Evaluation of Soil Applied *Arbuscular mycorrhiza* Along with Foliar Nutrition of Nitrogen, Iron and Zinc on, Mycorrhizal Colonization, Physiological Parameters, Growth and Yield of Rice under Aerobic Condition

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ABSTRACT

Background: In rice belts, cultivation of rice under aerobic conditions provides an alternative way to reduce the enormous amounts of water usage, by using 50 percent of the water when compared to traditional cultivation. However, micronutrient deficiencies are more common in aerobic rice during its early stages of growth. Mycorrhizal fungi were found to be efficient in mobilizing the nutrients under aerobic situations.

Methods: The field experiment was conducted in Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore. The experiment consisted of thirteen treatments which includes different recommended doses of VAM 50, 62.5 and 75kg ha^{-1} along with different combinations of foliar application of 0.5% urea, 0.5% $ZnSO_4$ and 1% $FeSO_4$.

Result: The study revealed that combined application of 150 % of recommended dose of VAM and foliar application of 0.5% urea + 0.5% $ZnSO_4$ + 1% $FeSO_4$ at 25 and 45 DAS significantly increased the VAM colonization area, photosynthetic pigments and growth parameters such as plant height, shoot and root weight, root growth, leaf area index and yield attributes and it was found to be a better optionin rice cultivation to overcome the micronutrient deficiencies in its earlier stages under aerobic conditions.

Key words: Growth and yield, Root morphology, Total chlorophyll content, Total colonization.

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food crop provides food for almost half of the population in the world (Ullah and Datta, 2018). Globally, Asiancountriesconsumes 90% of the freshwater for agricultural production. Conventional rice cultivation is practiced only under flooded condition and nearly 3000 to 5000 liters of water is required to produce 1 kg of rice (Anandan *et al.,*2015). Water scarcity is a major issue all over the world. Drought condition occurs more frequently and more severely than in the past due to rapid climate change, which would be a major constraint for rice cultivation under flooded conditions (Anupol Chareesri *et al.,* 2020). Hence, the conventional method of rice cultivation with continuous flooding need to be replaced by water saving cultivation methods without affecting the yield and grain quality. Aerobic system of rice cultivation needs special attention and operations to be taken care of including seed treatment, plant, soil, water, weed and nutrient management. Even though the aerobic system of rice cultivation has many advantages it has some disadvantages *viz.,* weed infestation and nutrient deficiencies. Due to the aerobic soil condition, most of the nutrients are unavailable which directly affects nutrient uptake in plant system (Anandan *et al*., 2021). Nutrients like phosphorous (P), iron (Fe), zinc (Zn), manganese (Mn) are the most limiting nutrients under aerobic soil condition. To overcome these constraints, foliar application of nutrients and soil application of nutrient mobilizing fungi species like (VAM) provides a way to manage the crop growth (Carvajal and Gloria, 2020). VAM fungi can act as a symbiosis relationship with plant roots and soil to increase nutrient and water uptake,

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especially in the dry season. Plants with mycorrhizal roots showed increased nutrient absorption compared to those without mycorrhizal roots (Berruti *et al*., 2016; Narwal *et al.,* 2018; Abdelhameed *et al*., 2018). Foliar nutrient application is the most effective method because it allows nutrients directly into plant metabolism and eliminates soil barriers and leaching losses that occur with soil-applied fertilizers (Fageria *et al.,* 2009; Burkhardt, 2010). Foliar application of nutrients boosts rice growth, yield and other yield attributes significantly (Shivay *et al*., 2015; Tuiwong *et al*., 2022; Saikh *et al.,* 2022; Mahmoud Soltani *et al.,* 2022). As a result, supplemental foliar nutrient application is the most effective method for plants grown in low-nutrient soil or under stress conditions, particularly in arid and semi-arid environments (Nadeem and Farooq 2019; Ishfaq *et al.,* 2021).

MATERIALS AND METHODS

Experimental site

The experiment was conducted at Wetland Farms, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore during January, 2022. The experimental site is geographically located at 11°N latitude and 77°E longitude at height of 426.72 m above mean sea level. The soil of the experimental site was clay loam in texture with pH of 7.84 and EC 0.51 dsm^{-1}. The initial soil fertility status showed low available $KMnO_4$ - N (225 kg ha^{-1}) (Asija and Subbiah, 1956), medium in available Olsen P (20.5 kg ha^{-1}) (Olsen, 1954) and high in available NH_4O Ac-K (740 kg ha^{-1}) (Stanford *et al.,* 1949).

Experimental design and treatment

The field experiment was laid out with 13 treatments replicating three times with individual plot sizes of 1 m × 1 m in a randomized block design. The treatments comprised of different doses of foliar application of urea, iron (Fe) and zinc (Zn) and different doses of VAM fungi as soil application under aerobic condition. (Table 1).

Crop management

Rice variety CO 53 (Duration-115 to 120 days, average yield -3.7 to 3.8 t ha^{-1}, white short bold rice) was used in this experiment. Rice seeds are directly sown in dry soil during Navarai season (Dec-Jan) with spacing of 20 × 10 cm. Recommended dose of fertilizers (150 : 50 : 50 Kg ha^{-1} of N, P and K) was given as a split application. N was given as four splits 20% at 15 DAS, 30% at tillering and panicle initiation stage and 20% at flowering. Full dose of P was given as basal application and K was given as 50 % at basal and 50% at panicle initiation stages. Foliar application of Urea, Iron (Fe) and Zinc (Zn) were given on 25th and 45th DAS. Application of 100%, 125%, 150% recommended dose of VAM spores (50, 62.5, 75 Kg ha^{-1}) along with farmyard manure was done before sowing. Crop was irrigated twice a week at depth of 3-4 cm. Weed management was done by the application of pre emergence herbicides (pendimethalin 1.0 kg a.i./ha) followed by early post emergence herbicides (bispyribac sodium 20 g a.i./ha) and one hand weeding. Pest management was carried out by application of insecticide (flubendiamide 39.35% SC 50 g) at tillering stage to control the stem borer (*Scirpophaga incertulas*) and malathion 50% EC 500 ml at milking stage to control the earhead bug (*Leptocorisa acuta* and *L. oratorius*). Fungicide tricyclozole 75 WP @ 500 g/ha was applied after observing initial infection of the rice blast disease (*Pyricularia grisea*). Crop was harvested when the grains attained full ripened stage.

Assessment of root colonization

Root colonization assessment of VAM was carried out by using root clearing method (Phillips and Haymann 1970).The percentage of VAM colonization was calculated by using the following formula:

$$\text{Colonization \%} = \frac{\text{No. of root segments colonized with VAM}}{\text{Total no. of root segments observed}} \times 100$$

Assessment of root morphology by using WinRHIZO software

The control and VAM inoculated plot root samples were collected from the experiment field without damage the root by using spade and then washed thoroughly with distilled water to remove the soil, dust and debris. The washed root samples were placed in the tray filled with water and the roots were arranged without overlapping. The tray was placed in the dual scan optical scanner attached with the system. The instrument WinRHIZO optical scanner (version 5.0) software was used to acquire the roots sample and the images was taken at 400 dpi resolution with color scale. The scanned root images of rice were analyzed for various root growth and developmental parameters including total root length (TRL), surface area (SA), average diameter (AD), root volume (RV), number of tips (TP), forks (FR) and crossings (CR).

Table 1: Treatment details.

Treatments
T_1 : Control
T_2 : Recommended dose of VAM*
T_3 : 125% recommended dose of VAM
T_4 : 150% recommended dose of VAM
T_5 : T_2 + foliar application of 0.5% urea at 25 and 45 DAS
T_6 : T_3 + foliar application of 0.5% urea at 25 and 45 DAS
T_7 : T_4 + foliar application of 0.5% urea at 25 and 45 DAS
T_8 : T_5+ foliar application of 0.5% $ZnSO_4$at 25 and 45 DAS
T_9 : T_6+ foliar application 0.5% $ZnSO_4$at 25 and 45 DAS
T_{10} : T_7+ foliar application of 0.5% $ZnSO_4$ at 25 and 45 DAS
T_{11} : T_8 + foliar application of 1% $FeSO_4$ at 25 and 45 DAS
T_{12} : T_9 + foliar application of 1% $FeSO_4$ at 25 and 45 DAS
T_{13} : T_{10} + foliar application of 1% $FeSO_4$ at 25 and 45 DAS

*Recommended dose of VAM 50 kg ha^{-1}, *Vesicular-arbuscular mycorrhiza* (VAM).

Analysis of photosynthetic pigments

Chlorophyll a, chlorophyll b and carotenoids were analysed by DMSO (Dimethyl Sulphoxide) methodHilscox and Israelstam (1979). The Chlorophyll 'a', 'b' total chlorophyll content and carotenoid content values are calculated by Yoshida, 1972.

Measurement of growth parameters

Plants from each treatment and replication were carefully pulled out, oven dried at 70°C for 48 hours and weighed for their dry weight. Shoot and root dry weight were also calculated separately and expressed in gram (g). Leaf Area Index of the plants were measured by using LI- COR 3000 leaf area meter.

Yield attributes

In each treatment, five plants were randomly selected for observing, number of filled grains, unfilled grains, 1000 grains weight, grain yield and straw yield and the mean value was arrived. The harvest index was calculated using following formula where economical yield was grain yield and biological yield sum of grain yield and straw yield Donald (1963).

$$\text{Harvest index} = \frac{\text{Economical yield}}{\text{Biological yield}}$$

Statistical analysis

Data were analysed for randomized block design by using SPSS 16.0. software to find the significance difference at 5% level was used to test for significant differences among 13 treatment means. The relationship between total VAM colonization percentage with growth and yield of rice was assessed by Pearson correlation analysis using R software version 4.2.0 (RStudio 2022.02.3+492).

RESULTS AND DISCUSSION

Effect of VAM and foliar application of nutrients on root morphology

Among the tested treatments, there was a significant difference in root morphology in all the treated plots at 30 DAS. Total root length (206 cm), surface area (241cm^2), average diameter (1.11 mm), root volume (1.37 cm^3), number of tips plant^{-1} (3700),number of forks plant^{-1} (3218) and crossings (79.2) were significantly increased with 150% recommended dose of VAM + foliar application of 0.5% urea + 0.5% $ZnSO_4$ + 1% $FeSO_4$ at 25 and 45 DAS (T_{13}) followed by 125% recommended dose of VAM + foliar application of 0.5% urea + 0.5% $ZnSO_4$ + 1% $FeSO_4$ at 25 and 45 DAS (T_{12}). Least values of root morphology was observed in control plot (T_1). Similar trend was observed at 50 DAS. Effect of different doses of soil applied VAM on rice root morphology by using winRHIZO root image analyser was showed in (Fig 1, 1a and 2). This might be due to the symbiosis between host plant roots and fungal hyphae which resulted in a well developed root system, increases water and nutrient uptake and leads to an increase the plant growth (Basu *et al*., 2018; Begum *et al*., 2019; Beura *et al.,* 2020; Iqbal *et al*., 2021).The increased solubilisation of phosphorous by VAM fungi and availability to rice plants resulted in increased root proliferation.

VAM colonization

Highest percentage of (87.3) VAM colonization was observed in the plot treated with 150% of recommended dose of VAM. The lowest percentage was observed in control plot (16.7). The number of vesicle, arbuscule and hyphal colonization was higher in 150% recommended dose of VAM applied plot compared to the other two doses of soil applied VAM fungi (Fig 3). Microscopic view of root colonized with VAM

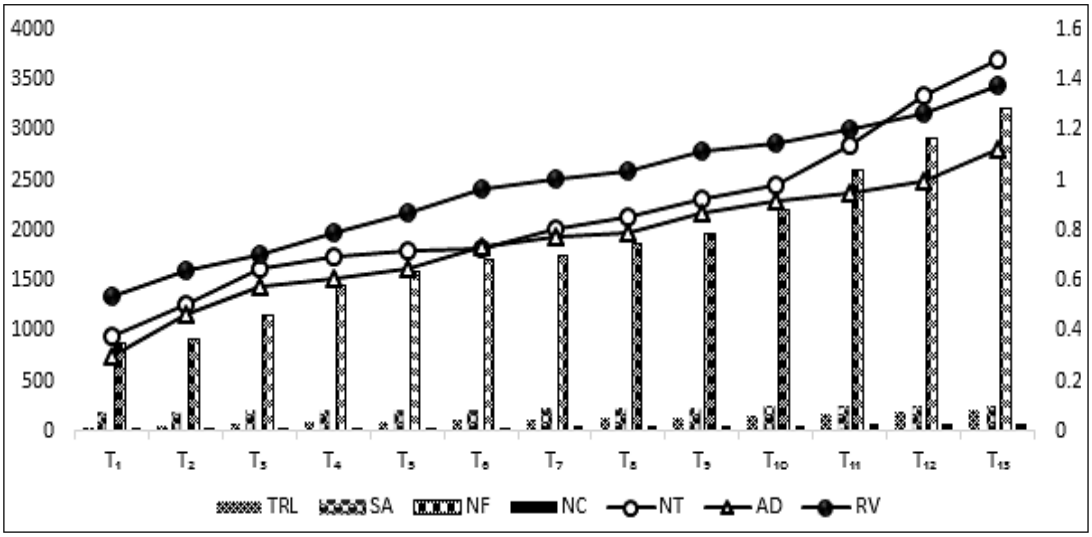


Fig 1: Effect of soil application of VAM and foliar application of nutrients on root morphology of aerobic rice at 30 DAS.

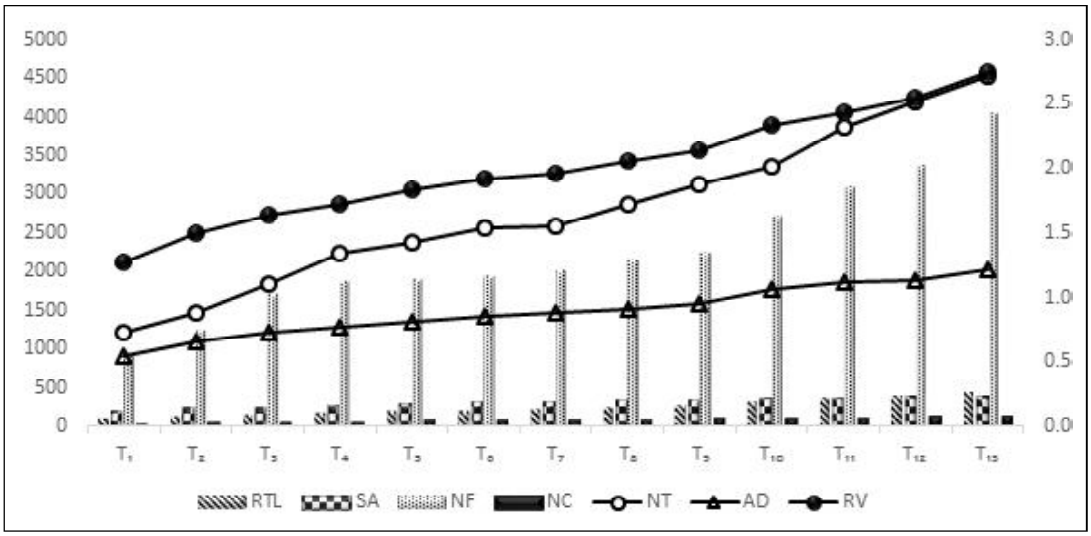


Fig 1: (a) Effect of soil application of VAM and foliar application of nutrients on root morphology of aerobic rice at 50 DAS.



Fig 2: Effect of different doses of soil applied VAM fungi on rice root morphology at 30 DAS by using win RHIZO root image analyser.

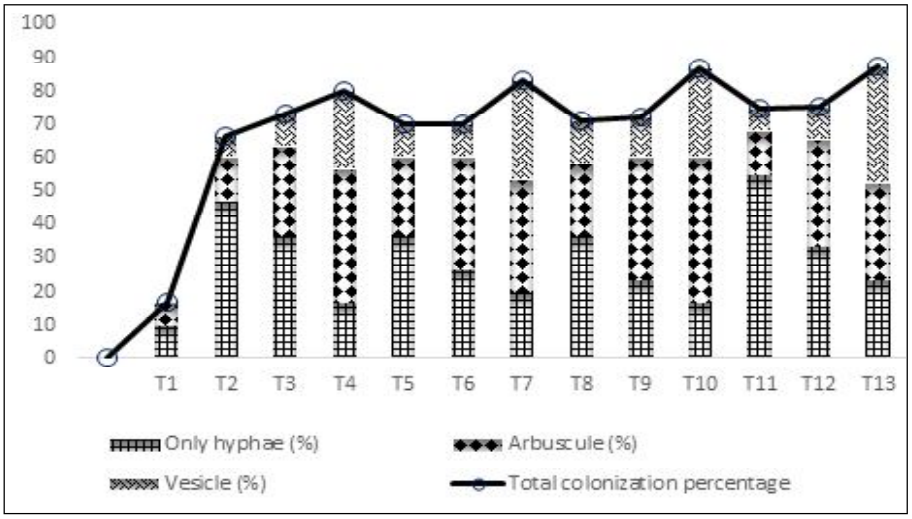


Fig 3: Effect of different dose of soil application of VAM and foliar application of nutrients on VAM colonization.

and without VAM infection was showed in Fig 4. There is no negative effect of foliar application of nutrients on VAM colonization where as VAM colonization was strongly influenced by the higher dose of VAM application of 150% (75 kg ha^{-1}). The highest root colonization might be due to more spore germination in rice roots. Application of VAM fungi had a positive effect on VAM colonization percentage, extraradical hyphal density (EHD), growth and yield of rice crops under non - flooded conditions as compared to flooding and shading conditions (Wangiyana *et al.,* 2006 ; Mitra *et al.,* 2021; Wang *et al.,* 2021).

Photosynthetic pigments

The photosynthetic pigments such as chlorophyll a, chlorophyll b and carotenoids significantly increased on 30 and 50 DAS. Plot which received 150% of recommended dose of VAM + foliar application of 0.5% urea + 0.5% $ZnSO_4$ + 1% $FeSO_4$ at 25 and 45 DAS (T_{13}) recorded significantly higher photosynthetic pigments followed by T_{12} except carotenoids. The carotenoids comparable with T_{12} on both stages of crop. The lowest photosynthetic pigments observed in control (T_1) (Fig 5, 5a). Similarly, combined application of Zn and VAM fungi significantly increased the leaf chlorophyll content and growth of the rice (Mohmoud *et al.,* 2022). Foliar nutrient application increased carbohydrate concentration in plants, resulting in increased biochemical activities and chlorophyll and carotenoid concentrations (Singh *et al.,* 2014; Mahmoodi *et al.,* 2020; Parvin *et al*., 2020). Increased enzymatic activity such as phosphoenolpyruvate carboxylase (PEPC) and rubisco by VAM fungi, as well as increased auxin metabolism in plants as a result of foliar nutrient application, may result in an increased photosynthetic.

Growth parameters

At 30 DAS plant height (33.1 cm), leaf area index (0.49) shoot weight (0.143 g), root dry weight (0.22 g) and total dry weight (0.36 g) was significantly higher in treatment

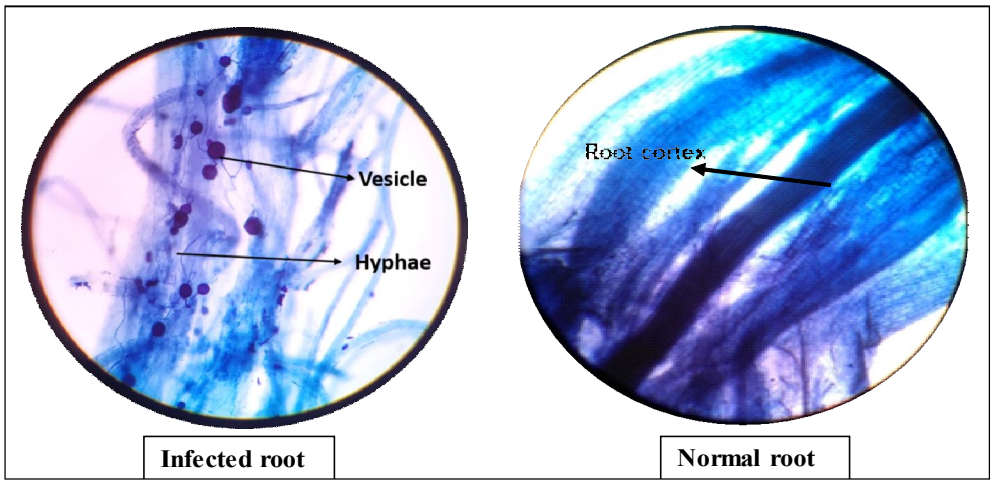


Fig 4: Microscopic view of root with and without VAM infection.

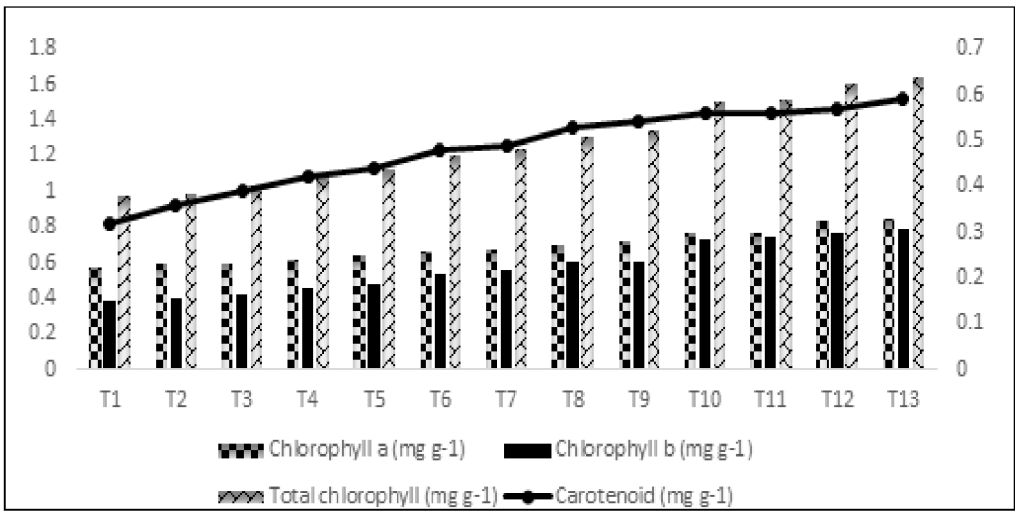


Fig 5: Effect of different dose of soil application of VAM and foliar application of nutrients on photosynthetic pigments at 30 DAS.

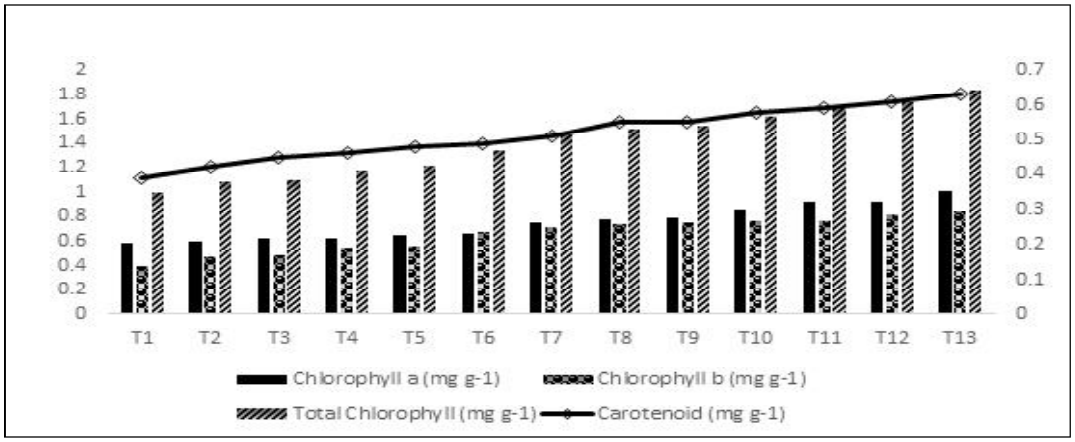


Fig 5(a): Effect of different dose of soil application of VAM and foliar application of nutrients on photosynthetic pigments at 50 DAS.

Table 2: Effect of different dose of soil application of VAM and foliar application of nutrients on growth parameters of aerobic rice at 30 and 50 DAS.

Treatments	30 DAS					50 DAS				
	Plant height (cm)	LAI	Root weight (g)	Shoot weight (g)	Total dry weight (g)	Plant height (cm)	LAI	Root weight (g)	Shoot weight (g)	Total dry weight (g)
T_1	20.4	0.14	0.009	0.02	0.03	32.1	0.70	0.42	0.47	0.86
T_2	21.2	0.20	0.02	0.02	0.05	32.7	0.88	0.46	0.48	0.94
T_3	21.5	0.23	0.03	0.04	0.07	34.2	1.00	0.52	0.58	1.00
T_4	22.3	0.25	0.04	0.06	0.10	35.2	1.09	0.57	0.63	1.21
T_5	23.0	0.26	0.05	0.06	0.12	36.4	1.22	0.65	0.71	1.35
T_6	24.8	0.29	0.06	0.07	0.13	36.5	1.28	0.70	0.80	1.50
T_7	26.1	0.32	0.07	0.08	0.15	37.0	1.31	0.78	1.04	1.81
T_8	27.4	0.32	0.07	0.08	0.16	37.8	1.37	0.90	1.18	2.00
T_9	27.7	0.33	0.08	0.09	0.18	38.1	1.49	1.01	1.49	2.50
T_{10}	28.7	0.34	0.09	0.10	0.20	38.2	1.58	1.14	1.72	2.82
T_{11}	29.1	0.41	0.10	0.10	0.21	39.5	1.75	1.17	1.79	2.96
T_{12}	30.3	0.47	0.11	0.12	0.23	41.6	1.90	1.45	1.90	3.31
T_{13}	33.1	0.49	0.22	0.14	0.36	41.8	2.00	1.74	2.15	4.01
SEd	0.4	0.02	0.001	0.001	0.0008	0.7	0.04	0.05	0.07	0.10
CD	0.9	0.05	0.002	0.003	0.0017	1.5	0.09	0.10	0.15	0.21

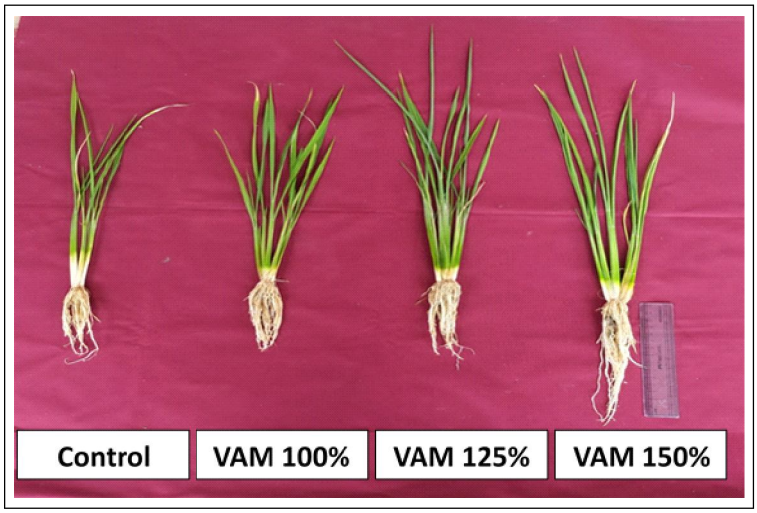


Fig 6: Effect of different doses of soil applied VAM fungi on growth of rice.

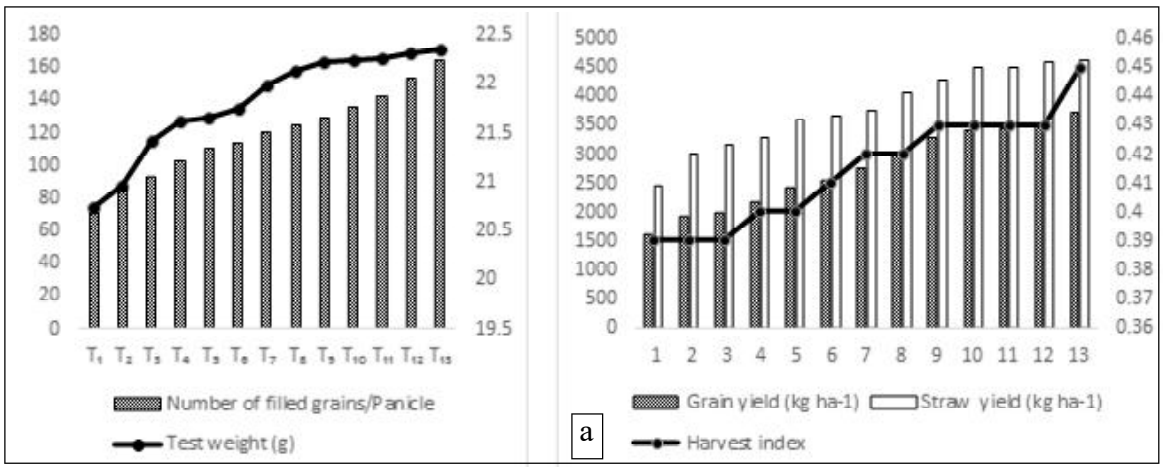


Fig 7 and 7a: Effect of different dose of soil application of VAM and foliar application of nutrients on yield attributes and yield of aerobic rice.

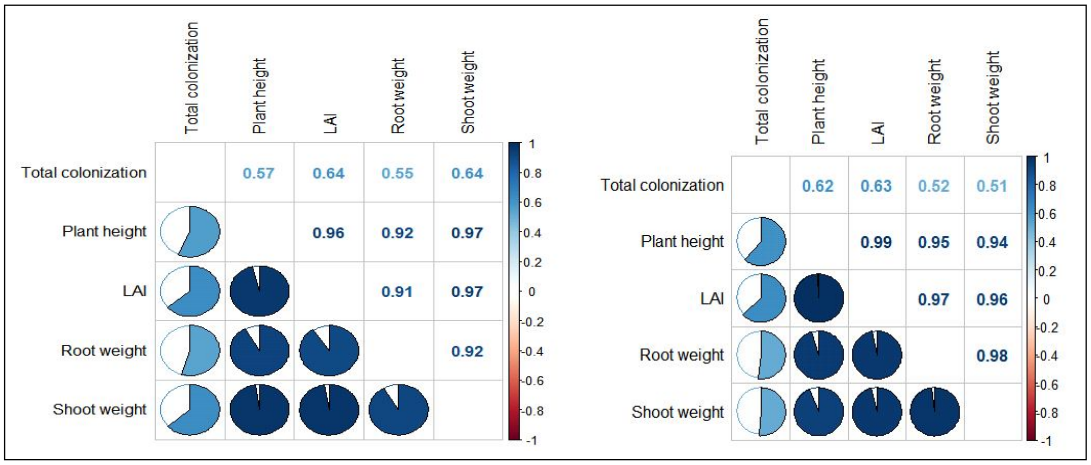


Fig 8: Correlation between total VAM colonization percent with growth attributes of aerobic rice at 30 and 50 DAS.

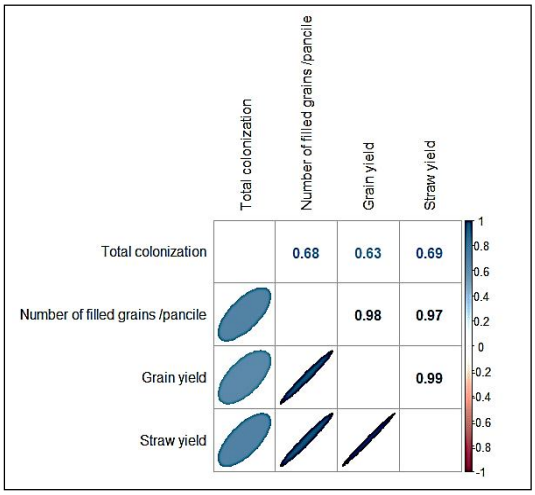


Fig 9: Correlation between total VAM colonization percent with yield attributes and yield of aerobic rice.

received 150% of recommended dose of VAM + foliar application of 0.5% urea + 0.5% $ZnSO_4$ + 1% $FeSO_4$ at 25 and 45 DAS (T_{13}) at 30 DAS. This was comparable with T_{12} with leaf area index of 0.47. Lowest plant height, leaf area index, shoot weight and root dry weight were observed in control plot (T_1) (Table 2).

At 50 DAS plant height (41.8 cm), leaf area index (2.0) shoot weight (2.15 g), root dry weight (1.74 g) and total dry weight (4.01 g) significantly increased with 150% of recommended dose of VAM + foliar application of 0.5% urea + 0.5% $ZnSO_4$ + 1% $FeSO_4$ at 25 and 45 DAS (T_{13}). However plant height was comparable with T_{12}. Lowest plant height, leaf area index, shoot and root dry weight was observed in control plot. Effect of different doses of soil applied VAM fungi on growth of rice showed in Fig 6. The positive effect on plant growth parameters might be due to VAM fungi and foliar application of nutrients to enhance nutrient availability for crop growth and development. Similar findings were

observed by Mostafa *et al.,* 2019; Lahijan *et al.,* 2020; Hussain *et al.,* 2021; Mohammad Hashim *et al.,* 2021; Patel *et al.,* 2022; Mahmoud Soltani *et al.,* 2022.

Yield attributes and yield

The yield attributes *viz.,* number of filled grains panicle^{-1} (164), grain yield (3702 kg ha^{-1}), straw yield (4608kg ha^{-1}) and harvest index were influenced by different dose of soil application of VAM with foliar application of Urea, Iron (Fe) and Zinc (Zn) (Fig 7 and 7a). All these parameters were higher values with 150% of recommended dose of VAM + foliar application of 0.5% urea + 0.5% $ZnSO_4$ + 1% $FeSO_4$ at 25 and 45 DAS (T_{13}). However straw yield and harvest index was on pair with T_{12}. The present study reported that VAM fungal colonization increased the transport of macro and micronutrients at the different growth stage of the crop which increased the rice yield. These results are in confirmation with the results of Khan *et al.,* 2022; Aziez *et al.,* 2022.

Correlation analysis

Correlation analysis was carried out for total VAM colonization percentage, growth and yield of the aerobic rice. Results showed that all growth and yield parameters observed were highly positively correlated with total VAM colonization percentage (Fig 8 and 9).

CONCLUSION

The study revealed that the application of 150% recommended dose of VAM (75 kg ha^{-1}) + foliar applications of 0.5% urea + 0.5% $ZnSO_4$ + 1% $FeSO_4$ at 25 and 45 DAS recorded significant improvements on root morphology, colonization of VAM, photosynthetic pigments, increased growth and yield of rice under aerobic conditions. Most of the nutrients are unavailable naturally due to scarcity of water, which lead to nutrient deficiency this could mitigated by the application of VAM fungi along with foliar nutrients in rice crop under aerobic condition.

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Conflict of interest

The authors declare that there is no conflict of interest.

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