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Effect of AM Fungi on Infectivity of *Pythium aphanidermatum* in *Curcuma longa* L.

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Abstract

Curcuma longa L. (Turmeric) a perennial herb belongs to Zingiberaceae. It's highly grown for its remarkable medicinal uses. Apart from medicinal properties and importance, Turmeric has not been emphasized much with respect to agricultural view. Sustainable agriculture is need of hour and hence it must be brought in practice on large scale. Arbuscular Mycorrhizal (AM) fungi has mutually beneficial relationship with the roots and support plants during biotic and abiotic stress. The function of AM fungi in different morphological parameters when Turmeric under biotic stress shows scanty records so far. Existing research studies are intended to evaluate an influence of AM fungi on Turmeric plant during biotic stress. During this investigation Turmeric plants were assessed for Growth and Vigour Index, relative mycorrhizal dependency, some biochemical contents and fresh weight of the rhizome. Statistical analysis of the results obtained showed strongly significant results.

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Introduction

Turmeric (*Curcuma longa* L.) a perennial herb belongs to family Zingiberaceae, it is extensively grown throughout Asian subcontinent; especially in India and China. The plant grows up to 1 to 1.5 meters in height and possess yellowish flowers. Rhizomes are underground stem which are fleshy, ringed with the persistent bases of older leaves, rhizome is a part of plant which has substantial ayurvedic medicinal properties. It is cultivated almost throughout India (Kapoor, 2000).[1] India is leading contributor for the supply of turmeric all over the globe (Leung and Foster,1996).[2] It is grown also in the countries like Taiwan, Japan, Burma, and Indonesia and African continent. Its rhizome powder in European countries is imported mainly from India and little from other south eastern Asian countries (Murugananthi *et al.*, 2008).[3] Dried rhizomes yield yellow powder which has a bitter flavour and spicy aromatic smell. Due to this it is used as colouring

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agent and a cooking spice. Apart from cooking spice it is ingested orally and applied on skin during various allergies, hepatitis, inflammation of joints, throat infections, remedies to cure acne, small wounds, boils, bruises, blistering, parasitic infections, hemorrhages and other skin diseases like herpes and wounds.[4,5] Oral administration of dried powder is best remedy for inflammatory swelling.[6] It also has anti carcinogenic properties[7] and free radicals scavenging activities.[8]

Although Turmeric is an important commercial crop and has many medicinal properties it is very adversely affected by the infection of fungus like *Pythium aphanidermatum*,[9] which affect the quality and commercial value of the crop. To control this disease, farmers use Metalaxyl and Mancozeb which affects microflora of soil and pollute soil as well as underground water. Hence there is need of finding proper and sustainable solution to control this with biological means. Plants possess natural capability to trigger defense mechanisms during infection of plant pathogens. AM fungi show mutually beneficial relationship with the plant roots. AM fungi influence growth vigour and productivity among terrestrial ecosystems[10] and its association with plant roots is highly supportive for the plants with respect to resistance as well as tolerance during biotic stresses. So far, the studies revealed that severity of diseases like root rot or wilting caused by diverse fungi such as *Fusarium, Rhizoctonia* and *Pythium*.[11] Considering *Pythium aphanidermatum* as a biggest threat to the turmeric plant and keeping idea in mind that AM fungi helps plants during such kind of infections present investigation has been undertaken.

Present studies are mainly commenced to evaluate the influence of AM fungi during infection of *Pythium aphanidermatum* for growth, yield and biochemical contents in Turmeric. These studies would certainly be helpful for establishing disease control mechanism using AM fungi and could be the best step towards environmental sustainability.

Materials and Methods

Set up of Experiment.

Medium for this experiment was made ready by mixing soil of pH 7.0 6 kg, fine sand, and farmyard manure 0.5 kg. This mixture was then autoclaved for 45 minutes at 15 lbs. pressure. After cooling this mixture, it was filled in square plastic pots of 12 x 12-inch size. The plastic pots were surface sterilized with absolute alcohol before filling the mixture of soil. The experiment was carried out in triplicate manner using six pots, out of that three were control and three were replicates. These all six pots were used to grow rhizomes of turmeric and further investigations.

Growing of Turmeric Rhizomes

Healthy and disease-free rhizomes of 'Karadi' variety of Turmeric were used for experimentation. The rhizomes of Turmeric of 'Karadi' variety were obtained from Satara district of Maharashtra state of India. Rhizomes selected for growing were placed in 0.1% $HgCl_2$ of laboratory grade by alpha chemica, for half hour and later washed under running tap water. The pots filled with 150 g of soil containing mixture of three species of AM fungi from pure culture. These included *Acaulospora dilatata*, *Glomus mosseae*, and *Scutellospora fulgida*.

Inoculation of *Pythium Aphanidermatum* and AM fungi in Pots

The pots after filling autoclave sterilized soil mixture, sand as well as FYM was ready to grow Turmeric rhizomes. Three different sets of six pots each were used for present investigation. The experimental plants of first set inoculated with the spores of *Acaulospora dilatata*, *Glomus mosseae*, and *Scutellospora fulgida* at the time of growing and *Pythium aphanidermatum* inoculated after 45 days of growing. Control plants were inoculated only with *Pythium aphanidermatum* after 45 days of growing. The experimental plants of second set were dual inoculated with *Acaulospora dilatata*, *Glomus mosseae*, and *Scutellospora fulgida* and *Pythium aphanidermatum* at the time of growing. The control plants were inoculated only with *Pythium aphanidermatum* at the time of growing. The third set was dual inoculated with *Acaulospora dilatata*, *Glomus mosseae*, and *Scutellospora fulgida* after 45 days after growing along with *Pythium aphanidermatum*. Similarly, like second set, the control plant of this set inoculated only with *Pythium aphanidermatum* after 45 days of growth.

Studies on Growth Parameters

The plants grown in different sets of pots were watered after every five days interval and supervised for different growth parameters. Number and surface area of leaves, shoot length etc. measured on 90th

day and root length, growth, and vigour index (GVI), fresh weight, dry weight and Relative mycorrhizal dependency (RMD) etc. was measured at the time of harvesting i.e., on 180th day.

Measuring of Growth Parameters

The GVI was calculated by using the formula as given in.[12]

(GVI) =Root length + Shoot length x germination %
The relative mycorrhizal dependency was calculated using the following formula as given in 13.
Relative mycorrhizal dependency = (Mycorrhizal dry weight-Non mycorrhizal dry weight)/(Mycorrhizal dry weight) x 100

Root length and shoot length was measured using measuring tape. Germination percentage was calculated by counting number of rhizomes germinated and number of rhizomes grown.

Recording Fresh Weigh and Dry Weight

Turmeric grown for 180 days, at the time of harvesting, their fresh weights were recorded. The fresh wt. was weighed after soaking in water and blot drying the rhizomes and entire shoot. The dry weights were recorded after drying the shoot and Rhizome for four days in a hot air oven with 50º C temperature.

Estimation of Chlorophylls

Estimation of chlorophylls done from leaves of control as well as experimental plants of all three sets of an experiment by and the calculations were done using formula given by.[14]

Estimation of Proteins

Turmeric Rhizomes were used to estimate the proteins using method of.[15] The rhizomes from control and experimental plants of all three sets were used for protein estimation. 0.5 g rhizomes were crushed in 5 ml, 0.1 M phosphate buffer (pH 7.0). This mixture centrifuged for 15 minutes with 10,000 rpm. The pellet then mixed in 2 ml of 1.0 N NaOH. From this sample, 0.2 ml was used for proteins estimation. The plant extract and working standard of BSA filled in series of test tubes and raised the final volume to 1 ml in each tube. Each tube later topped with 5 ml of reagent C and incubated the tubes for 10 minutes. Each tube now added with 0.5 ml of folin ciocalteau reagent and incubated in dark for half hour. Reaction mixture turned blue was read at 660 nm on UV-visible spectrophotometer. BSA (fraction V) used at the concentration of 50 mg for dissolving in 50 ml distilled water and readings used to plot standard graph. Am amount of protein calculated using standard graph.

Estimation of Total Soluble Carbohydrates

Total soluble carbohydrates were determined as per[16] method. Rhizomes were used for this estimation. 100 mg of rhizomes taken in a boiling test tube. The tubes after adding 2.5 N HCl placed in boiling water for 3 hours for hydrolyzation. All tubes later cooled at room temperature. Nacl was used to neutralize it and confirmed it after effervescence ceased. Tubes centrifuged after adding 10 ml distilled water. 0.5 ml and 1 ml of supernatant was used as aliquots. The working standards prepared by making 0, 0.2, 0.4, 0.6, 0.8- and 1-ml reaction samples. Non-working standard solution containing tube treated as a blank. The volume in tubes raised till 1 ml including blank using distilled water. 4 ml Anthrone reagent was mixed in all the tubes and tubes heated in boiling water for 8 minutes. After cooling the tubes rapidly absorbance read at 630 nm. The amount of total soluble carbohydrates was then calculated using standard graph.

Statistical Analysis

Analysis of the data was done using MS office excel 2016. Variance between control and experimental plants was calculated using t. Test. Comparison of the data done using Pearson's correlation coefficients. All determinations done in triplicate and averaged. The confidence limits used in this study were based on 95% ($P < 0.05$).

Results

Growth Parameters

Root length

The root length of all controlled plants was recorded less as compared with all the experimental plants. The first set of experiment where controlled plants inoculated with *Pythium aphanidermatum* after growth for 45 days. An experimental set initially inoculated with of *Acaulospora dilatata*, *Glomus mosseae*, and *Scutellospora fulgida* at the time of growing and *Pythium aphanidermatum* inoculated after 45 days of growth showed more growth of roots as compared with controlled plants. Table 1.

Table 1: Effect of *Acaulospora dilatata, Glomus mosseae*, and *Scutellospora fulgida* and *Pythium aphanidermatum* on root length, shoot length, number and surface area of leaves, germination percentage and Growth Vigour Index (GVI) of Turmeric.

Growth parameter	Set 1		Set 2		Set 3	
	Control	Experimental	Control	Experimental	Control	Experimental
Root length cm	24.06 ± 0.8	31.26 ± 0.9***	24.83 ± 0.6	27.66 ± 0.9***	23.76 ± 0.4	28.56 ± 0.2**
Shoot length cm	89.86 ± 0.8	97.83 ± 0.4***	84.63 ± 0.7	90.76 ± 0.9***	86.43 ± 0.6	95.90 ± 0.3**
No. of leaves	9.66 ± 0.09	11.66 ± 0.11***	9.33 ± 0.11	11.66 ± 0.11***	8.66 ± 0.01	12.00 ± 0.01***
Leaf surface area cm^2	2085.3 ± 59.2	2229.33 ± 33.4***	2101.67 ± 47.8	2264,66 ± 38.9***	2113.33 ± 53.1	2374.66 ± 19.1***
Germination %	100	100	100	100	100	100
GVI	9010.06	9814.26	8487.83	9103.66	8666.76	9618.56

Results are given as mean ± SD. t test with significant differences (P < 0.05) between means are indicated by different * signs. (** significant at <0.001 level and *** significant at <0.0001 level)

Shoot length

Similar trend was recorded for the shoot length as in case of root length. This might be due to differences in the root length. More root length has increased area of absorption of nutrients and more length supported more colonization of AM fungi in root cortex which might have facilitated more mobilization of immobile plant nutrients and due to these experimental plants have showed growth enhancement. Highest shoot length was recorded in experimental plants of first set of experiment. Highest difference in shoot length was recorded in third set of experiment where both control and experimental plants were inoculated after 45 days of growth, Table 1.

Number of leaves

The average number of leaves were ranging between 8.66 to 12. There were a greater number of leaves among experimental plants of all sets as compared to control plants. Most difference in the leaves was recorded in third set of experiment. This might be because of infection of P. *aphanidermatum* with turmeric plants after 45 days of growth, Table 1.

Leaf Surface Area

Surface area of leaves were ranging between 2085.03 to 2374.66. There was significant difference between surface area of leaves in controlled and experimental plants in all the sets of experiments, Table 1.

Germination %

Germination percentage was recorded cent percent in all experimental as well as controlled plants Table 1.

Growth and Vigour Index (GVI)

GVI showed similar trends like root and shoot length. The growth and vigour Index are the measure dependent on root and shoot length along with germination percentage. Highest GVI was recorded in experimental plants of first set of experiment. Whereas it was lowest in controlled plants of second set where control plants were inoculated with P. *aphanidermatum* at the time of growing where control plants got infected right from the beginning of growth and fungus colonized the cortical zone which resulted in stunted growth, Table 1.

Fresh and Dry Weight of Pseudo Stem and Rhizome

Fresh wt.t of pseudo stem was recorded highest (104.96 g) in the experimental plants of first set of an experiment. Similarly, the dry weight was recorded highest (6.66 g) in the experimental plants of first set of experiment. Whereas, the fresh wt. and dry wt. of rhizome was highest in the set of experiment where the set of an experiment was dual inoculated with *Acaulospora dilatata*, *Glomus mosseae*, and *Scutellospora fulgida* after 45 days after growing along with *Pythium aphanidermatum*, Table 2.

Table 2: Effect of *Acaulospora dilatata*, *Glomus mosseae*, and *Scutellospora fulgida* and *Pythium aphanidermatum* on fresh wt. of pseudo stem, dry wt. of pseudo stem, fresh wt. of rhizome and dry wt. of Turmeric.

Growth parameter	Set 1		Set 2		Set 3	
	Control	Experimental	Control	Experimental	Control	Experimental
Fresh wt. Pseudo stem	82.76 ± 1.04	104.96 ± 1.48***	84.13 ± 0.53	97.36 ± 0.75***	83.66 ± 0.44	97.03 ± 0.46**
Dry wt. Pseudo stem	6.09 ± 0.03	6.66 ± 0.06***	6.12 ± 0.02	6.54 ± 0.04***	6.18 ± 0.02	6.59 ± 0.01**
Fresh wt. Rhizome	96.8 ± 0.37	108.5 ± 0.64***	95.5 ± 0.16	104.7 ± 0.48***	92.43 ± 0.57	109.4 ± 0.53***
Dry wt. Rhizome	5.12 ± 0.01	5.49 ± 0.01***	5.08 ± 0.04	5.85 ± 0.01***	5.07 ± 0.02	5.95 ± 0.01***

Results are given as mean ± SD. t test with significant differences (P < 0.05) between means are indicated by different * signs. (** significant at <0.001 level and *** significant at <0.0001 level)

Chlorophyll content

Chlorophyll a was recorded highest (1.05 mg) in the first and third set of experimental plants. Amount of chlorophyll b was also recorded more in experimental plants than control plants and it was 1.32 mg/g. Total chlorophyll content was recorded maximum in the third set of an experiment, Table 3.

Table 3: Effect of *Acaulospora dilatata*, *Glomus mosseae*, and *Scutellospora fulgida* and *Pythium aphanidermatum* on chlorophyll a, b and total chlorophyll content in Turmeric.

	Chlorophyll a (mg/g)		Chlorophyll b (mg/g)		Total Chlorophylls (mg/g)	
	Control	Experimental	Control	Experimental	Control	Experimental
Set 1	0.92 ± 0.008	1.05 ± 0.03***	1.12 ± 0.008	1.32 ± 0.008*	2.03 ± 0.01	2.36 ± 0.03**
Set 2	0.73 ± 0.02	0.94 ± 0.02***	1.00 ± 0.004	0.97 ± 0.008***	1.74 ± 0.01	1.90 ± 0.02***
Set 3	0.92 ± 0.01	1.05 ± 0.02***	1.12 ± 0.01	1.32 ± 0.01***	2.05 ± 0.002	2.38 ± 0.01***

Results are given as mean ± SD. t test with significant differences (P < 0.05) between means are indicated by different * signs. (*significant at <0.05 level, ** significant at <0.001 level, *** significant at <0.0001 level)

Protein Content

Amount of protein were more among control plants in comparison with experimental plants. It was recorded highest in first set of experiment. And it was recorded lowest in experimental plants of third set of experimental plants, Table 4.

Table 4 Effect of *Acaulospora dilatata*, *Glomus mosseae*, and *Scutellospora fulgida* and *Pythium aphanidermatum* on chlorophyll a, b and total chlorophyll content in Turmeric.

Results are given as mean ± SD. t test with significant differences (P < 0.05) between means are indicated by different * signs. (*significant at <0.05 level, ** significant at <0.001 level, *** significant at <0.0001 level)

Total Soluble Carbohydrates Content

Unlike protein content the total soluble carbohydrate contents were more in experimental plants and it was recorded highest (177.33 mg) in experimental plants of third set of an experiment. Table 4.

Table 4: Effect of *Acaulospora dilatata*, *Glomus mosseae*, and *Scutellospora fulgida* and *Pythium aphanidermatum* on chlorophyll a, b and total chlorophyll content in Turmeric.

Growth parameter	Set 1		Set 2		Set 3	
	Control	Experimental	Control	Experimental	Control	Experimental
Protein	1.18 ± 0.01	1.09 ± 0.01***	1.17 ± 0.01	1.11 ± 0.02***	1.15 ± 0.01	1.11 ± 0.02**
Total soluble carbohydrates	159.66 ± 1.24	176.66 ± 1. 69***	144.33 ± 2.05	155.66 ± 1. 24***	165.00 ± 3.26	177.33 ± 2. 49**

Discussion

Growth Parameters

Root Length

Growth enhancement of root might be due of association of AM fungi right from the beginning of the growth. The colonization of AM fungi with the roots of Turmeric might have occupied entire cortex so that *Pythium aphanidermatum* did not get place for infection in cortical zone. Similarly, AM fungi also has antagonistic effect over other soil born fungi 17, which has positive effect on growth parameters. Parallel findings also noted by 18, 19. There was substantial difference between the growth between controlled and experimental plants. Pearson's correlation test showed positive correlation of addition of AM fungi and root length. Table 1.

Shoot length

Improved shoot length had positive effect of improved root length. More root length has increased area of absorption of nutrients and more length supported more colonization of AM fungi in root cortex which might have facilitated more mobilization of immobile plant nutrients and due to these experimental plants have showed growth enhancement. Highest shoot length was noted in experimental plants of first set of experiment. Highest difference in shoot length was recorded in third set of experiment where both control and experimental plants were inoculated after 45 days of growth. Similar results were obtained by[20,21] and[22] All the results were statistically significant and Pearson's correlation test showed positive correlation of addition of AM fungi and increased shoot length

Number of leaves

This might be because of infection of P. *aphanidermatum* with turmeric plants after 45 days of growth. As these plants have got more time for growth and after their proper growth there was fungal inoculation which least affected the plants of the third sets. Inoculation AM fungi mobilize soil nutrients and make it readily available for the plant roots. This resulted in vigorous growth of plants and thus there was increased number of leaves. Similar results were recorded by[23,24] and.[25]

Leaf Surface Area

Vigorous growth due to amendment of AM resulted increased leaf number as well as increased surface area. Accumulation of more nutrients due to presence of AM fungi had more absorption of soil nutrients which ultimately had more efficacy with respect to increased surface area of leaves. Our findings are corroborating with[26, 27] and.[28]

Germination %

Germination percentage was recorded cent percent in all experimental as well as controlled plants because all the plants were grown by vegetative method using pieces of rhizome.

Growth and Vigour Index (GVI)

The growth and vigour Index are the measure dependent on root and shoot length along with germination percentage. Highest GVI was recorded in experimental plants of first set of experiment. Whereas it was lowest in controlled plants of second set where control plants were inoculated with P. *aphanidermatum* at the time of growing where control plants got infected right from the beginning of growth and fungus colonized the cortical zone which resulted in stunted growth. It was significantly different in the same set, in experimental plants because dual inoculation resulted in anticipated colonization of AM fungi before P. *aphanidermatum* and resulted in more GVI.

Fresh and Dry Weight of Pseudo Stem and Rhizome

Fresh wt. as well as dry wt. of pseudo stem was recorded highest in all the experimental plants of first set of an experiment. There was overall positive effect of association of AM fungi on all the experimental plants.

Chlorophyll Content

Chlorophyll a, b and total chlorophylls was more in all experimental plants than control because of inoculation of AM fungi, which helped the plant roots to absorb more soil nutrients and this resulted in vigorous growth of experimental plants. The vigorous growth of experimental plants is one of the factors which increased surface area and other growth parameters of the experimental plants which resulted in more chlorophyll content. Similar results were obtained by[29] in maize plants,[30] in Ocimum plant. t test for Chlorophyll a, b and total chlorophylls contents in all experimental plants were found significant at $P < 0.05$ level. Pearson's correlation test showed positive correlation of chlorophyll content in experimental plants, Table 3.

Protein Content

Amount of protein in all experimental plants have utilized protein so that protein content was more in control plants as compared with experimental plants. Among experimental plants the protein content were highest in second and third set of an experiment. This is the first report on protein content in turmeric with respect to Am fungi.

Total Soluble Carbohydrates Content

The increased content of total soluble carbohydrates among experimental plants than all control plants might be due to the reason that plants make carbohydrates available to associated AM fungi in their symbiotic association. Similarly, these results obtained also reported very first time with respect to Am fungi in Turmeric plants.

Conclusions

Addition of mycorrhizal fungal spores at the time of growing Turmeric plants leads to establish their colonization in early-stage of growth. This in fact does not allow to establish relationship of soil born fungi with the rhizomes as mycorrhizal fungi are already takes the place of cortex and start the mutually beneficial association with plant roots. This research work is an outcome which is highly beneficial for the growth of Turmeric plants and similar research work can be carried out with respect to rhizomatous and cormatous plants.

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Conflicts of Interest

The undertake that no financial or intellectual conflicts of interest associated with this research work.

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