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Influence of Temperature, pH and Light on Growth and Sporulation of *Alternaria solani* (Ellis and Martin) Jones and Grout Causing Early Blight of Tomato (*Solanum lycopersicum* L.) under *in vitro* Condition

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A B S T R A C T

Effect of different temperature, pH levels, and light intensity were tested against the growth of *Alternaria solani* under *in vitro* conditions. Mycelium growth and sporulation of *Alternaria solani* was significantly influenced with temperature. Maximum colony growth was recorded at 25°C followed by 30°C and minimum colony growth was recorded at 40°C. Excellent sporulation was noticed at 20°C and 25°C temperature. However, no sporulation was found at 40°C temperature. Effect of different pH level on colony diameter, sporulation and mycelium dry weight of *Alternaria solani* on PDA medium reveal that, the maximum colony diameter, dry weight were observed at 6.5 pH level followed by 6.0 pH level, while minimum colony diameter and dry weight were observed at 8.5 pH. Excellent sporulation was observed at pH level of 6.0 to 7.0, while poor sporulation was recorded at 4.0, 8.0 and 8.5 pH level. Light also influenced the colony growth and sporulation of *Alternaria solani* on PDA medium. Maximum colony growth and excellent sporulation was noticed at 12 hrs dark and 12 hrs light, minimum colony growth was observed at 8 hrs dark and 16 hrs light, good sporulation at 24 hrs light and 24 hrs dark and poor sporulation was observed at 8 hrs dark and16 hrs light and 8 hrs light and 16 hrs dark.

Introduction

Tomato [*Solanum lycopersicum* L.] also known as Love apple, Tomate, Tomat, Tomatar, Rangam and Tomati in different parts of the world, and also popularly called as 'Poormen's orange'. It is a small annual or short-lived perennial herb belongs to the family *Solanaceae*, probably native of 'Peru-Equador'. Tomato is diploid plant with 2n =

24 chromosomes and grown extensively and marketed throughout the world. It ranks second largest vegetable crop after potato in the world. It is a traditional vegetable crop commercially cultivated with a large area and higher production and productivity in India.

Cultivated in an area of 4.73 million hectares all over the world with production of 163.96 million tonnes and an average yield of 34.66 tones ha-1. The major tomato producing states are Gujarat, Bihar, Karnataka, Uttar Pradesh, Orissa, Andhra Pradesh, Maharashtra, Madhya Pradesh, and West Bengal. In Karnataka, Madhya Pradesh and Chhattisgarh, accounting total production of 18732 thousand tonnes from an area of774 thousand hectares with an average productivity of 24.20 tonnes ha-1 during2015-16 (Anonymous, 2016).Andhra Pradesh ranked first in terms of area and production of tomato in India which occupying 296300 hectares of land with a production of 33, 54,470 metric tons with the productivity of 20.0 tonnes/ha. Tomato is found to suffer from a variety of disease caused by fungi, bacteria, viruses and nematodes. The important diseases are damping off, early blight, late blight, *Fusarium* wilt, *Verticillium* wilt, bacterial wilt and tomato mosaic virus. Among the diseases early blight caused by *Alternaria solani* is one of the most important limiting factors lowering down the quantity and quality both in India. The symptoms of the early blight disease appear as brown to dark leathery necrotic spots first on the leaflets producing target board effect. (Locke, 1949).The early blight was the most catastrophic diseases incurring loss both at pre and post-harvest stages causing 35 to 78 per cent reduction in yield (Jones *et al.,* 1993).Impact of different temperature, pH and light on growth and sporulation of *Alternaria solani*has been observed by, Albert *et al.,* (2012); Taware *et al.,* (2014); Kumar *et al.,* (2015); Kumawat *et al.,* (2016) and Kumar *et al.,* (2017).In the present research work experiments were done to observe the effect of different Temperature, pH and Light on growth and sporulation of *Alternaria solani*.

Materials and Methods

Isolation of the pathogen from diseased samples

The infected portions of leaves showing typical early blight symptoms was used for isolation of pathogen. The diseased plant's leaves were taken from Student Instructional Farm, Net house complex of the Department of Plant Pathology and Vegetable Research Farm, C.S.A. University of Agriculture and Technology, Kanpur, and washed thoroughly with tape water and finely with distilled water to remove all dust particles. The diseased part of the leaves was cut into small bits (infected portions along with some healthy parts) by sterilized knife and surface sterilized in 0.15% $HgCl_2$ (mercuric chloride) solution for 30 seconds. The bits were washed repeatedly thrice in sterilized distilled water to remove trace amount of $HgCl_2$. The pieces were then placed in between two folds of sterilized blotter paper under aseptic condition to remove excess amount of water. Petri plates were taken and sterilized at 165°Cfor 2hrs.in hot air oven. These plates were placed in laminar air flow chamber and 20ml PDA was poured in the Petri dishes. After solidification of media, the surface sterilized leaves pieces were placed in to position in each plate with the help of sterilized forceps. The plates were finally sealed with Para film tape and were incubated at 25±1°Cin a BOD incubator for 7 days and observed periodically for fungal growth and sporulation. Colonies, which developed from the bits, were identified by microscopic observation on the basis of mycelial and spore characters. After identification they were transferred to PDA slants and incubated at 25±1^{o}C for further use.

Purification of pathogen

The culture was purified by both hyphal tip method (Pathak, 1972) and single spore technique described.As soon as the mycelia growth was observed in Petri plates. The mycelial bits of *Alternaria solani* was removed from the margin of fungal colony and then transferred to another Petriplates which was already poured with sterilized Potato-Dextrose-Agar medium. After purification the pure culture of *Alternaria solani* were sub cultured at monthly intervals and maintained on Potato-Dextrose-Agar slants under refrigeration at 6 to 8 °C for further studies.

Identification of the pathogen

The pathogen was identified on the basis of its cultural and morphological characteristics. The spores and hyphae of the fungus were observed under compound microscope. A pure culture of *A. solani* was obtained from colony showed black mycelia and surrounded by white young tip of hyphae at initial stage turning later to ash brown and finally grey colour in the potato dextrose agar medium. The inter and intra - cellular mycelium of *A. solani* consisted of septate, branched, light brown hyphae, which turned darker with age. The conidiophores are septate, simple or sometimes branched; emerge through the stomata from the dead centers of the spot. Conidia are usually in chains (acropetal) and sometimes solitary also. Conidia were 150-300µm length x 15-20 µm thick in size. The broadest part with 4-9 transverse and 0-4 longitudinal septa. Conidia are dark coloured and borne singly (Plate-1).

Effect of different temperature on growth and sporulation of *A. solani*

Potato Dextrose broth was used as a basal medium to study the effect of temperatures on the growth of *A. solani.* 15-20 ml. of medium was poured in sterilized Petri plates. These plates were inoculated with 5 mm. mycelia bit of 9 days old isolates of *A. solani* were inoculated and incubated at different temperature *viz.,* 15, 20, 25, 30, 35 and 40°Cin BOD incubators for 9 days. Three replications were maintained for each treatment. The observations on radial growth were recorded with the help of metric scale, sporulation and mycelium dry weight (mg) were recorded and data were analyzed statistically using completely randomized design.

Effect of different hydrogen ion concentration (pH) on growth and sporulation of *A. solani*

Potato dextrose broth was used as a basal medium to study the effect of pH on the growth of *A. solani*. The pH of the medium was adjusted to various levels namely 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 by adding 0.1N sodium hydroxide and 0.1N hydrochloric acid and it was determined by electronic pH meter. 15-20 ml. of medium was poured in sterilized Petri plates. These plates were inoculated with 5 mm. discs taken from 9 days old isolates of *A. solani* were inoculated and incubated at 27±1°C for 9 days. Three replications were maintained for each treatment. The observations on radial growth were recorded with the help of metric scale, sporulation and mycelium dry weight (mg) recorded and the data were analysed statistically.

Impact of light on the growth and sporulation of *A. solani*

The effect of light on growth of *A. solani*was studied on Potato Dextrose Agar medium by exposing the pure cultures to 4 hours dark 20 hours light, 12 hours dark 12 hours light, 8 hours dark 16 hours light, 8 hours light 16 hours dark and 24 hours dark 24 hours light. The inoculation of culture to Petri plates

containing PDA was done as explained earlier. The plates were incubated at 26±1°C for nine days. Observations on colony diameter and sporulation were recorded and data were analysed statistically.

Degree of sporulation

In case of sporulation, sporulation was observed from 10 days old culture of each isolate by making the spore suspension. A single block of 5 mm diameter was cut out from the fungal colony near the margin by sterilized corkborer and was transferred to 5 ml sterile distilled water in a test tube, where it was mixed thoroughly to make a uniform spore suspension. One small drop of spore suspension was taken on a slide and average spore count of three microscopic fields under low power (10X) objective of the microscope. Sporulation was categorized as below:

No. of spore Per microscopic field	Designation
0	- (nil)
1- 10	+ (poor)
11-20	++ (moderate)
21-30	+++ (good)
31 and above	++++ (excellent

Results and Discussion

Effect of different temperature on the colony growth, sporulation and mycelium dry weight of pathogen

To find out optimum as well as the range of temperature on the colony growth sporulation and dry weight of pathogen, fungus was grown at six different temperatures on PDA. After 9 days of incubation, the average colony growth, mycelial dry weight and sporulation were noted below in Table 1. The results obtained and presented in Table 1 and its corresponding bar diagram Figure 1 and also represented with photograph Plate-2 revealed that the mycelial growth of *Alternaria solani* was significantly influenced with temperature. Maximum colony growth of 87.47 mm was recorded at 25ºC followed by 30ºC (74.77mm). The mycelial growth 56.71mm, 32.01mm, 26.45mm was recorded at temperature 20ºC, 35 ºC and 15 ºC respectively. Minimum colony growth (17.78mm) was recorded at 40 ºC. However, mycelium dry weight was observed maximum at 25ºC (613.90mg). It was found at par with 30ºC (523.76mg) and significant higher over rest of temperature levels. Minimum mycelium dry weight (185.05 mg) was obtained at 40ºC.Temperature also influenced the sporulation of *Alternaria solani* on PDA medium. Excellent sporulation was noticed at 20ºC and 25ºC temperature, good sporulation at 30 ºC and poor sporulation was observed at 15 and 35 ºC. However, no sporulation was found at 40ºC temperature.

The present findings are similar with the result of Kaul and Saxena (1988), who reported that the maximum growth of *A. solani* was obtained at 25°C followed by 20, 15, 10 and 5°C and least growth at 35°C temperature. Similar types of results were also obtained by Arunakumara (2006), reported *A. solani* produced maximum growth at 25 to 30°C temperature. Similar results were also reported by several workers (Kemmitt, 2002; Rodrigues *et al.*, 2010; Hubballi *et al.*, 2010).

Effect of different hydrogen ion concentration (pH) on the colony growth, sporulation and mycelium dry weight of pathogen

The growth of the fungus might be inhibited or prevented by the medium which are acidic or saline. Satisfactory medium may he prepared by addition of N/10 HCl and NaOH for lower and higher pH values, respectively. In order to find out the range of pH suitable

for growth of the pathogen 10 different pH levels were tried for the fungus. This study was made in liquid form of Potato dextrose agar medium. Effect of different pH level on colony diameter, sporulation and mycelium dry weight of *Alternaria solani* on PDA medium have been presented in Table 2.

It is indicated from the above Table 2 and its corresponding bar diagram Figure 2 and also represented with photograph Plate 3 that all treatment allowed better response. Maximum colony diameter (85.87mm) was observed at 6.5 pH level followed by 6.0 pH level (75.00mm), and it was found at par with pH 7.0(74.74 mm), while minimum colony diameter (32.38mm) was observed at 8.5 pH. As for as, mycelium dry weight is concerned it that was observed maximum mycelia dry weight (349.55 mg) was found at pH 6.5. It was found at par with pH 6.0 (328.29mg) and significant higher over rest of pH levels. However, minimum mycelium dry weight (119.38 mg) was obtained at 8.5 pH level. In case sporulation, pH level influenced the sporulation of *A. solani*.

Excellent sporulation was observed at pH level 6.0 to 7.0, good sporulation at 5.0, 5.5 and 7.5 pH levels and moderate sporulation was observed at 4.5pH, while poor sporulation was recorded at 4.0, 8.0 and 8.5 pH level.

The present finding confirming with the results obtained by Alhussaen (2012), they observed that the optimum pH level of *Alternaria solani* grow *in vitro* were 6-7.

Similar types of result were also obtained by several workers. Samuel and Govindaswamy (1972), demonstrated that good mycelial growth and sporulation of *A. solani* between pH 4.0 to 8.0 and pH 5.0 was the best for mycelia growth and pH 7.0 for sporulation. Gemawat and Ghosh (1980), observed that the *A. solani* was capable to grow on wide range of pH (4.0 to 9.5) and maximum growth and sporulation were observed at 6.3 pH.

Impact of light on the colony growth and sporulation of pathogen

To furnish the effect of light on growth and sporulation of *A. solani* an experiment was conducted. The fungus was exposed to alternate cycles of dark and light and continuous light and continuous darkness for different period of time up to nine days as described in material and methods. The maximum growth of 87.86mm was noticed when pathogen exposed to 12 hrs dark and 12 hrs light followed by 24 hrs light and 24 hrs dark, 4 hrs dark and 20 hrs light, 8 hrs dark and 16 hour light and least radial growth of 78.25mm was recorded in treatment with exposure to 8 hrs light and 16 hrs dark (Table 3).

It is clearly indicated from above Table 3 and its corresponding bar diagram Figure 3 light has profound effect on growth and sporulation of fungi. The preliminary studies carried out in the present investigation with *A. solani* indicating a maximum growth and sporulation when inoculated plates were exposed to alternate light and dark condition (12 hrs light alternated with 12 hrs dark) followed by continuous dark. Light also influenced the sporulation of *Alternaria solani*on PDA medium. Excellent sporulation was noticed at 12 hrs dark and 12 hrs light, good sporulation at 24 hrs light and 24 hrs dark and poor sporulation was observed at 8 hrs dark and16 hrs light and 8 hrs light and 16 hrs dark.

Table.1 Effect of different temperature on the colony growth, sporulation and mycelium dry weight of *A. solani*

S. No.	Temperature (^{0}C)	Average colony diameter(mm)	Sporulation	Average mycelium dry weight(mg)
T1	15	26.45	+	216.63
T2	20	56.71	++++	358.19
T3	25	87.47	++++	613.90
T4	30	74.77	+++	523.76
T5	35	32.01	+	263.62
T6	40	17.78	-	185.05
	CV (%)	**6.6070**		**2.4603**
	SE.m.±	**1.8767**		**5.1163**
	CD @ 0.01%	**5.7826**		**15.7651**

Table.2 Effect of different pH on colony growth, sporulation and mycelium dry weight of *A. solani*

S.No.	pH	Average colony diameter(mm)	Sporulation	Average mycelium dry weight(mg)
P1	4.0	41.13	+	142.29
P2	4.5	45.09	++	188.04
P3	5.0	45.47	+++	230.68
P4	5.5	62.17	+++	264.36
P5	6.0	75.00	++++	328.29
P6	6.5	85.87	++++	349.55
P7	7.0	74.74	++++	312.16
P8	7.5	58.25	+++	276.46
P9	8.0	42.47	+	135.54
P10	8.5	32.38	+	119.38
	CV (%)	**7.1292**		**3.5463**
	SE.m.±	**2.3537**		**4.8049**
	CD @ 0.01%	**6.9434**		**14.1746**

Table.3 Effect of light on growth and sporulation of *A. solani*

S.No.	Exposed intervals (h)	Average colony diameter(mm)	Sporulation
1.	4 h dark 20 h light	82.23	++
2.	12 h dark 12 h light	87.86	++++
3.	8 h dark 16 h light	81.31	+
4.	8 h light 16 h dark	78.25	+
5.	24 h light 24 h dark	85.52	+++
	CV (%)	**2.8383**	
	SE.m.±	**1.3607**	
	CD @ 0.01%	**4.2877**	

Plate.1 Pure culture of *A. solani* and its conidia

A. Pure culture of *A. solani* in culture tubes

B. conidia of *Alternaria solani*

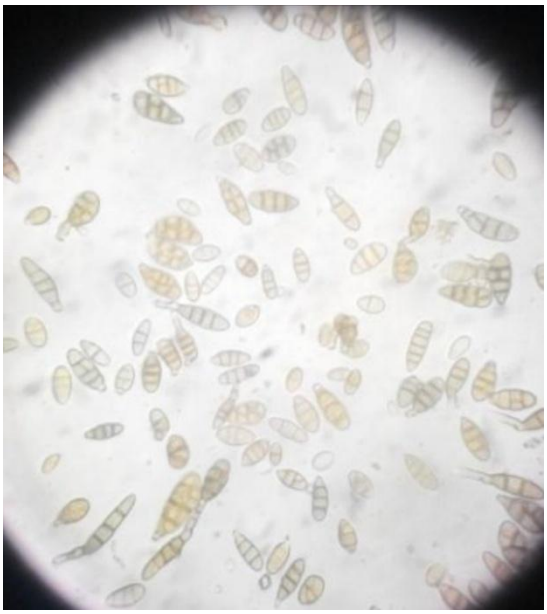


Plate.2 Effect of temperature on *Alternaria solani* on PDA medium

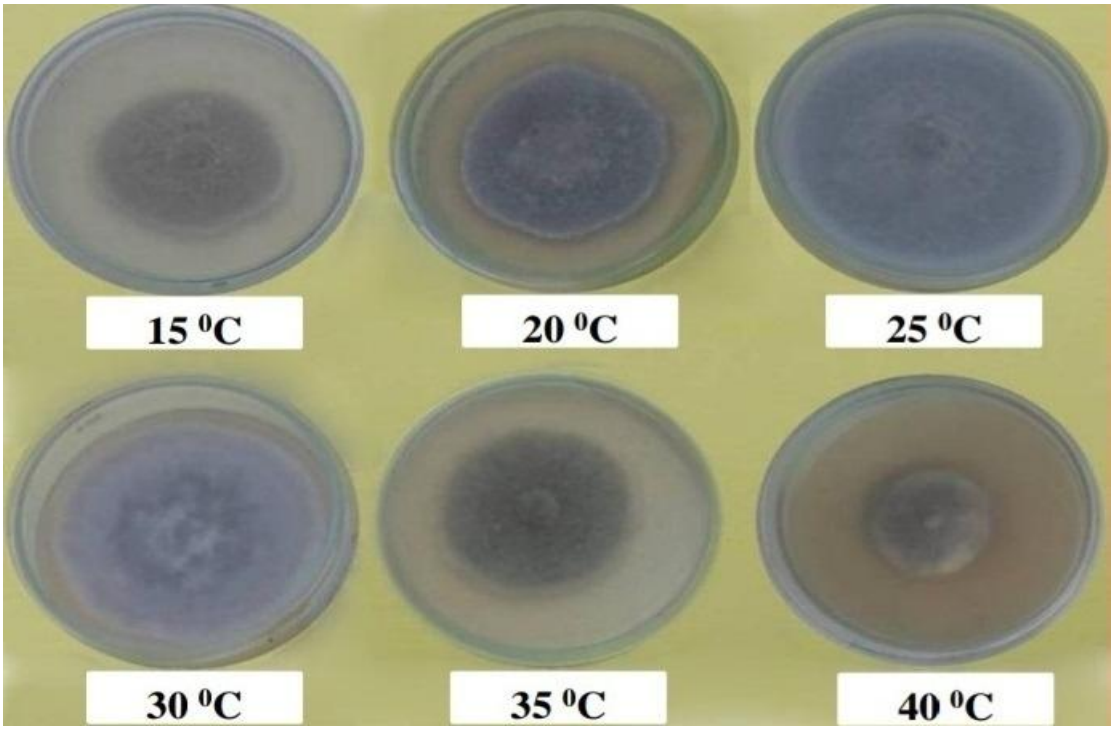


Plate.3 Effect of pH on *Alternaria solani* on PDA medium

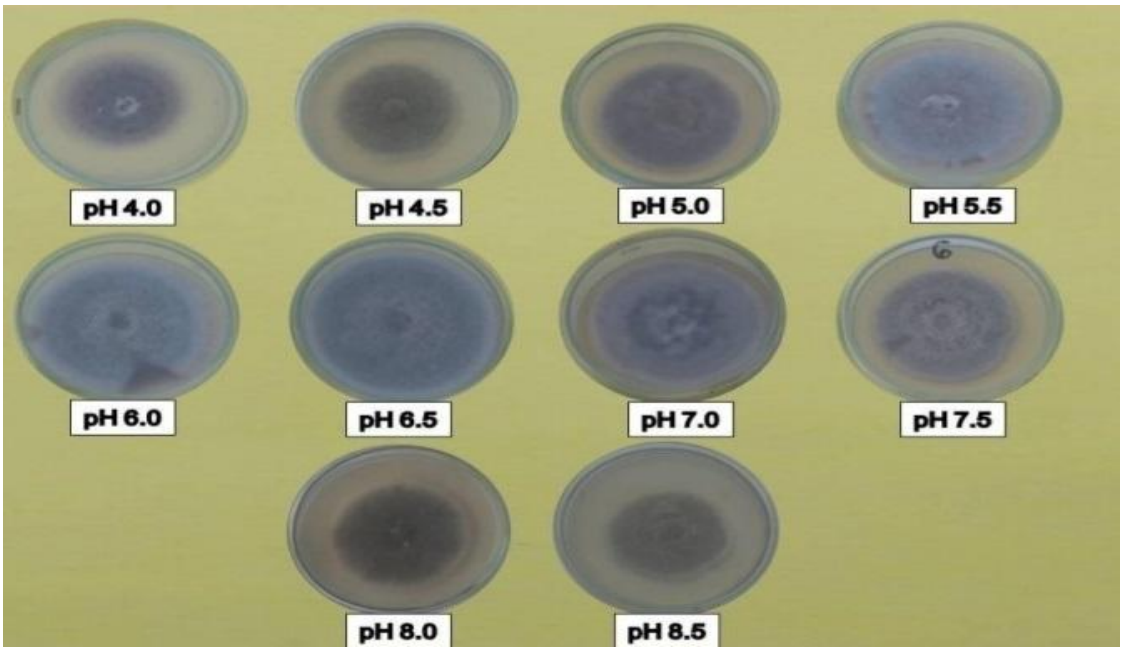


Fig.1 Average colony growth and dry weight of the fungal mat of *Alternaria solani* at different temperatures

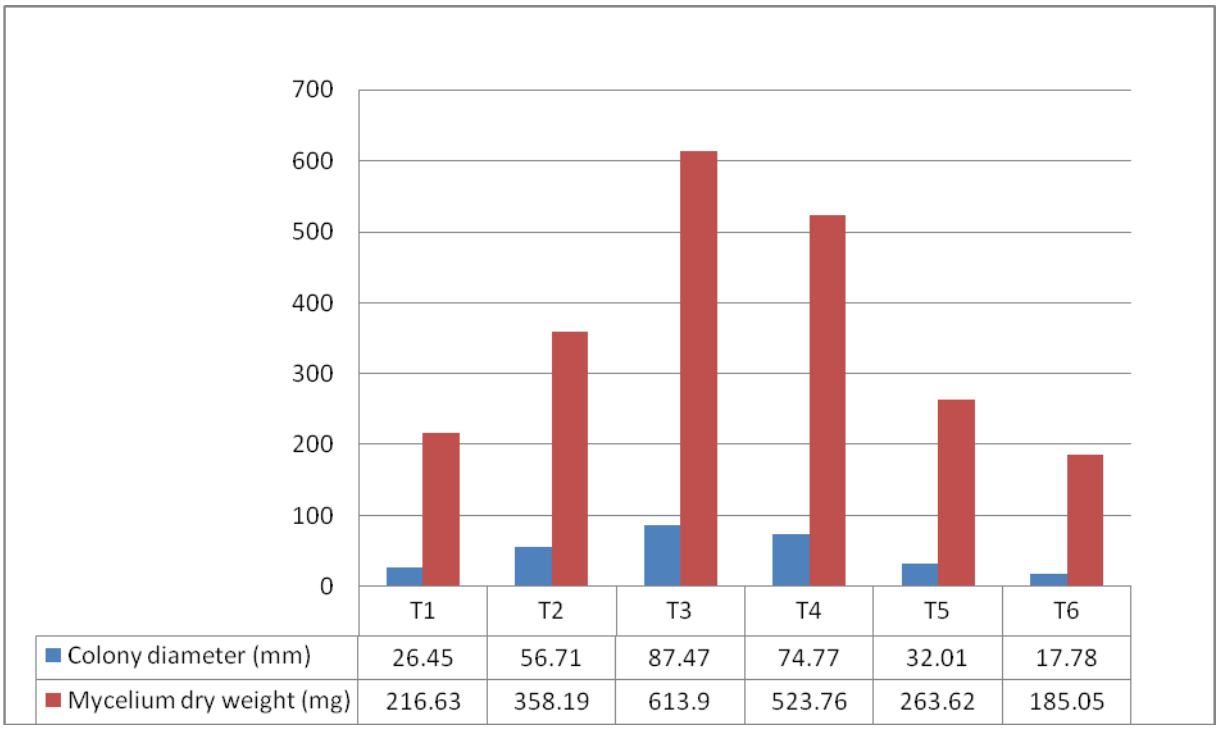


Fig.2 Effect of different pH level on colony growth and mycelium dry weight of pathogen

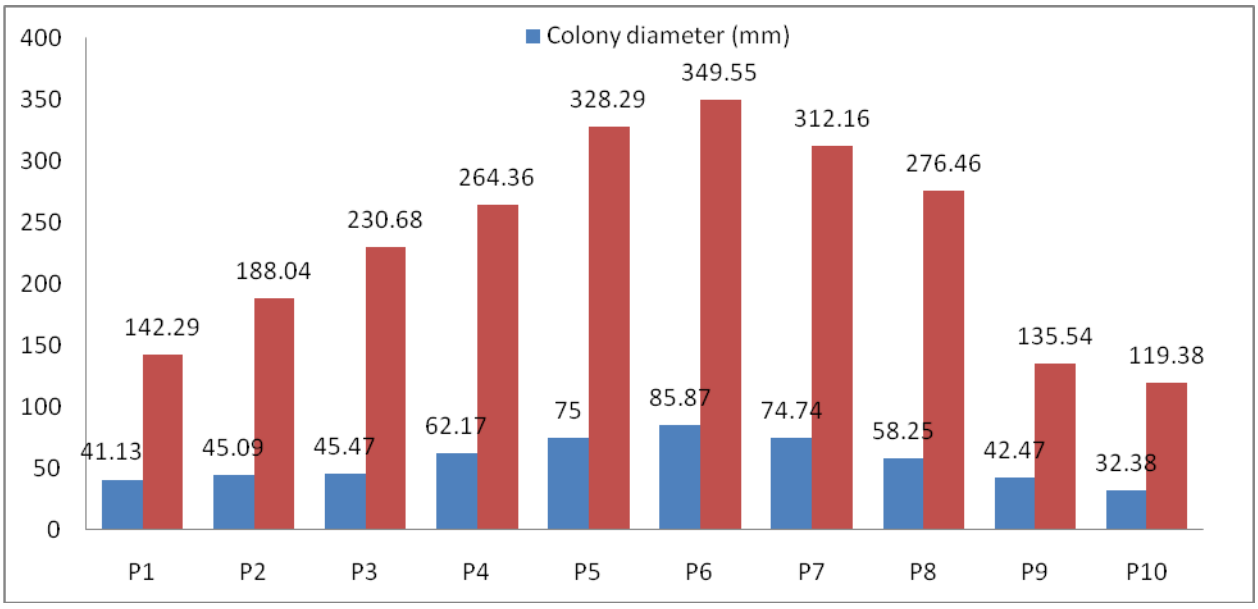
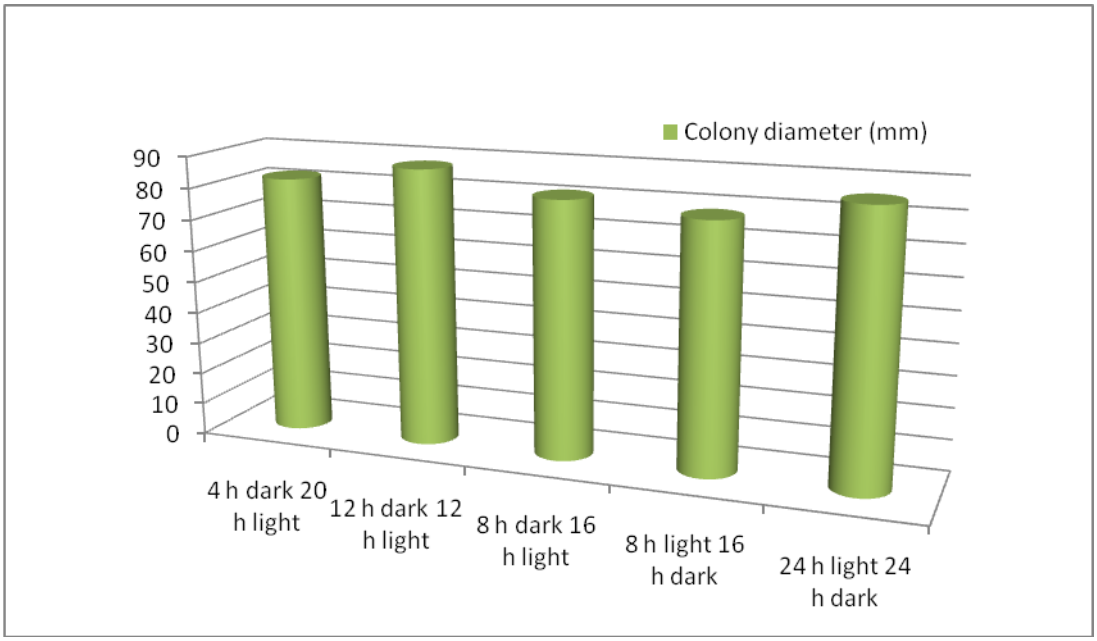


Fig.3 Effect of light on growth of *A. solani*



The present results supported the observations of Arunakumara *et al.*, (2014) give the detail observation with *A. solani* indicating a maximum growth and sporulation when inoculated plates were exposed to alternate light and dark condition (12 hrs light alternated with 12 hrs dark) followed by continuous dark. Lukens (1963), reported that the conidia of *A. solani* normally formed after incubation for 6 hours in the dark

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