RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

Tomato fruits are rich in antioxidant compounds that have been recognized to be beneficial for human health. Tomatoes are rich in nutrients, especially potassium, folic acid and vitamin C and contain a mixture of different carotenoids, including vitamin A, β-carotene as well as lycopene (Ulrichs *et al.*, 2008).

A number of diseases have been reported from nursery stage to maturity in tomatoes of which the damping-off disease is the most common during the nursery stage (Dar *et al.*, 2012). Among the fungal diseases, damping-off caused by *Pythium* species causes more than 60 percent mortality of seedlings both in the nursery and the main field (Muthukumar *et al.*, 2011).

Fluorescent pseudomonads comprise an important group of bacteria used for biological control of micro fungi of the plant rhizosphere and play an important role in the suppression of fungal diseases. Plants develop an enhanced defensive capacity against a broad spectrum of plant pathogens after colonization of the roots by selected strains of non-pathogenic fluorescent Pseudomonas spp. (Amara *et al.*, 2009).

In the present study, the selected tomato seedlings were subjected to four treatments (with talc based formulation of only bacterial isolate AUPF8, talc based formulation of bacterial isolate AUPF8 challenge inoculated with *Pythium aphanidermatum*, talc powder alone and *Pythium* alone) and then allowed to grow. The defense - related compounds, namely, phenylalanine ammonia lyase, peroxidase, polyphenol oxidase and total phenols, growth hormones such as indole acetic acid and gibberellic acid and biometric parameters like root length, shoot length and chlorophyll content were studied in the plantlets on the 0^{th}, 3^{rd}, 5^{th} and 8^{th} days of treatment. The results obtained from the above are discussed under the following heads:

4.1. Defense – related compounds

4.1.1. Phenylalanine ammonia lyase

4.1.2. Peroxidase

4.1.3. Polyphenol oxidase

4.1.4. Total phenols

4.1.5. Analysis of Native polyacrylamide gel electrophoretogram

4.2. Growth hormones

4.2.1. Indole acetic acid

4.2.2. Gibberellic acid

4.3. Biometric parameters

4.3.1. Root length

4.3.2. Shoot length

4.3.3. Chlorophyll content

4.1. Defense - Related Compounds

The *Pseudomonas fluorescens* $AUPF_8$ is known to induce defense mechanisms in plant systems such as production of defense - related compounds, namely, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and total phenols. These parameters are generally elevated during infection in order to resist the pathogens.

4.1.1. Phenylalanine ammonia lyase

Phenylalanine ammonia lyase (PAL) has been extensively studied because of its role in plant development and its response to a wide variety of environmental stimuli. The importance of this enzyme in plant metabolism is demonstrated by the huge diversity and large quantities of phenylpropanoid products found in plant materials (Hyun *et al.*, 2011).

Table 1 and Figure 5 record the phenylalanine ammonia lyase activity in *Pseudomonas fluorescens* (AUPF8) treated tomato plants on different days of treatment.

Table 1

Phenylalanine ammonia lyase activity in *Pseudomonas fluorescens* (AUPF8) treated tomato plants

S.No.	Treatment	Phenylalanine ammonia lyase activity (nmol transcinnamic acid / min/ g of tissue)			
		0th day	3rd day	5th day	8th day
1.	AUPf8 + *Pythium aphanidermatum* (T1)	39.70 ai	42.20 ai	45.40 aj	49.36 aj
2.	AUPf8 (T2)	37.24 bi	40.48 abj	43.48 bk	44.31 bl
3.	Talc powder (T3)	38.52 abi	39.51 bcj	41.73 bck	43.22 bi
4.	*Pythium* only (T4)	34.44 ci	37.74 cj	40.35 cdk	33.60 dk
5.	Control	33.26 ci	35.63 dj	39.53 dk	40.55 ck
CD ($p<0.05$) = 1.88					

Means followed by a common letter are not significantly different at the 5 % level by DMRT.

From the table and figure, it can be said that the phenylalanine ammonia lyase activity is significantly higher ($p<0.05$) in T1 (39.70 units) on the 0th day when compared to the other samples. This was followed by T3 (38.52).

On the 3rd day, the level of phenylalanine ammonia lyase was the maximum in T1 (42.20 units) which was significantly higher ($p<0.05$) than the other samples. This was followed by T2 (40.48 units).

The enzyme activity on the 5th day showed a similar pattern of change with the T1 sample recording significantly ($p<0.05$) the highest value (45.40 units) followed by the T2 sample (43.48 units).

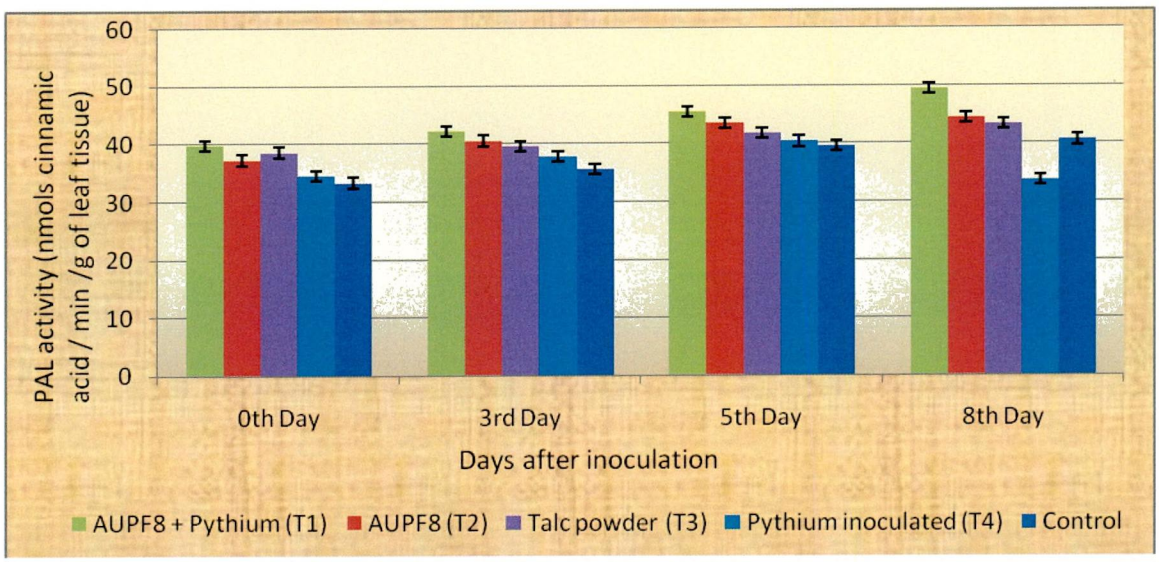
On the 8th day of treatment, the T1 sample exhibited significantly ($p<0.05$) the highest enzyme activity (49.36 units) on comparison with the other samples. Here again, this was followed by the T2 and T3 samples which recorded similar values (44.31 and 43.22 units respectively).

It can also be noticed from the table and figure that T1 sample showed a significantly ($p<0.05$) gradual increase in phenylalanine ammonia lyase activity from the 0^{th} to the 8^{th} day of treatment.

Hence, from the above observations, it can be stated that the best treatment for the tomato seedlings studied is T1 (AUPF8 + *Pythium aphanidrmatum*) since it induced the maximum production of the defense – related enzyme phenylalanine ammonia lyase from the 0^{th} day upto the 8^{th} day of treatment.

Figure 5

Phenylalanine ammonia lyase activity in *Pseudomonas fluorescens* (AUPF8) treated tomato plants



The above findings are supported by the study of Manonmani *et al.*, (2009), who stated that PAL induction was lower in *Xanthomonas axonopodispv. citri* (Xac) infected leaf tissues than in the healthy leaves due to pathogen challenge. Similiarly, Liang *et al.*, (2011), reported the levels of PAL activity in the roots of cucumber seedlings to display a wave-like induction pattern after treatment with *Pythium* + *Bacillus megatricum* and showed two peaks on the 5^{th} and 11^{th} days respectively after treatment. According to El-Beltagi *et al.*, (2012), phenylalanine ammonia lyase activity was increased

with the treatment of tomato plant with biofertilizers when compared to nematode infected plants and healthy tomato plants. Christopher *et al.*, (2010), reported that the PAL activity was significantly increased in plants treated with *T. virens* followed by challenge inoculation with *F.oxysporum* f.sp. *Lycopersici*, on comparison with other treatments with the PAL induction reaching its maximum on the 9th day of germination and thereafter decreasing. Generally enzyme activities gradually increased upto the 9th day from germination and thereafter declines gradually in all the treatments.

Hence, it can be concluded from the Table 1 and Figure 5 that treatment of *Pythium* infected tomato plants with T1 recorded maximum activity of Phenylalanine ammonia lyase activity than other treatment groups.

4.1.1.2. Peroxidase

Peroxidase enzymes participate in hormone catabolism, phenol oxidation, polysaccharide and cell wall protein intercrossing, lignin polymerization, fruit ripening and defense against pathogens. During fruit ripening and particularly during climacterium, peroxidase activity increases alongwith polygalacturonase and cellulose enzymes (Ortiz *et al.*, 2007).

Table 2 and Figure 6 represents the activity of peroxidase enzymes in *Pseudomonas fluorescens* (AUPF8) treated tomato plants on different days of treatment.

Table 2

Peroxidase activity in *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants

S.No.	Treatment	Peroxidase activity (change in absorbance / min / g of tissue)			
		0th day	**3rd day**	**5th day**	**8th day**
1.	AUPf8 (T1)	0.072 ai	0.084 bj	0.094 ak	0.098 bk
2.	AUPf8 + *Pythium aphanidermatum* (T2)	0.074 ai	0.089 aj	0.096 ak	0.118 al
3.	Talc powder (T3)	0.066 bi	0.072 dj	0.081 bk	0.080 ck
4.	*Pythium* only (T4)	0.053 ci	0.054 ei	0.068 cj	0.053 cj
5.	Control	0.075 ai	0.078 ci	0.081 bj	0.083 cj
CD ($p<0.05$) = 0.004					

Means followed by a common letter are not significantly different at the 5 % level by DMRT.

From the table and figure, it can be inferrred that the levels of the enzyme were not significant in all the samples on the 0th day.

However, on the 3rd day of treatment, the enzyme activity showed significantly ($p<0.05$) the highest value (0.089 units) for T1 followed by T2 (0.084 units).

On the 5th day of treatment, T1 exhibited the highest peroxidase activity (0.096 units) followed by T2 (0.094 units) which were significant ($p<0.05$) on comparison with the other samples.

A similar trend was recorded on the 8th day of treatment also were T1 and T2 depicted the highest activity of the enzyme (0.118 and 0.098 units respectively) when compared to the other samples.

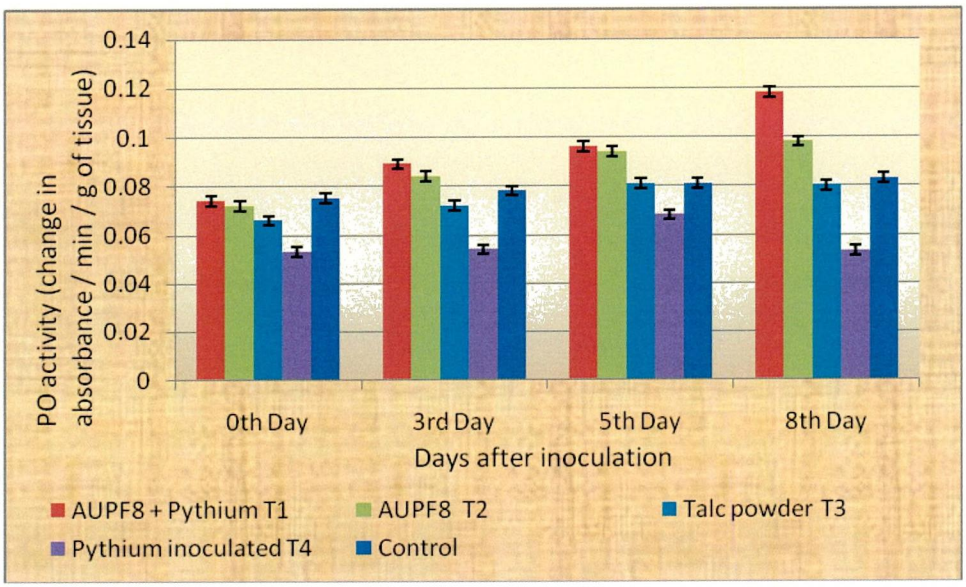
It can be also be observed from the table and figure that T4 (*Pythium* inoculated plants) recorded the least values for the enzyme on all the days since these plants were infected.

It can also be observed from the table and figure that T1 sample showed a significantly ($p<0.05$) gradual increase in peroxidase activity from the 0^{th} to the 8^{th} day of treatment.

Thus, it can be inferred from Table 2 and Figure 6 that T1 is the best treatment for *Pythium* infected tomato plants since they induced the maximum production of the defense- related enzyme peroxidase.

Figure 6

Peroxidase activity in *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants



The above findings are in accordance with the reports of Ojha and Chaterjee (2012) who reported that there was a gradual increase in the activity of peroxidase from the 7th day upto the 28th day in tomato plants infected with *F. oxysporum* and treated with salicylic acid and this increase was due to the defense related compound salicylic acid.

According to Bhagat and Chakraborty (2010) also, the peroxidase activity was increased in all the tested varieties of tea which were treated with the plant growth promoting rhizobacteria *Sclerotium rolfsii* with the highest value exhibited by the HV-39 variety of the tea plant.

4.1.1.3. Polyphenol oxidase

Plant Polyphenol oxidases (PPOs) are ubiquitous plastid-localized enzymes. A precise analysis of PPO function in plants has been complicated by the presence of several family members with immunological cross reactivity (Newman *et al.*, 2011). In addition, systemic induction of PPO expression in response to wounding and pathogens might provide an additional line of defense to protect plants against further attack by pathogens and insects (Raju *et al.*, 2008).

Table 3 and Figure 7 depict the activity of polyphenol oxidase in *Pseudomonas fluorescens* (AUPF8) treated tomato plants on different days of treatment.

Table 3

Polyphenol Oxidase Activity in *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants

S.No.	Treatment	PPO activity (change in absorbance / min / g of tissue)			
		0th day	**3rd day**	**5th day**	**8th day**
1.	AUPf8 (T1)	0.72 ci	0.82 cj	1.21 bk	1.33 al
2.	AUPf8 + *Pythium aphanidermatum* (T2)	0.82 ai	0.92 aj	1.32 ak	1.34 ak
3.	Talc powder (T3)	0.82 ai	0.87 bj	0.91 dk	1.07 bl
4.	*Pythium* only (T4)	0.86 ai	0.90 abi	0.97 cj	0.85 di
5.	Control	0.77 bi	0.82 cj	0.86 ej	0.92 ck
CD ($p<0.05$) = 0.04					

Means followed by a common letter are not significantly different at the 5 % level by DMRT.

It is clear from the table and figure that on the 3rd day of treatment, T1 showed the highest activity of polyphenol oxidase (0.92 units) which was significant ($p<0.05$) compared to the other samples.

On the 5th day of treatment, T1 recorded the highest value (1.32 units) followed by T2 (1.21 units) both of which were significant ($p<0.05$) when compared to the other samples.

A similar trend was followed on the 8th day also when T1 showed the highest value (1.34 units) for polyphenol oxidase which was significant ($p<0.05$).

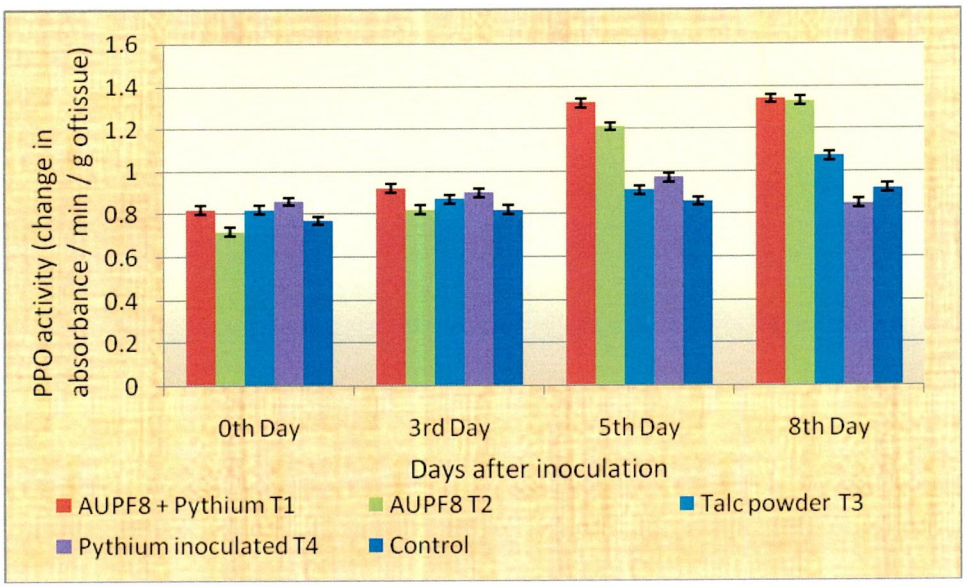
It can also be noticed from the table that though the T4 (*Pythium* inoculated) sample recorded the highest value on the 0th day (0.86 units) when compared to the other samples, this value decreased to the minimum by the 8th day.

It can also obvious from the table and figure that T1 sample showed a significantly ($p<0.05$) gradual increase in polyphenol oxidase activity from the 0th to the 8th day of treatment.

Here again, it can be concluded that T1 is the best treatment for *Pythium* infected tomato plants since it induced maximum production of the enzyme polyphenol oxidase.

Figure 7

Polyphenol oxidase activity in *Pseudomonas fluorescens* (AUPF8) treated tomato plants



The above findings are in agreement with the report of Raju *et al.*, (2008), who mentioned that the PPO over expressing transgenic tomato plants exhibited high resistance to *Pseudomonas syringae*, the causative agent of speck disease compared with control plants. Localized inoculation of tomato leaflets with *P. syringae* induced a significant increase in PPO activity and led to systemic resistance to the subsequent infection by *P. syringae*. Ardebili *et al.*, (2011), stated that, inoculation of tomato plant with *Fusarium oxysporum* f.sp. lycopersici and *Pseudomonas fluorescens* species CHA0, showed a significant increase in the PPO activity.

4.1.1.4. Total phenols

Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contribution to plants colours. They are ubiquitous in all plant organs and are therefore an integral part of the human diet (Dai and Mumper, 2010)

Table 4 and Figure 8 represent the Total phenol content in *Pseudomonas fluorescens* (AUPF8) treated tomato plants on the different days of treatment.

Table 4

Total Phenol Content in *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants

S.No.	Treatment	Total phenol content (µg of catechol / min / g of tissue)			
		0th day	3rd day	5th day	8th day
1.	AUPf8 (T1)	32.57 di	37.03 cj	45.83 bk	50.07 bk
2.	AUPf8 + *Pythium aphanidermatum* (T2)	47.15 ai	49.62 aij	52.39 aj	60.62 ak
3.	Talc powder (T3)	40.50 bi	43.05 bi	44.52 bi	50.68 bj
4.	*Pythium* only (T4)	38.15 bci	38.30 bci	44.60 bj	38.82 ci
5.	Control	34.87 cdi	42.46 bj	49.44 abk	50.35 bk
CD ($p<0.05$) = 4.65					

Means followed by a common letter are not significantly different at the 5 % level by DMRT.

It is clearly understood from the table and figure that the total phenol content of T1 recorded significantly ($p<0.05$) the maximum value (47.15 units) on the 0^{th} day when compared to the other samples.

In the case of the 3^{rd} day samples, T1 marked significantly ($p<0.05$) the highest content of phenols (49.62 units) on comparison with the other samples.

On the 5^{th} day of treatment, the T1 sample recorded significantly ($p<0.05$) the maximum total phenol content (52.39 units) followed by T2 (45.83 units) when compared to the other samples.

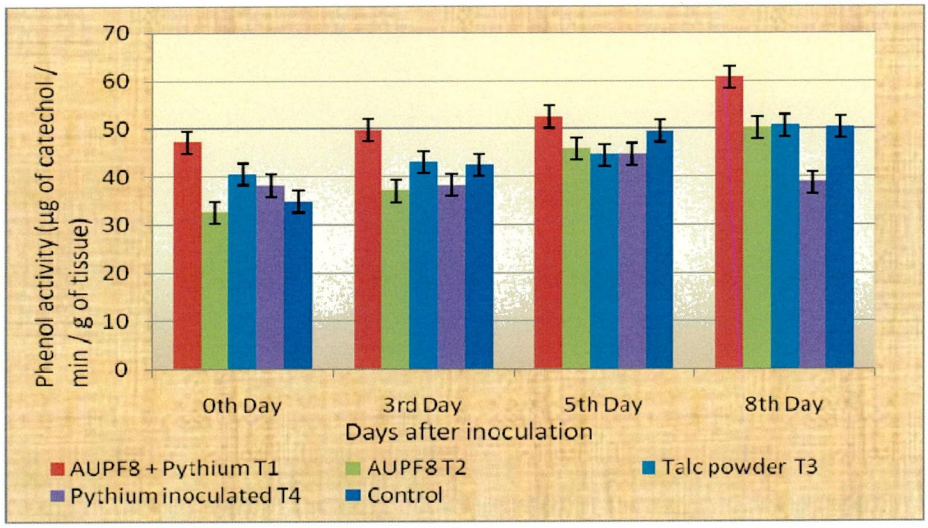
The 8^{th} day of treatment showed the T1 sample to have the highest phenol content (60.62 units) the value of which was significant ($p<0.05$).

It can also clear from the table and figure that T1 sample showed a significantly ($p<0.05$) gradual increase in total phenol content from the 0^{th} to the 8^{th} day of treatment.

Thus, from the above findings, it can be stated that T1 is the best treatment for *Pythium* infected tomato plants as it increases the level of defense related compounds like total phenols.

Figure 8

Total Phenol content in *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants



The above mentioned results are similar to those reported by other researchers. Umasankari and Sekar, (2011) stated the induction of phenolic

content of rice plant due to *Pseudomonas* inoculation and challenge inoculation with *P.oryzae*. Raju *et al.*, (2008), reported a higher phenolic accumulation in tomato plants treated with salicylic acid + pathogen. The levels of phenolics increased by 1.3, 1.4 and 1.5 folds in roots and 1.3, 1.4, and 1.7 fold in shoots for a period of 10 days in response to the said chemical treatment. In another study, Bhagat and Chakraborty (2010) reported that there was a greater accumulation of orthodihydroxy phenols in tea plants inoculated with *S.rolfsii*.

4.1.1.5. Native PAGE Analysis

Plate 1 reveals the banding pattern of peroxidase in *Pseudomonas fluorescens* (AUPF8) treated tomato plants on native – PAGE.

From the plate, it is clearly understood that single bands were obtained for T3, T4 and control, two bands for T2 and three bands for T1. This showed that the bands for T2 were that of peroxidase isoforms 1and 2 and for T1 peroxidase isoforms 1, 2 and 3. This also shows that there was maximum induction of peroxidase in T1 plants (AUPF8 + *Pythium aphanidermatum*) followed by T2 plants (AUPF8 only).

Thus it can be concluded from the above that the treatment group T1 was the best among the other treatment groups since it revealed more number of the isoforms of peroxidase on native-PAGE.

The above findings are in accordance with the findings of other research workers. Anita and Samiyappan, (2012) reported that the Native PAGE analysis of peroxidase revealed 5 isoforms designated as PO1 to PO5 in *P.fluorescens* treated root tissues challenged with the root-knot nematode *M. graminicola* and four isoforms PO2, PO3, PO4 and PO5 in bacterized plants challenge inoculated with the nematode compared to other treatments and two isoforms PO4 and PO5 in healthy plants. Sarwar *et al.*, (2011) stated that, though Native PAGE analysis of chickpea plants both after induction treatment and the control showed the peroxidase to have 3 isozymes, the activity of peroxidase was significantly increased in chickpea plants after induction treatments when compared to control.

Plate 1
Peroxidase Isoforms in *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants

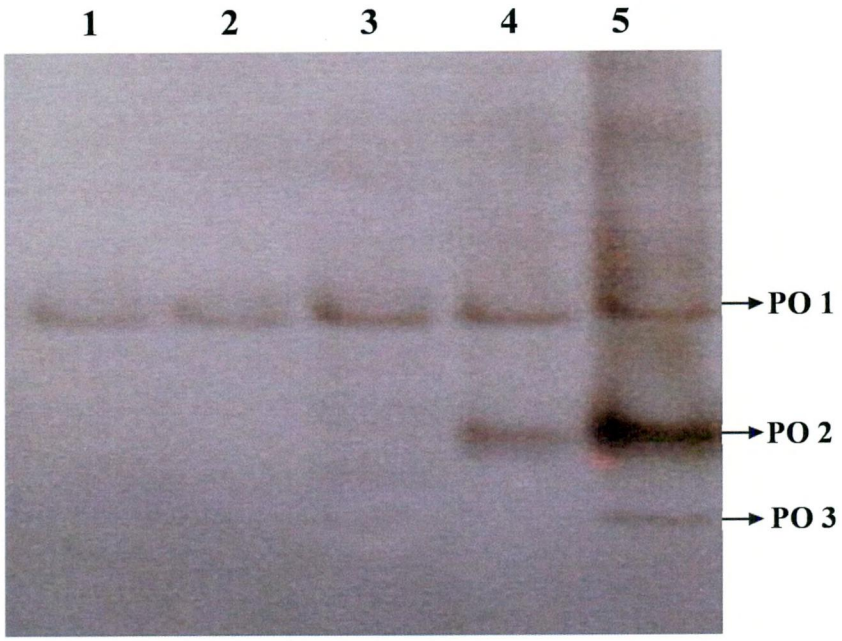
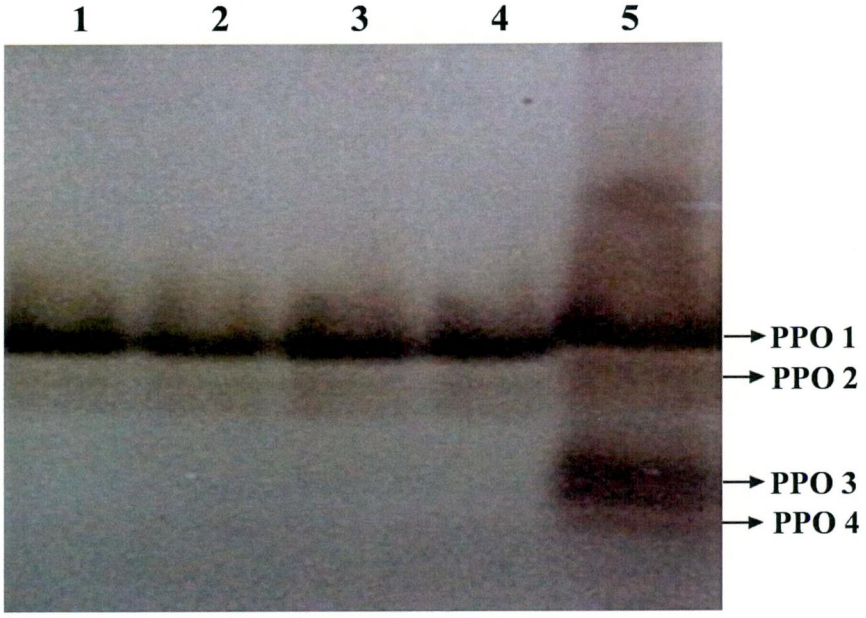


Plate 2
Polyphenol oxidse isoforms in *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants



Lane 1 – Control; Lane 2 – Pythium inoculated (T4)
Lane 3 – Talc powder (T3); Lane 4 – AUPF8 inoculated (T2)
Lane 5 – AUPF 8 challenged with Pythium (T1)

Plate 2 records the native PAGE analysis of polyphenol oxidase in *Pseudomonas fluorescens* (AUPF8) treated tomato plants.

From the plate, it can be seen that single bands of polyphenol oxidase were present in control, T2, T3 and T4 samples, while in T1, four prominent bands were present indicating the higher induction of polyphenol oxidase. The four bands were that PPO 1, PPO 2, PPO 3 and PPO 4.

Thus it can be deduced from the above observations that of the various treatments, T1 (AUPF8 + *Pythium aphanidermatum*) was the best as it produced more number of polyphenol oxidase isoforms in native- PAGE.

The above data agrees with that of Anita and Samiyappan (2012), who revealed that the five PPO isoforms, PPO1, PPO2, PPO3, PPO4 and PPO5 were observed in bacterized rice root tissues inoculated with a root-knot nematode. The induction of isoforms PPO1and PPO2 were observed in all the treatments except in healthy plants. Isoforms, PPO3 and PPO4 were detected both in bacterized root tissues and root knot infected roots but it was more prominent in root tissues treated with *P. fluorescens* and challenge inoculated with the nematode.

4.2. Analysis of Growth hormones

The exogenous application of growth hormones has an effect on the bio-productivity, growth, photosynthesis, water relations and various enzyme activities in plants and also on those plants that are exposed to various biotic and abiotic stresses (Ahemed *et al.*, 2012).

The plant growth hormones, namely, Cytokinins (CKs) and Gibberellins (GAs) are found in actively dividing tissues of seeds. They are important in breaking dormancy and allowing germination and growth of dormant embryoes (Bakrim *et al.*, 2007).

4.2.1. Indole Acetic Acid (IAA)

Indole-3-Acetic Acid (IAA) at the lowest concentration stimulates stem elongation as well as root numbers in plants. It is an essential factor for growth

of floral stems and shoot cuttings treated with IAA form more roots (Khan *et al.*, 2011).

Biosynthesis of IAA is not limited to higher plants. Organisms such as bacteria, fungi and algae are also able to make physiologically active IAA that may have pronounced effects on plant growth and development. Many bacteria isolated from the rhizosphere have the capacity to synthesize IAA *in vitro* in the presence or absence of physiological precursors mainly tryptophan (Shahab *et al.*, 2009).

Table 5 and Figure 9 record the levels of Indole acetic acid in *Pseudomonas fluorescens* treated tomato plants on the 8th day of treatment.

From Table 5 and Figure 9 it is evident that the Indole acetic acid level is significantly ($p<0.05$) the highest (64.74 units) in T1 as compared to the other samples. The level of indole acetic acid in T4 recorded the least value (22.73 units) which was significantly ($p<0.05$) the lowest since these plants were infected with damping off disease caused by *Pythium*.

Table 5

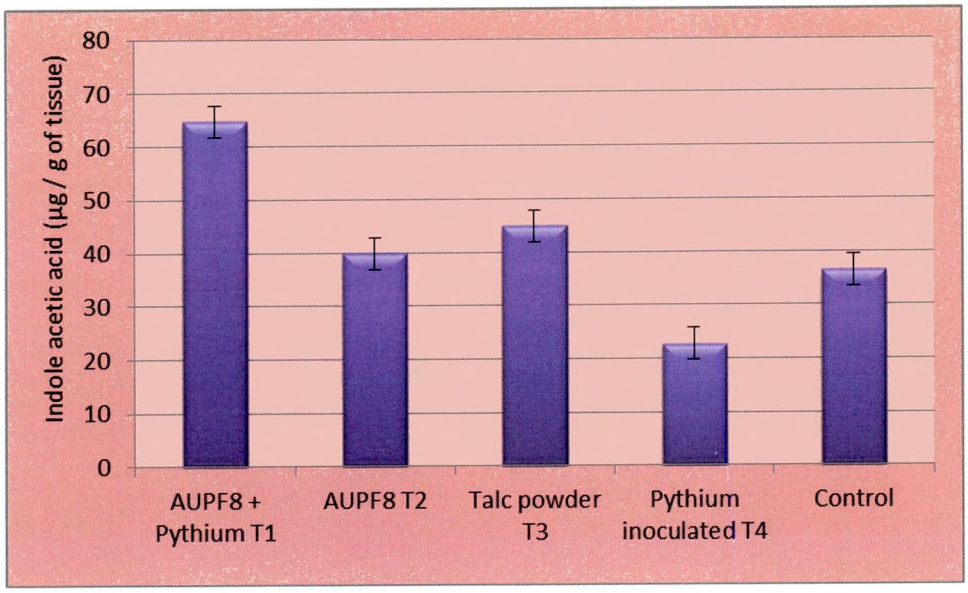
Indole Acetic Acid Level in *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants

S.No	Treatments	Indole acetic acid (µg /g of tissue)
1.	AUPF8 + *Pythium*	64.74 d
2.	AUPF8 alone	39.85 c
3.	Talc powder alone	41.81 c
4.	*Pythium* alone	22.73 a
5.	Control	36.44 b
	CD ($p<0.05$) = 3.02	

Means followed by common letters are not significantly different at 5 % level by DMRT

Figure 9

Indole Acetic Acid Level in *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants



The *Pythium* inoculated (T4) showed lowest production of indole acetic acid among the other treatment groups.

The above studies are on parr with the works of Babu *et al.*, (2012) who reported that the endogenous bioactive IAA content in tomato plants significantly increased with application of sodium chloride. This suggests that the growth and development of tomato plants under salt stress is supported by increased production of IAA. Shahab *et al.*, (2009) also observed that induction of longer roots with increased number of root hairs and root laterals is a growth response attributed to IAA production by rhizobacteria.

4.2.2. Gibberellic acid

Gibberellic acid promotes synthesis of enzymes that convert stored nutrients like starch into sugars needed for rapid cell respiration and germination (Bakrim *et al.*, 2007).

Gibberellic acid is a naturally occurring plant hormone that is produced in higher quantities in warmer months. During the colder months, its production is low, Hence plant growth is slower. The idea behind the

application of the hormone is that it stimulates cell expansion resulting in leaf and stem elongation. (Bolto and Rohrlach, 2010).

Table 6 and Figure 10 represent the level of Gibberellic acid in *Pseudomonas fluorescens* (AUPF8) treated tomato plants on the 8th day of treatment.

Table 6

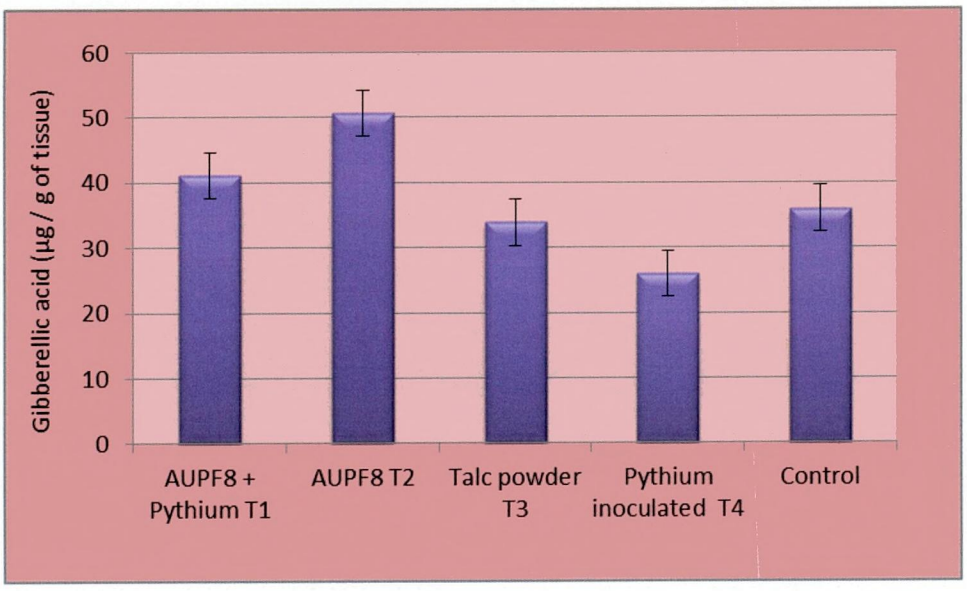
Gibberellic Acid Level in *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants

S.No	Treatments	Gibberellic acid (µg /g of tissue)
1.	AUPF8 + *Pythium*	50.64 d
2.	AUPF8 alone	41.10 c
3.	Talc powder alone	33.85 b
4.	*Pythium* alone	25.92 a
5.	Control	35.97 b
	CD (p<0.05) = 3.54	

Means followed by common letters are not significantly different at 5 % level by DMRT

Figure 10

Gibberellic Acid Level in *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants



It can be understood from the table and figure that the level of gibberellic acid is significantly ($p<0.05$) the highest in T1 (50.64 µg/g of tissue) followed by T2 (41.10 µg/g of tissue) in tomato plants when compared to the other samples. It can also be seen that T4 recorded the least value (25.92 µg/g of tissue) indicating a decrease in the hormone level due to infection by *Pythium.*

4.3. Analysis of Biometric Parameters

4.3.1. Root Length

Figure 11 depicts the root lengths of tomato seedlings of *Pseudomonas fluorescens* (AUPF8) treated tomato plants on different days of treatment.

From the figure, it is understood that the root length of tomato seedlings was the highest and the same in T1 and T2 plants on the 3rd day of treatment.

On the 5th day of treatment, the root length of T2 plants were higher than that of the T1 plants.

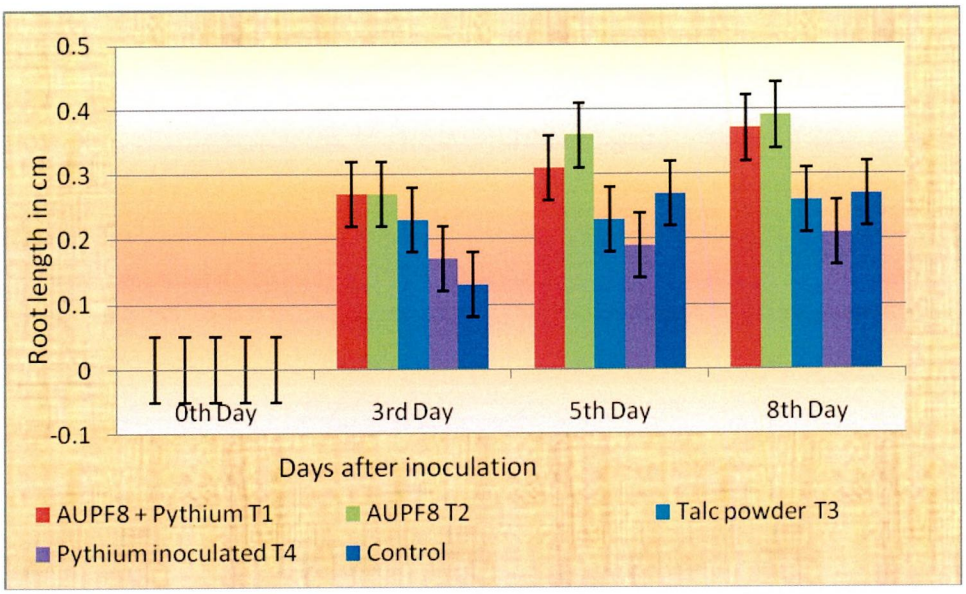
A similar trend was followed on the 8th day of treatment also with the highest root length registered by T2 followed by T1 plants.

Yet another observation of the figure indicates that with increase in the number of days of treatment the length of the roots increased.

The root lengths of the control and T4 (*Pythium* inoculated) plants were lesser when compared to the other plants.

Figure 11

Root Length of *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants



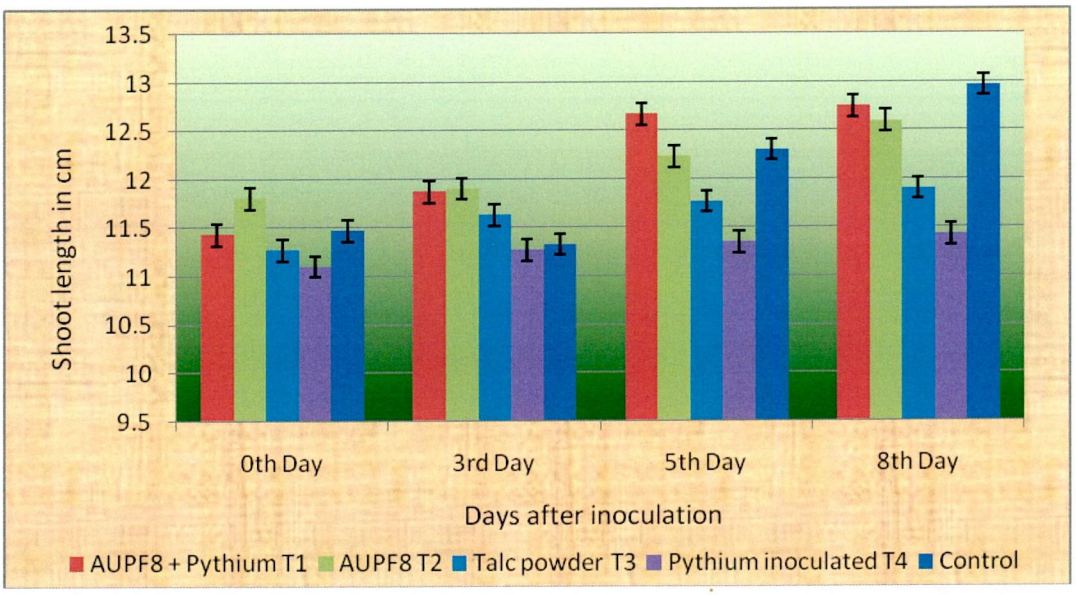
The above study is supported by the work of Ashrafuzzaman *et al.*, (2009) who reported that PGPR isolates significantly increased the root length of rice seedlings. The *Bacillus* isolate PGB4 produced the highest root length when compared to the other isolates.

4.3.2. Shoot Length

Figure 12 reveals that the shoot length of tomato seedlings of *Pseudomonas fluorescens* (AUPF8) treated tomato plants on different days of treatment.

Figure 12

Shoot Length *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants



From the figure it can be noted that on the 0th day of treatment, the shoot length of T2 tomato seedlings recorded significantly ($p<0.05$) the highest value followed by T1 tomato seedlings.

On the 3rd day of treatment, T1 and T2 showed a more or less similar shoot length which was significant when compared to the other samples.

The 5th day of treatment shows the T1 samples to have a higher shoot length followed by T2.

The above pattern was noticed on the 8th day of treatment also.

The shoot length of T4 plants did not show much of a variation from the 0th to the 8th day of treatment.

A similar work by Salaheddin *et al.*, (2010) also reported a maximum increase in root length and shoot length on treatment of cotton seeds with a mixture of Pf32, Pf93 and B49 bacterial isolates. Maisuria and Patel, (2009) recorded the shoot length to be the least in case of *P. aphanidermatum* infected plants and the highest in case of plants inoculated with *T. viride*.

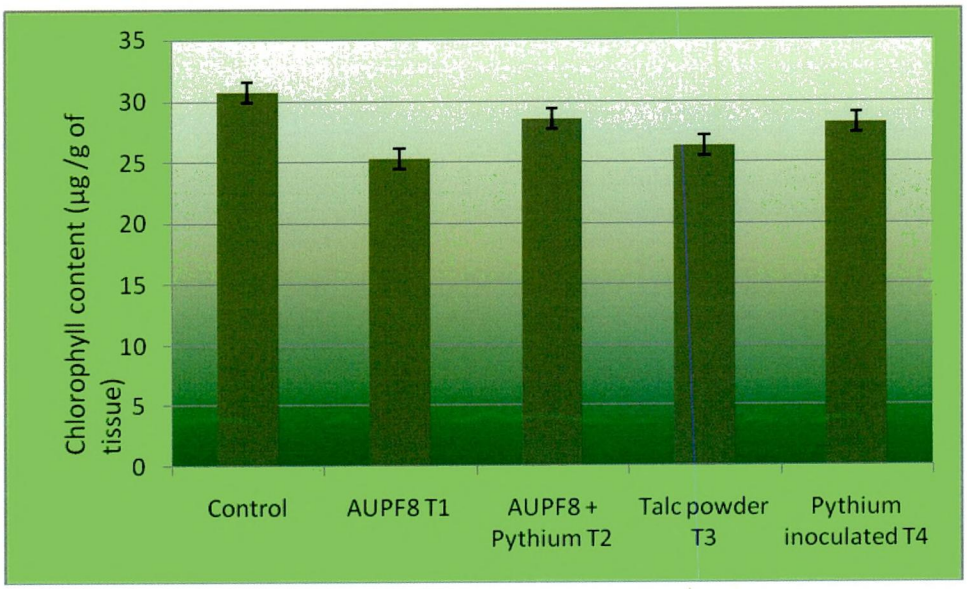
4.3.3. Chlorophyll content

Leaf chlorophyll content provides valuable information about the physiological status of plants. Usually, the first symptom produced by a shortage of any mineral element is a loss of chlorophyll (chlorosis), which results in an alteration of the chloroplast structure (Gladden *et al.*, 2012).

Figure 13 records the chlorophyll content of tomato seedlings treated with *P.fluroscens* (AUPF8) on the 8th day of treatment.

Figure 13

Total Chlorophyll Content of *Pseudomonas fluorescens* (AUPF8) Treated Tomato Plants



The results of Figure 13 reveal that among the treated samples, T1 recorded the highest content of chlorophyll, whereas, T4 recorded the least content of chlorophyll which may be due to infection of the plants by *Pythium.*

Amara *et al.*, (2009) reported that the chlorophyll content of plants increased when grown in soil treated with a mixture of *P. aeruginosa* and *P. putida*, Kavina *et al.*, (2011) also stated that treatment of Mentha plants with hormones like abcisic acid and Gibberellic acid significantly increased the total chlorophyll contents.

Highlights of the Study

- Significant increases in the amounts of defense related compounds like PAL,PPO,PO and total phenols in tomato plants infected with damping off disease (caused by *Pythium aphanidermatm)* and treated with the Plant growth promoting rhizobacteria *Pseudomonas fluorescens* (AUPF8).
- Significant increases in the levels of hormones like IAA and GA in tomato plants infected with damping off disease (caused by *Pythium aphanidermatm)* and treated with the Plant growth promoting rhizobacteria *Pseudomonas fluorescens* (AUPF8).
- Increase in biometric parameters like root length and shoot length in tomato plants infected with damping off disease (caused by *Pythium aphanidermatm)* and treated with the Plant growth promoting rhizobacteria *Pseudomonas fluorescens* (AUPF8).
- Plant growth promoting rhizobacteria *Pseudomonas fluorescens* (AUPF8) is thus effective in controlling damping off disease in tomato plants.