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INFLUENCE OF AM FUNGI ON BIOMASS AND ROOT GROWTH IN CUSTARD APPLE (*ANNONA SQUAMOSA* L.) SEEDLINGS

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ABSTRACT

The influence of arbuscular mycorrhizal (AM) fungi on the biomass and root growth of custard apple (Annona squamosa L.) seedlings was investigated in a study conducted from October 2021 to May 2022 at the College of Horticulture, Venkataramannagudem, Andhra Pradesh. The experiment utilized a Completely Randomized Design (CRD) with four treatments: T1 (*G. fasciculatum* @ 3g), T2 (*G. leptotichum* @ 3g), T3 (*G. fasciculatum* @ 1.5g + *G. leptotichum* @ 1.5g), and T4 (non-mycorrhizal control), each replicated five times. Results indicated significant increases in root length, number of roots, fresh and dry weights of both shoots and roots in mycorrhizal treatments compared to the control. Treatment T3 exhibited the highest growth parameters across all metrics, suggesting synergistic effects of the two AM fungi species. The enhanced growth observed is attributed to improved nutrient uptake, particularly phosphorus, and increased water retention facilitated by AM fungi. These findings support the potential of AM fungi in promoting the growth of custard apple seedlings, which could benefit cultivation practices in arid and semi-arid regions.
Keywords : AM Fungi, Custard apple, Root length, Biomass, Shoot

Introduction

Custard apple (*Annona squamosa* L.) is one of the important minor fruit crops grown in sub-tropical and tropical conditions. Its chromosome number is 2n=14 and belongs to the family Annonaceae. (George and Nissen, 1993) In India, it is cultivated in Andhra Pradesh, Bihar, Madhya Pradesh, Maharashtra, Gujarat, Rajasthan and Odisha in an area of 48 thousand hectares with 435 thousand MT production (Anon, 2021). The custard apple tree grows to a height of 5 to 9 feet. It has branched tap root system. Leaves are oblong, 12-14 cm long and 2-4 cm wide, alternately arranged on short petioles, young leaves are slightly hairy, solitary and clustered crystals occur in epidermal cells (Kumar *et al.*, 2005). The flowers are penicillate, actinomorphic, protogynous, spiro cyclic and bisexual (Olesen and Muldoon, 2012). Trees start to bear fruit at 3–4-year-old. In India fruits are produced in July-August. Arbuscular mycorrhizal (AM) fungi are symbiotic biotrophs that form close relationships with a host plant via intracellular fungal structures, namely arbuscules, in root cortical cells (Rillig et *al.*, 2002). It is currently estimated that AM fungi began their associations with host plants between 400 and 480 million years ago, contributing to the initial land colonization by terrestrial plants. Approximately 80% of terrestrial plant species are in close symbiotic relations with AM fungi (Kottke and Nebel,2005) for several plant-promoting properties, such as nutrient acquisition, increases in crop mass and yield, as well as reduced stress from abiotic pressures, e.g., soil salinity and drought. In general, very limited and scattered work was done on influence of AM fungi species *viz., Glomus fasiculatum* and *Glomus leptotichum* on growth and biomass production.

Material and Methods

The present investigation entitled "Influence of am fungi on biomass and root growth in custard apple (*Annona squamosa* L.) seedlings was conducted from October 2021 to May 2022 at the College of Horticulture, Venkataramannagudem, Dr.Y.S.R. Horticulture University, West Godavari District, Andhra Pradesh.

Treatment details

Location of work	:	Centre of Excellence for Protected Cultivation, Venkataramannagudem
Crop	:	Custard apple (*Annona squamosa.* L)
Variety	:	Local cultivar
Statistical design	:	CRD
Number of treatments	:	4
Number of replications	:	5
Number of seedlings per treatment	:	30
Pre -soaking of seeds	:	GA_3 @ 500ppm for 24 hours
Season	:	2021-22 (October to May)

Treatments:

T_1 : (Inoculation with *G. fasciculatum* @ 3g)
T_2 : (Inoculation with *G. leptotichum* @ 3g)
T_3 : (Inoculation with *G. fasciculatum* @ 1.5g + *G. leptotichum* @ 1.5g)
T_4 : (Non mycorrhizal)

Material used

Potting mixture comprises of 2:1:1 ratio of red earth, FYM and vermicompost. They are mixed well and sterilized in autoclave for 15 minutes at 121^0C and 15 psi pressure.

Arbuscular Mycorrhiza fungi inoculation

The soil culture of *G. fasiculatum* and *G. leptotichum* were obtained from Centre for Natural Biological Resources and Community Development, Bangalore. *G. fasiculatum* and *G. leptotichum* consisted of 48 spores and 58 spores respectively per 5 gram of inoculation culture. The AM fungi inoculums was applied just before seed sowing at 3cm below the surface of potting mixture as per the treatments.

Observations Recorded

Five seedlings were randomly selected and tagged in each replication of were carefully removed from polybags at each time of recording observations (at 70, 90, 110, 130, 150 DAS) in polybag. The polybags were profusely watered before removing the seedlings which were uprooted with the ball of earth and placed in water, then washed thoroughly to remove the soil particles adhered to the root system.

Root length of seedling (cm) and Number of roots per seedling

Root length was measured from the collar region to the tip of the longest root at 70, 90, 110, 130 and 150 days after sowing, average value was computed and expressed in centimeters. Number of roots produced per seedling were counted in each treatment at 70, 90, 110, 130 and 150 days after sowing. The mean values were calculated and expressed as number of roots per seedling.

Fresh weight of shoots (g) and root (g)

The shoot and root portion of each selected seedling was separated and the fresh weight was recorded at 70, 90, 110, 130 and 150 days after sowing with the help of an electronic balance and mean shoot fresh weight was computed and expressed in grams**.**

Dry weight of shoots (g) and root (g)

At 70, 90, 110, 130 and 150 days after sowing the shoots and roots of each seedling taken for recording fresh weight were kept in brown cover were completely dried in hot air oven at 60˚C until they attain a constant weight and dry weights were recorded with electronic balance. The mean values were calculated and expressed in grams.

Statistical Analysis

The data was analyzed using the method outlined by Panse and Sukhatme (1985) for a Completely Randomized Design (CRD). The means were compared at a 5% level of significance to determine statistical differences between treatments.

Results and discussion

Root length of seedling (cm)

The data on seedling root length of custard apple, recorded at 70, 90, 110, 130, and 150 Days After Sowing (DAS), is shown in Figure1. The results indicate that root length increased consistently over time across all treatments. The highest root length at each interval (17.03, 21.97, 26.56, 31.78, and 36.87 cm) was observed in treatment T3, which involved the application of *Glomus fasiculatum* (1.5g) and *Glomus leptotichum* (1.5 g). This was followed by treatment T2 (G. leptotichum @ 3g), which showed root lengths of 14.56, 19.65, 24.76, 27.08, and 31.90 cm. The lowest root length (7.94, 10.89, 14.45, 17.67, and 21.34 cm) was recorded in the non-mycorrhizal control group (T4). The increase in root length is likely due to the enhanced soil health facilitated by arbuscular mycorrhizal (AM) fungi, which is consistent with findings in sweet orange (Citrus sinensis cv. Mosambi) and pomegranate, as reported by Singh *et al.* (2000) and Rupnawar and Navale (2000), respectively.

Number of roots per seedling

The data show that AM fungi significantly increased the number of roots in custard apple seedlings. Treatment T3 (*G. fasiculatum* + *G. leptotichum*) produced the highest root numbers, while T4 (non-mycorrhizal) had the lowest. AM fungi likely enhanced root growth by improving water retention and phosphorus availability, supporting past findings. Production of plant growth substances like cytokinins, gibberellins and auxins increased the water holding capacity and the readily available phosphorus in AM fungi applied seedlings could have promoted the root elongation which leads to increased size and number of root hairs (Edward and Lofty, 1980). These results were supported by Bopaiah and Khader (1989) in black pepper.

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Fresh weight of shoot (g)

The data in Table 2 show that AM fungi significantly influenced the fresh weight of custard apple seedling shoots at 70, 90, 110, 130, and 150 DAS. Treatment T3 (*G. fasiculatum* + *G. leptotichum*) recorded the highest fresh weight, while T4 (non-mycorrhizal) had the lowest. AM fungi helped the plant by increasing the rate of sucrose translocation from expanded leaves through enhanced photosynthesis. The increased photosynthesis might be the reason for increase in fresh weight of shoot. Increased leaf area in inoculated plants resulted in higher photosynthetic efficiency. This might be the reason for more production of plant biomass (Mathews *et al.*, 2003). The present results were in consonance with findings of Bhuiyan (2015) in tomato seedlings and Boyer *et al.* (2014) in strawberry

Fresh weight of root (g)

The data in Table 3 show that root fresh weight of custard apple seedlings increased at 70, 90, 110, 130, and 150 DAS across treatments. AM fungi significantly enhanced root fresh weight, with T3 (*G. fasiculatum* @ 1.5 g + *G. leptotichum* @ 1.5 g) recording the highest values, followed by T2 (*G. leptotichum* @ 3 g). T4 (non-mycorrhizal) had the lowest fresh root weights. Root fresh weight was highest in AM fungi inoculated seedlings where mycorrhizal infection was more. It was expected that more mycorrhizal infection expands the roots hairs and increased the root volume which led to increase in root biomass. These results were in corroboration with the findings of Bagheri *et al.* (2018) in zinna, Bhuiyan *et al.* (2015) in tomato seedlings and Boyer *et al.* (2014) in strawberry.

Dry weight of shoot (g)

It is evident from the data that irrespective of the treatments, the dry weight of shoot per seedling increased at 70, 90, 110, 130 and 150 DAS (Table 4.8).

Increase in dry weight of shoot might be due to production of a greater number of leaves per seedling and higher seedling height. In AM fungi inoculated seedlings more accumulation of biomass was recorded due to increased nutrient uptake and more photosynthesis. The present results were in consonance with findings of Bagheri *et al.* (2018) in zinna, Jiang *et al.* (2013) in bamboo seedlings and Ortas *et al.* (2011) in pepper seedlings.

Dry weight of root (g)

Maximum dry weight of root (0.75, 1.45, 2.34, 3.12 and 3.98) was recorded in T_3 (*G. fasiculatum* @ 1.5 g + *G. leptotichum* @ 1.5 g) followed by T_2 - *G. leptotichum* @ 3g (0.43, 0.99, 1.80, 2.34 and 3.11) and lowest dry weight of root (0.25, 0.45, 0.98, 1.25 and 1.67) was recorded in T_4 (non mycorrhizal) at 70, 90, 110, 130 and 150 DAS respectively.

Increased number of roots, root length and more fresh weight of roots resulted in higher dry weight of root. Similar results were reported in bamboo seedlings (Jiang *et al.*, 2013), pepper seedlings (Ortas *et al.*, 2011), zinna (Bagheri *et al.*, 2018) and cucumber (Chen *et al.*, 2017).

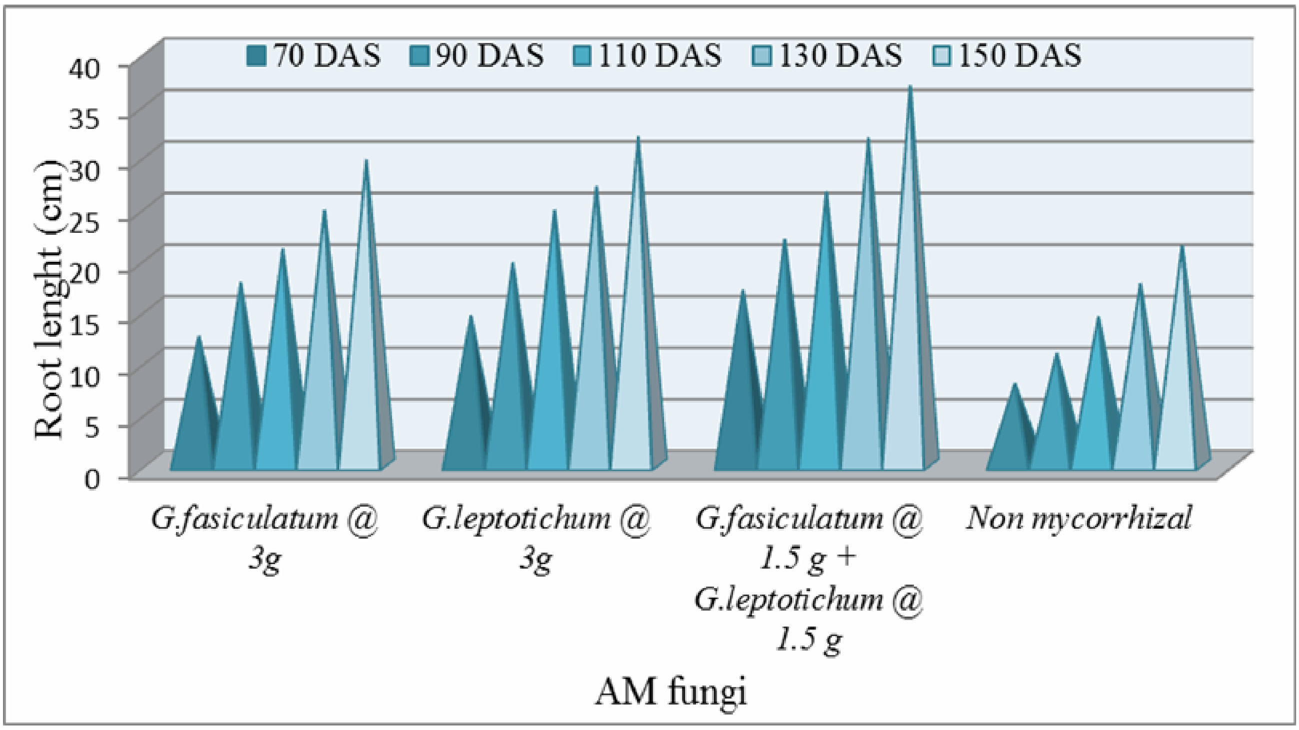

Fig. 1: Influence of AM Fungi on root length in custard apple (*Annona squamosa* L.) seedlings.

Table 1: Influence of AM Fungi on number of roots produced in custard apple (*Annona squamosa* L.) seedlings.

Treatment	70 DAS	90 DAS	110 DAS	130 DAS	150 DAS
T_1 (*G. fasiculatum* @ 3g)	44.12	48.67	51.89	63.02	77.98
T_2 (*G. leptotichum* @ 3g)	47.12	50.14	54.89	64.78	79.67
T_3 (*G. fasiculatum* @ 1.5 g + *G. leptotichum* @ 1.5 g)	57.14	60.12	65.32	71.34	89.31
T_4 (Non mycorrhizal)	29.56	33.45	38.12	42.31	50.34
SE m +	0.64	0.69	0.75	0.87	1.07
CD at 5%	1.95	2.10	2.28	2.62	3.24

Table 2 : Influence of AM Fungi on fresh weight of shoot (g) in custard apple (*Annona squamosa* L.) seedlings.

Treatment	70 DAS	90 DAS	110 DAS	130 DAS	150 DAS
T_1 (*G. fasiculatum* @ 3g)	3.54	4.47	5.24	6.16	7.53
T_2 (*G. leptotichum* @ 3g)	3.79	5.11	5.67	6.89	8.23
T_3 (*G. fasiculatum* @ 1.5 g + *G. leptotichum* @ 1.5 g)	4.36	6.13	7.87	9.56	12.24
T_4 (Non mycorrhizal)	2.09	2.89	3.67	4.34	5.93
SE m +	0.02	0.03	0.04	0.05	0.06
CD at 5%	0.07	0.10	0.12	0.15	0.18

Table 3 : Influence of AM Fungi on fresh weight of root (g) in custard apple (*Annona squamosa* L.) seedlings.

Treatment	70 DAS	90 DAS	110 DAS	130 DAS	150 DAS
T_1 (*G. fasiculatum* @ 3g)	1.37	2.02	3.45	4.06	4.78
T_2 (*G. leptotichum* @ 3g)	1.48	2.24	3.67	4.30	5.13
T_3 (*G. fasiculatum* @ 1.5 g + *G. leptotichum* @ 1.5 g)	2.20	3.89	4.98	5.67	6.56
T_4 (Non mycorrhizal)	1.12	1.67	2.01	2.45	3.45
SE m +	0.01	0.02	0.03	0.03	0.03
CD at 5%	0.03	0.05	0.08	0.09	0.11

Table 4: Influence of AM Fungi on dry weight of shoot (g) in custard apple (*Annona squamosa* L.) seedlings.

Treatment	70 DAS	90 DAS	110 DAS	130 DAS	150 DAS
T_1 (*G. fasiculatum* @ 3g)	1.02	2.12	3.57	4.32	5.42
T_2 (*G. leptotichum* @ 3g)	1.23	2.59	3.98	4.89	5.97
T_3 (*G. fasiculatum* @ 1.5 g + *G. leptotichum* @ 1.5 g)	2.01	3.21	4.13	5.32	6.63
T_4 (Non mycorrhizal)	0.86	1.47	2.23	2.79	3.11
SE m +	0.09	0.10	0.13	0.14	0.16
CD at 5%	0.26	0.28	0.37	0.39	0.46

Table 5 : Influence of AM Fungi on dry weight of root (g) in custard apple (*Annona squamosa* L.) seedlings.

Treatment	70 DAS	90 DAS	110 DAS	130 DAS	150 DAS
T_1 (*G. fasiculatum* @ 3g)	0.31	0.78	1.57	1.97	2.56
T_2 (*G. leptotichum* @ 3g)	0.43	0.99	1.80	2.34	3.11
T_3 (*G. fasiculatum* @ 1.5 g + *G. leptotichum* @ 1.5 g)	0.75	1.45	2.34	3.12	3.98
T_4 (Non mycorrhizal)	0.25	0.45	0.98	1.25	1.67
SE m +	0.07	0.07	0.14	0.20	0.20
CD at 5%	0.10	0.20	0.30	0.60	0.60

Conclusions

The study concludes that the application of AM fungi, particularly in a combined form, significantly enhances the growth parameters of custard apple seedlings. This includes increased root length, number of roots, and both fresh and dry weights of shoots and roots. The findings support the potential use of AM fungi to improve plant growth under suboptimal conditions, thereby contributing to more sustainable agricultural practices for fruit crops like custard apple. This research highlights the beneficial role of AM fungi in enhancing root and shoot biomass, which can be crucial for improving fruit crop productivity, particularly in regions prone to water stress.

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