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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 04

Res Jr of Agril Sci (2021) 12: 1187–1190

CARAS

Res. Jr. of Agril. Sci. (July-Aug 2021) 12(4): 1187–1190
www.rjas.org

Full Length Research Article

Influence of VAM on Seed Germination, Seedling Growth and Nutrient Content of Some Leafy Vegetables

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Received: 12 May 2021 | Revised accepted: 20 Jun 2021 | Published online: 13 July 2021

A B S T R A C T

Fertilizer plays a significant role in production of any agricultural crops but continuous and improper use of chemical fertilizer will have an adverse effect on the environment and soil health. VAM offer an eco-friendly biological alternative to chemical fertilizers for enhancing plant quality and productivity in agriculture. The present study was carried out to study the influence of VAM on seed germination, seedling growth and nutrient contents of some selected leafy vegetables such as *Amaranthus dubius*, *Anethum graveolens, Hibiscus sabdariffa, Spinacia oleracea* and *Coriandrum sativum*. The results show that seeds of leafy vegetables inoculated with VAM had a significant effect on seed germination, seedling growth and nutrient contents of all the five selected leafy vegetables.

***Key words*:** Fertilizer, VAM, Germination, Leafy vegetables, Seedling growth

Considering the hazardous effects of chemical fertilizers, biofertilizers are supposed to be a safe alternative to chemical inputs and minimizes ecological disturbance to a great extent. Biofertilizers are cost-effective, ecofriendly in nature, and their prolonged use enhances soil fertility substantially [1]. Bio-fertilizers are a mixture of naturally occurring substances that are used to improve soil fertility. These fertilizers are very useful for soil health as well as for plant growth and development [2].

Different research studies conducted on AMF during the past two decades have highlighted their countless benefits on soil health and crop productivity. Therefore, it is widely believed that AMF could be considered as a replacement of inorganic fertilizers in the near future, because mycorrhizal application can effectively reduce the quantitative use of chemical fertilizer input especially of phosphorus [3]. AMF are also very effective in helping plants to take up nutrients from the nutrient-deficient soils [4].

Green leafy vegetables are considered as excellent source of vitamins, minerals and phenolic compounds. Mineral nutrients like iron and calcium are high in leafy vegetables than staple food grains. Also, leafy vegetables are the only natural sources of folic acid. It is also recommended that one of the five servings of vegetables should be green leafy vegetables. The Present study was conducted to investigate the effect of VAM on seed germination, seedling growth and nutrient status of some selected leafy vegetables.

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MATERIALS AND METHODS

Pot experiment was conducted using leafy vegetables. Seeds of the above said leafy vegetables procured from local nursery at Tenkasi, Tamil Nadu, India. Fifty seeds of each plant sample were inoculated with 100 gm inoculum of VAM. There was no addition of VAM inoculums to control. The earthen pots were filled with garden soil. Plants were allowed to grow in the greenhouse. Seedlings were harvested after 15days of planting. Ten seedlings of different leafy vegetables control and VAM treated were randomly selected for the measurement of root and shoot length, fresh and dry weight. Germination percentage and seedling growth recorded up to 15 days.

Germination percentage was calculated by:

$$\text{Percentage of germination} = \frac{\text{No. of seeds germinated}}{\text{No of seeds sown}} \times 100$$

Biochemical constituents like nitrogen, sodium, potassium, phosphorous, and iron were also measured in studied plant samples.

Growth parameters

The various growth parameters were measured on 15 days after seeding.

Root and shoot length

Root and shoot length was recorded from randomly selected ten plants in each treated pot.

Vigour Index (VI) was calculated according to the method suggested by Abdul-Baki and Anderson [5].

Vigour Index = (Root length + shoot length) × Germination percentage

Fresh weight and dry weight per plant

For this purpose, ten plants from each treatment were uprooted cleaned and fresh weight was taken. The respective plants were kept in oven separately at 80ºC in oven for 72 hours and then dry weights were recorded.

Sample preparation

To the powdered plant sample, 5ml of 65% HNO_3 was added and then the mixture was boiled gently for 30-45 minutes. After cooling, 2.5ml of 70% $Hclo_4$ was added and the mixture was gently boiled until dense white fumes appeared. Later the mixture was allowed to cool and 10ml of deionized water was added followed by further boiling until the fumes were totally released [6]. The contents were allowed to cool and then filtered through what man No_4 filter paper in a flask. The filtrate was diluted to 50ml with deionized H_2O and stored for further analysis.

Determination of Ash, N, Na, K, P and Fe

Ash content

20g fresh sample (W1) was taken in porcelain dish. The weighed sample with porcelain was weighed (W2) and was heated at 90ºC for 1hr. It was then kept in muffle furnace about 525ºC until it was converted in to white ash. It was cool in desiccator and weighed (W3). Ash content was obtained by following formula:

$$\text{Ash content (g /100 g)} = \frac{(W2 - W3) - W1}{\text{Wt. of sample}} \times 100$$

Nitrogen (Nessler's method)

Pipette out 1-2ml of aliquote in 25ml volumetric flask and add 2-5ml of distilled water to it. Now add 1ml of 10% sodium silicate solution. Wash the neck of the flask thoroughly from inside. Add required quantity of 10% sodium hydroxide. Wash the neck of flask thoroughly once again. Now add 2ml of Nessler's reagent. Yellow colour appears. Make the volume and take the reading at 440nm.

Sodium (APHA [7])

The quantity of sodium in the sample was determined. The solution under analysis is sprayed in Flame photometer using appropriate filter. The flame photometer was standardized using the known standard sodium solution, 5ml of the solution was fed directly into the flame photometer. The sodium content was read as ppm. For samples containing high concentration of sodium appropriate dilution with deionized distilled water were made before feeding into the flame photometer.

Potassium (APHA [7])

5ml of the digested sample was neutralized with equal amount of ammonia solution and fed into the flame photometer. The potassium content was directly read out as ppm. The instrument was standardized using different concentration of working standard solution.

Phosphorous (Fiske and Subbarow [8])

Various volumes of working standard solution 1.0, 2.0, 3.0, 4.0 and 5.0ml were pipette out into a series of test tubes. The concentrations of above solution were 8, 16, 24, 32 and 40mg of phosphorous respectively. One millilitre of the digested sample was pipette in to another test tube. ANSA (0.4ml) and 1.0 ml of molybdate I were added to standard and 1.0 ml of molybdate II was pipette into the unknown samples respectively. The volume was made up to 10ml with distilled water. It was mixed well and the developed color was read in spectrometer at 660nm after 20 minutes. The standard graph was drawn using the standard values.

RESULTS AND DISCUSSION

Green leafy vegetables are the rich sources of essential minerals and vitamins. The natural compositions of such vegetables have huge importance as nutrient supplement and in treatment of various diseases. The results show that seed inoculated with VAM had a significant effect on seed germination, seedling growth and nutrient contents of all the five selected leafy vegetables. Table-I shows the effect of VAM on seed germination. Except *Amaranthus dubius*, there was no significant effect on the germination percentage of selected leafy vegetables.

Table 1 Effect of VAM on Seed germination, Shoot and Root length of some selected leafy vegetables

Plant samples	Percent of seed germination		Shoot length in cm		Root length in cm	
	Control	Treated	Control	Treated	Control	Treated
Amaranthus dubius	76%	98%	2.87	3.54	2.13	2.51
Anethum graveolens	83%	77%	2.23	3.30	2.08	2.73
Hibiscus sabdariffa	77%	75%	7.29	10.44	6.86	9.38
Spinacia oleracea	87%	71%	2.53	3.36	2.25	3.17
Coriandrum sativum	76%	78%	3.30	4.83	2.66	3.63

Length of both root and shoot of all VAM infected leafy vegetables significantly increased when compared to control. Highest shoot and root length was also recorded for VAM infected seeds of *Hibiscus sabdariffa* (Table 1). The test (mycorrhizal) plants of maize, rice and wheat had increased plant heights by 43.5, 65.1 and 41.4% respectively as compared to the plant heights of control (non-mycorrhizal) plants [9]. Significant increase in chlorophyll content, shoot and root length and total biomass of different plants was observed following inoculation with *Glomus* sp. [10-12].

VAM inoculation also increased the seed vigour index (Table 2) of all studied leafy vegetables compared to control. Similarly, VAM inoculation increased fresh and dry weights (Table 3) of all the three seedlings. *Casuarina equisetifolia* seedlings inoculated with a combination of

Frankia and VAM fungi increased growth and biomass [13]. VAM inoculation had a significant effect on biochemical constituents such as ash, nitrogen, sodium, potassium and phosphorus (Table 4). The ash content is generally recognized as a measure of quality for the assessment of the functional properties of foods [14]. Ash content of treated seedlings ranged from 5.51% to 27.7% which was higher than Control (4.45%-24%).

Table 2 Effect of VAM on seed vigour index of some selected leafy vegetables

Plant samples	Vigour Index	
	Control	Treated
Amaranthus dubius	380	592.9
Anethum graveolens	357.73	464.31
Hibiscus sabdariffa	1.089.5	1,486.5
Spinacia oleracea	415.86	463.63
Coriandrum sativum	452.96	659.88

Table 3 Effect of VAM on fresh and dry weight of seedlings of some selected leafy vegetables

Plant samples	Fresh weight in grams		Dry weight in grams	
	Control	Treated	Control	Treated
Amaranthus dubius	0.053	0.071	0.008	0.01
Anethum graveolens	0.045	0.062	0.001	0.003
Hibiscus sabdariffa	0.73	1.90	0.027	0.126
Spinacia oleracea	0.35	1.61	0.032	0.095
Coriandrum sativum	0.06	0.92	0.005	0.008

Table 4 Effect of VAM on biochemical composition of some selected leafy vegetables

Compositions	*Amaranthus dubius*		*Anethum graveolens*		*Hibiscus sabdariffa*		*Spinacia oleracea*		*Coriandrum sativum*	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Ash (%)	20.3	25	8.9	9.5	24.0	27.7	4.45	5.51	10.5	11.21
Nitrogen (mg/g)	2.99	4.03	3.19	3.39	2.26	3.34	2.21	2.91	2.66	2.88
Sodium (ppm)	10.3	12.0	77.7	86.2	18.6	31.1	90.3	166	1.60	4.60
Potassium (ppm)	30.6	144	36.7	130	15.5	86.6	45.2	51.4	2.1	6.2
Phosporous (mg/g)	0.467	0.631	0.383	0.497	0.165	0.280	0.239	0.262	0.288	0.326

Highest nitrogen content was recorded in VAM treated seedlings of *Amaranthus dubius.* All the treated samples showed higher content of nitrogen than control group. Percent content of nitrogen, phosphorus and potassium and their uptake was higher in soybean plants inoculated with VA mycorrhizal fungi compared to uninoculated plants The increase in the uptake of three elements was best when the VA-mycorrhizal inoculum was given at the time of sowing. The uptake was reduced with delay in VA-mycorrhizal inoculation [15]. Not only the uptake of P is enhanced by VAM colonization of plant roots, the uptake of other macro and micronutrients like N, Ca, Mg, S, Cu, Fe, Zn and B have also been enhanced [16-17]. Several studies have demonstrated the transport of inorganic Nitrogen (N) by VAM fungi [18].

The value of Na content in treated plant samples varied in a range of 4.60ppm-166ppm. Lowest amount was recorded in control sample of *Coriandrum sativum.* Vast difference established in potassium content of all the treated samples compared to control. Highest value (144ppm) was recorded for the seeds of *Amaranthus dubius.* The earlier reports revealed that mycorrhization raised the K+ level in root [19].

Highest concentration of phosphorus (0.631mg/g) was recorded in the treated samples of *Amaranthus dubius.* Similar observations pertaining to the increased phosphorus uptake by VAM treated plants have been reported by earlier workers [20]. The external VAM hyphae reach beyond the depletion zone around the root hairs, absorb soil P and translocate it, perhaps in the form of polyphosphate granules, to the arbuscules where P is transferred to the plant cell in exchange of carbon [21]. All the four tested nutrients such as nitrogen, sodium, potassium and phosphorus were higher in the VAM inoculated samples compared to control group.

CONCLUSION

The Present study indicates that all the growth parameters such as seed germination, shoot and root length, seed vigour, fresh and dry weight, nutrient contents of selected leafy vegetables were recorded to be significantly higher in VAM treated plants than the control sets. Thus, it is evidenced from the result that vesicular arbuscular mycorrhizal association have beneficial effects on plant growth and development.

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