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Effect of AM fungi on growth of custard apple (*Annona squamosa* L.) seedlings

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Abstract
The present investigation entitled "Effect of AM fungi on growth of custard apple (*Annona squamosa* L.) Seedlings" was carried out during December 2021 to May 2022 at center of excellence for protected cultivation (CEPC), Dr. Y.S.R Horticultural University, Venkataramannagudem, West Godavari. The experiment was laid out in Completely Randomized Design (CRD) with four treatments *Glomus fasiculatum* @ 3 g, *Glomus leptotichum* @ 3 g, *Glomus fasiculatum* @ 1.5 g + *Glomus leptotichum* @ 1.5 g, non mycorrhizal) and five replication.The results revealed that the application of *Glomus fasiculatum* @ 1.5 g+ *Glomus leptotichum* @ 1.5 g resulted in significant improvement of seedling height (19.27, 27.61, 38.98, 45.67 and 53.17 cm), number of leaves per seedling (11.88, 16.38, 19.23, 22.34 and 25.12), stem diameter (3.65, 4.42, 4.85, 5.45 and 6.13 mm) and total chlorophyll content (8.91, 9.56, 10.74, 11.00 and 12.16 mg/g^{-1}) at 70, 90, 110, 130, 150 days after sowing.

Keywords: Custard apple, AM fungi, Seedling height, number of leaves, stem diameter, total chlorophyll

Introduction
Custard apple (*Annona squamosa* L.) is an important minor fruit crop grown under tropical and sub tropical climatic conditions. The main commercial species of the genus *Annona* are *A.cherimola* Mill, *A. squamosa* L, *A. muricata* L, *A. reticulata* and *A. glabra*. The symbiosis of mycorrhizal fungi with plant roots has been linked to increased plant growth and development (Hall, 1988) [7], and increased P contents in tissues (Johnson & Hummel, 1985) [8]. VA mycorrhizas can increase the drought resistance of host plants (Nelson and Safir, 1985) [12]. Hitherto several mechanisms have been proposed to explain drought protection by AMF, such as changes in plant hormones levels (Goicoechea *et al*., 1995) [6], increased photosynthesis (RuizLozano *et al*., 1996), enhanced activity of enzymes involved in anti-oxidant defense (RuizLozano *et al*., 1996), enhanced water uptake through improved hydraulic conductivity and increase in leaf conductance and photosynthetic activity (Amico *et al*., 2002) [5], osmotic adjustment and changes in cell-wall elasticity (Auge *et al*., 1987) [1].
By keeping the above aspects in view, the investigations were undertaken to study the effect of AM fungi *viz., Glomus fasiculatum* and *Glomus leptotichum* on growth of custard apple (*Annona squamosa* L.) seedlings.

Materials and Methods
The present investigation entitled "Effect of AM fungi on growth of custard apple (*Annona squamosa* L.) Seedlings" was carried out during December 2021 to May 2022 at center of excellence for protected cultivation (CEPC), Dr. Y.S.R Horticultural University, Venkataramannagudem, West Godavari. The experiment was laid out in Completely Randomized Design (CRD) with four treatments *Glomus fasciculatum* @ 3 g, *Glomus leptotichum* @ 3 g, *Glomus fasiculatum* @ 1.5 g+*Glomus leptotichum* @ 1.5 g and non mycorrhizal) and five replications. Potting mixture used comprises of 2:1:1 ratio of red earth soil, FYM and vermicompost. They are mixed well and sterilized in autoclave for 15 minutes at 121 ºC and 15 psi pressure. Polythene bags of 20 cm × 17 cm size and 300-gauge thickness were used for filling the potting mixture after surface sterilization with 1% formaldehyde.AM fungi was inoculated in poly bags before sowing of seed. Five seedlings were randomly selected and tagged in each replication for recording the non-destructive observations on shoot parameters throughout the study (at 70, 90, 110, 130 and 150 DAS) in polybag. For total chlorophyll content (mg/g^{-1} fresh weight) four fresh leaves from middle part of seedling

region in each treatment were plucked on 70, 90, 110, 130 and 150 days after sowing for estimating chlorophyll content. The chlorophyll content was estimated as per the procedure outlined by Arnon (1949) [17]. The collected fresh leaves are macerated with 80% acetone and centrifuged at 3000 rpm for 15 minutes then O.D values are taken at wave lengths of 645 and 663 nm using spectrophotometer.
Total chlorophyll is calculated based on the following formula.

Total cholorophyll mg/g^{-1}tissue = [20. 2(A645)-8.02(A663)] x V /1000 xW

where,
A = Absorbance of specific wave lengths (645 and 663 nm)
V = Final volume of chlorophyll extractant in 80 per cent acetone in 100 ml.
W =Fresh weight of leaf sample (1 g)

Results and Discussion
The data pertaining to custard apple seedling height(cm) from table 1 revealed that significant maximum height of seedling (19.27, 27.61, 38.98, 45.67 and 53.17) were recorded in T3 (*Glomus fasiculatum* @ 1.5 g + *Glomus leptotichum* @ 1.5 g) followed by T1 (*Glomus fasiculatum* @ 1.5g) with 18.14, 24.68, 33.65, 39.90 and 45.35 where as minimum height of seedling (13.35, 18.58, 22.89, 28.45 and 33.89) were registered in T4 (non mycorrhizal) at 70, 90, 110, 130 and 150 DAS respectively. Increase in height of seedlings might be attributed due to synthesis of beneficial hormones by AM fungi which results in increase in cell division and cell multiplication. (Miller, 1971 [10], Crafts and Miller, 1974 [4], and Slankis, 1975. The results are in harmony with the findings of Rupnawar and Navale, 2000 [16] in pomegranate and Ojha *et al*., 2008 in custard apple seedlings.

Number of leaves per seedling
From the data on number of leaves produced in table 2 it is evident that irrespective of the treatments imposed, the number of leaves produced per seedling increased during the period of study *i.e*. up to 150 DAS. However, due to influence of AM fungi the number of leaves produced per seedling differed significantly. Highest number of leaves (11.88, 16.38, 19.23, 22.34 and 25.12) were produced in T3 (*Glomus fasiculatum*@ 1.5 g + *Glomus leptotichum* @ 1.5 g) followed by T2 (*Glomus leptotichum* @ 3 g) with 10.72, 14.74, 16.98, 20.89 and 22.13 whereas lowest number of leaves (8.48, 12.67, 15.12, 17.98 and 19.78) were observed in T4 (non mycorrhizal) at 70, 90, 110, 130 and 150 DAS respectively. The absorption and mobilization of nutrients like nitrogen, phosphorus, copper, boron, zinc, sulphur and maintenance of soil moisture around the plant roots by AM fungi might be responsible for the production of more leaves in AM fungi treated seedling (Mosse *et al*., 1981) [11]. A similar effect of AM fungi on number of leaves was also reported by Reena and Bagyraj (1990) [14] in tamarind and Rupnawar and Navale (2000) [16] in orange.

Stem diameter (mm)
The data depicted in table 3 and showed maximum stem diameter (mm) (3.65, 4.42, 4.85, 5.45 and 6.13) in T3 (*Glomusfasiculatum* @ 1.5 g + *Glomus leptotichum* @ 1.5 g) followed by T2 (*Glomus leptotichum*) with 3.12, 4.06, 4.45, 5.01and 5.87 whereas minimum stem diameter (2.25, 2.90, 3.34, 3.89 and 4.04) was found in T4 (non mycorrhizal) at 70, 90, 110, 130 and 150 DAS respectively. Increase in stem diameter in AM inoculated treatments might be due to increase in availability of macro and micro nutrients, which helps in production of more number of photosynthecially active leaves which in turn helps in development of better stem diameter (Borah *et al*., 1994) [3]. Similiar findings were reported in sweet orange cv. mosambi (Singh *et al*., 2000).

Total chlorophyll (mg/g^{-1} fresh weight)
From the data presented in table 4 it is evident that total chlorophyll (mg/g-1 fresh weight) content per seedling increased with increase in days after sowing in all the treatments however chlorophyll content differed significantly among the treatments. Maximum chlorophyll content (8.91, 9.56, 10.74, 11.00 and 12.16) were recorded in T3 (*Glomus fasiculatum* @ 1.5 g + *Glomus leptotichum* @ 1.5 g) followed by T2 (*Glomus leptotichum*) with 8.32, 9.47, 10.07, 10.43 and 10.84 and minimum chlorophyll content (6.75, 7.89, 8.50, 9.09, 9.74) were reported in T4 (non mycorrhizal) at 70, 90, 110, 130 and 150 DAS respectively. Enhanced total chlorophyll content in AM fungi inoculated seedlings might be due to the direct effect of symbiotic association between host and AM fungi which led to higher water and nutrient uptake thereby results in higher biosynthesis of chlorophyll. AM fungi induced nitrogen uptake may increase chlorophyll content in inoculated seedling. The present results are in accordance with the findings of Manoharan *et al*. (2010) [9] in *Erythrina variegata*.

Table 1: Influence of AM Fungi on seedling height (cm) in custard apple (*Annona squamosa* L.)

Treatment	Seedling height (cm)				
	70 DAS	90 DAS	110 DAS	130 DAS	150 DAS
T_1 (*G. fasiculatum* @ 3g)	18.14	24.68	33.65	39.90	45.35
T_2 (*G. leptotichum* @ 3g)	17.09	22.93	30.03	36.87	42.78
T_3 (*G. fasiculatum* @ 1.5 g + *G. leptotichum* @ 1.5 g)	19.27	27.61	38.98	45.67	53.17
T_4 (Non mycorrhizal)	13.35	18.58	22.89	28.45	33.89
SE(m) ±	0.09	0.14	0.25	0.24	0.32
CD at 5%	0.29	0.42	0.75	0.73	0.96

Table 2: Influence of AM Fungi on number of leaves per seedling in custard apple (*Annona squamosa* L.)

Treatment	Number of Leaves				
	70 DAS	90 DAS	110 DAS	130 DAS	150 DAS
T_1 (*G. fasiculatum* @ 3 g)	10.42	14.56	16.85	19.58	21.89
T_2 (*G. leptotichum* @ 3 g)	10.72	14.74	16.98	20.89	22.13
T_3 (*G. fasiculatum* @ 1.5 g + *G. leptotichum* @ 1.5 g)	11.88	16.38	19.23	22.34	25.12
T_4 (Non mycorrhizal)	8.48	12.67	15.12	17.98	19.78
SE(m)±	0.47	0.43	0.21	0.23	0.27
CD at 5%	1.42	1.32	0.63	0.70	0.81

Table 3: Influence of AM Fungi on stem diameter (mm) in custard apple (*Annona squamosa* L.) seedlings.

Treatment	Stem diameter(mm)				
	70 DAS	90 DAS	110 DAS	130 DAS	150 DAS
T_1 (*G. fasiculatum* @ 3 g)	3.04	3.75	4.09	4.56	4.99
T_2 (*G. leptotichum* @ 3 g)	3.12	4.06	4.45	5.01	5.87
T_3 (*G. fasiculatum* @ 1.5 g + *G. leptotichum* @ 1.5 g)	3.65	4.42	4.85	5.45	6.13
T_4 (Non mycorrhizal)	2.25	2.90	3.34	3.89	4.04
SE(m) ±	0.02	0.03	0.03	0.03	0.04
CD at 5%	0.06	0.08	0.09	0.10	0.11

Table 4: Influence of AM Fungi on chlorophyll content in custard apple (*Annona squamosa* L.) seedlings.

Treatment	Chlorophyll content (mg/g^{-1})				
	70 DAS	90 DAS	110 DAS	130 DAS	150 DAS
G. fasiculatum @ 3 g	8.23	9.35	9.98	10.12	10.81
G. leptotichum @ 3 g	8.32	9.47	10.07	10.43	10.84
G. fasiculatum @ 1.5 g + *G. leptotichum* @ 1.5 g	8.91	9.56	10.74	11.00	12.16
Non mycorrhizal	6.75	7.89	8.50	9.09	9.74
SE(m)±	0.06	0.07	0.09	0.11	0.28
C.D.	0.19	0.22	0.29	0.33	0.86

Conclusion

The present investigation on the effect of AM fungi on seedling growth of custard apple (*Annona squamosa* L.)" revealed that combined application of AM fungi species *i.e. Glomus fasiculatum* @ 1.5 g + *Glomus leptotichum* @ 1.5 g followed by application of AM fungi species *Glomus leptotichum* @ 3 g alone showed superior performance in terms of growth parameters. The results indicated the positive effect of AM fungi on growth of custard apple (*Annona squamosa* L.) seedlings.

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