated with T5 which received triple inoculation rather than dual or single inoculation showed positive influence on growth parameters, is seen due to increased percent of root colonization, number of Mycorrhizal spores increased biomass production, phosphorus and nitrogen content in shoot, harvesting parameters, chlorophyll content of the wheat. These findings were consistent with early workers contribution [5] & Shashidhar et al., [20] on Lavender plants and chilies. Increase in chlorophyll content in VAM inoculated plants reported by Devi and Reddy [7] in groundnut, Shivaputra et al., [21] in papaya found increased in the experimental plants compared to uninoculated plants. Experimental data in field study suggests the two strains of selected AM inoculants along with *Azotobacter* as a triple inoculum helps to increase the nutrient availability and restores the soil fertility and make it biologically alive which improves the soil structure and texture and helps the soil to hold

water better than before. The yield of late sowing can be improved by the use of bio-inoculant. M, Jalaluddin [14] conducted a study to know the effect of inoculation with VAM Fungi and *Bradyrhizobium*in Sindh on growth and yield of Soybean. Wheat sown with narrow spacing has given higher yields, healthy and vigorous growth of wheat plants which helps in well establishment of crops when planted in agricultural fields can be used as a bio-enhancer.

In order to augmentation of growth and yield of crop plants, it is necessary to choose the most effective fungal species. The GW496 wheat variety produced about average 6 to 6.66 tons of grains per hectare under irrigation conditions. Result of this study showed that triple inoculum proved economical as well as beneficial with respect to saving 50% cost on fertilizers and VAM colonization improved many characteristics of wheat like soil fertility, leaf quality, growth and harvesting parameters. Thus, this technology can be recommended for agricultural farmers.

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