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Effect of Light intensities on the growth of Fungal plant pathogen *Alternaria solani*, and Initiation of Leaf spot Disease symptoms in Tomato Plants: A New Study

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Abstract

The natural visible lights emitted by the sun and moon are a driving force for life on our planet. Though life begins in the dark, both darkness and sunlight illumination are necessarily required for the life processes and several metabolic cycles in living beings. This light illumination in terms of light intensities varies in the day, months of the year, and at a given latitude and longitude of a location on planet Earth. Its role in the growth of plant pathogenic fungi and plant disease initiation has not yet been studied and reported.

In the present investigation, we studied the effect of light intensities on the growth of fungal plant pathogen *Alternaria solani* under *in vitro* conditions and its effect on initiation of leaf spot disease symptoms in tomato plants. Further, the sunlight and moonlight intensities at locations having 19.392677 latitude and 74.648827 longitude in India were measured in the agricultural field and in tomato plant canopies to relate these with *A. solani* leaf spot disease development.

The natural light intensities were measured in lux (Lx) units with the help of a photometric Lux meter during three agricultural crop seasons of a year (rainy season: June – September; winter season: October – January, and summer season: February – May). The natural sunlight intensities varied during the day and months over this period. The sunlight intensities of 72,000 – 1, 08, 100 received from 12.00-2.00 pm and 45, 000 – 1, 05, 200 Lx received from 2.00- 4.00 pm have a detrimental effect on the growth of a tomato leaf spot fungal pathogen *Alternaria solani*. The light intensities lower than 72, 000 Lx were favourable for the growth of this fungal pathogen. The lunar light intensities were observed very negligible i.e. 0.1 to 0.7 Lx during the full-moon nights, otherwise, these were 0.0 Lx during other nights. The effect of lunar light intensities on the growth of this fungal pathogen was negligible as compared to total darkness which produced more fungal growth. The diurnal treatment of 6 hours of light + 18 hours of darkness produced more fungal growth of *A. solani* as compared to continuous light without any dark period.

To initiate *Alternaria* leaf spot disease symptoms in tomato plant leaves, the lower light intensities up to 2000 Lx were much more helpful than the higher light intensities under *in vitro* studies. Such lower light intensities were available in the lower-leaf canopies of the tomato plants under field conditions, which may be one of the reasons for the first initiation and development of *Alternaria* leaf spot and blight symptoms in lower leaves of tomato plants. The minimum light intensity of 180 Lx and 420 Lx was observed in the rainy and winter seasons respectively while the minimum light intensity of 3200 Lx was observed in the summer season. The varied light intensities in different months may be the reason for the prevalence of this fungus prominently during particular periods of the months of the year.

These results of light intensities are useful to determine the structure of the shade-net or poly-houses, which can regulate the light intensities in the protected cultivations to hamper fungal growth and disease development by this pathogen.

Keywords

Light intensities, Sunlight, Moonlight, Lux unit, fungal growth, *Alternaria solani*, disease development, tomato crop, protected cultivation.

Introduction

The natural lights emitted by the sun and moon are a driving force for life on our planet. Though life begins in the dark, both darkness and sunlight illumination are necessarily required for the life processes and several metabolic cycles in living beings. This light illumination in terms of light intensities varies in the day, months of the year, and at a given latitude and longitude of a location on planet Earth. Therefore, in the fungal kingdom also light may have a role in regulating fungal growth, the direction of growth, asexual and sexual reproduction, and pigment formation all of which are important aspects for the survival and reproduction of fungal species. Similarly, it may also affect the infection process of the fungal plant pathogen in the host plant.

The word light usually refers to visible light which is visible to the human eye and is responsible for the sense of sight. Visible light is usually defined as having wavelengths in the range of 400-700 nano-meters (nm) between the infrared (with longer wavelength) and the ultralight (with shorter wavelength) (Pal and Pal, 2001). The lux (symbol: Lx) is the SI unit of illuminance and luminous emittance, measuring luminous flux per unit area i. e nothing but light intensity. It is equal to 1 Lx per square meter (SI-derived units, National Institute of Standards and Technology). Light is electromagnetic radiation within a certain portion of the electromagnetic spectrum.

When the light reaches the plant canopy, its intensity/illuminance varies at different places in the canopy. At the top of the plant surface, the illuminance (Lx) of light may be more as compared to Lx in the middle canopy and at the lower canopy. In the process of fungal infection of plants to cause plant disease, how this light illuminance (Lx) plays a role is not yet studied and understood. What we know at present is that the alternate cycle of darkness and light enhances fungal growth. Cotty and Mishagi (1985) observed more lesions on inoculated plants kept in dark humid chambers than in illuminated humidity chambers. Determination of the role of light intensity (Lx) on fungal growth and plant infection will enhance our basic understanding and knowledge of plant pathology. With these objectives, the basic studies on the effect of Light intensities on the growth of the fungal plant pathogen *Alternaria solani*, and its initiation of Leaf spot disease symptoms in Tomato Plants were undertaken.

Material and Methods

1. Measuring Light Intensities in Experimental designs in Laboratory and Open Crop Fields

1.1. Use of Photometric Lux meter to measure light intensities

The photometric lux meter was used to measure light intensities in lux (Lx) units. The lux meter having a range of 100 Lx to 4, 00, 000 Lx was used to measure visible light intensities during the day and moon-light intensities at night (where the specificity of the lux meter was up to 0.1 Lx).

1.2. Creation of different light intensities for laboratory studies.

Different light intensities regulatory mechanism was developed for *in vitro* studies to adjust different light intensities. Also, electric bulbs of different watt power were used to obtain different light intensities.

1.2.1. *In vitro,* the arrangement of the experimental setup to obtain different light intensities and their measurements.

The experimental unit was set up in a dark room to avoid external light interference for *in vitro* studies. The light intensity measurement unit consisted of an electric board with regulators for adjustment of light intensities. Initially, the bulbs of different watts (viz. 0, 40, 60, 100, and 200 watts) were used to obtain different light intensities. However, it was observed that a 200-watt bulb alone was sufficient for the experimental set-up, as the bulb shows an intensity of 25,000 Lx when the regulator turns at maximum and 100 Lx when the regulator turns at minimum, and therefore, a 200-watt bulb was used. The experimental set-up was prepared based on the observation that as the distance increased from the source of light, the intensity of light decreased and that it remained the same at a particular point/location when there was no environmental change (fig. 1). By using this observation, the light intensities set up were formed. For this, first, the white papers were spread over the tabletop, and electric boards with a 200-watt bulb were kept at a fixed position at one end of the paper. The light intensities at various points/locations from the source of the bulb were measured by using a photometric lux meter to fix the spots for a particular light intensity, which was used to keep the fungal-inoculated plates/tomato plants, for the fungal growth/disease initiation, at that particular light intensity.

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Fig 1. *In vitro* experimental setup for obtaining different light intensities.

Based on the effect of light intensities observations on the fungus growth (fig 2) under *in vitro* studies up to 11 days, only five light intensities viz. 2000 Lx, 1000 Lx, 600 Lx, 200 Lx, and 100 Lx were selected for the *in vitro* studies. The remaining intensities were discarded as these were not feasible for the experiment.

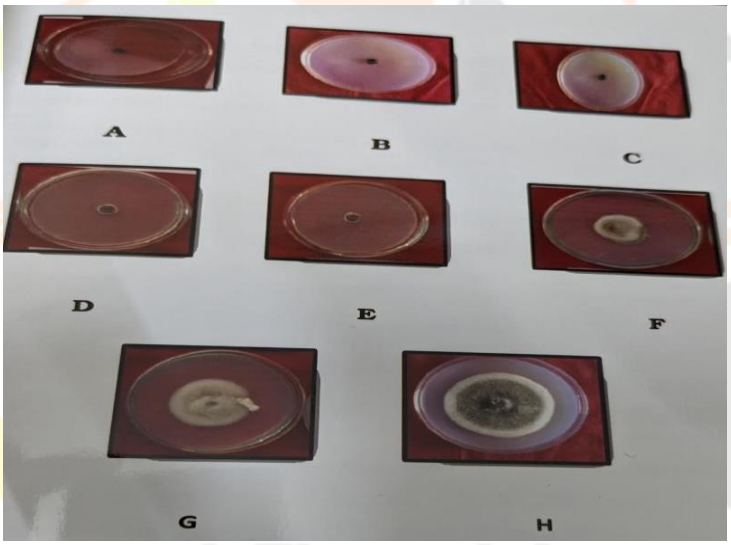

Fig 2. Selection of light intensities for *in vitro* studies based on *Alternaria solani* growth

A= at 20000 Lx, B= at 10000 Lx, C= at 5000 Lx, D= at 2000 Lx, E= at 1000 Lx, F= at 600 Lx, G= at 200 Lx, and H= at 100 Lx.

Note: The natural room light has 140 Lx. Light intensity.

The PDA plates inoculated with *A. solani* fungal disc (1cm) at the center (fig 3) were kept on white paper at a particular spot of light intensities for *in vitro* studies and observed daily for the growth of fungal mycelium. Similarly, separate inoculated plates were exposed to different light intensities in the open field for *in vivo* studies to determine the role of light intensities on the growth of fungal pathogen *A. solani*.

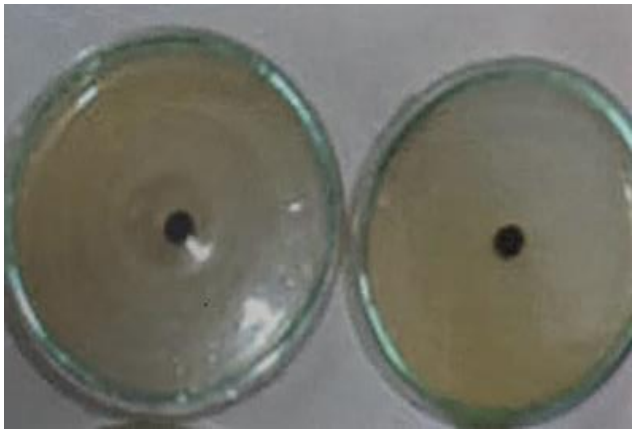

Fig.3. Preparation of *Alternaria* fungal disc plates to be exposed to various light intensities.

2. Data collection for sun-light and moon-light intensities

2.1. Collection of Open-field data for visible light intensity over a period of the year

The readings for the daylight intensities in open fields and in tomato plant canopies were recorded during three agricultural crop seasons of a year (rainy season: June – September; winter season: October – January, and summer season: February – May) at a place having 19.392677 latitude and 74.648827 longitude at Maharashtra, India. Observations were taken at weekly intervals and for 4 times a day i.e. at 9.00 am, at 12.00 pm, at 3.00 pm, and at 6.00 pm. The location for taking readings for visible light intensities in open fields was free from trees, walls, and any such things that interrupt the light intensity. The sensor of the photometric lux meter was held in such a way that it was directed towards the sun so that sunlight fell directly on it. The average reading shown by the photometric lux meter was noted.

2.2. Collection of light intensity data in Tomato Crop Canopy in tomato field over a period of year

The same procedure was followed for measuring light intensity data in the tomato plant canopy. First, the tomato plant canopy was differentiated as an upper canopy, middle canopy, and lower canopy based on the tomato plant height and the light intensities prevalent at particular canopy levels were measured. The sensor of the lux meter was held in the respective canopy place and the light intensity was recorded.

2.3. Collection of data for lunar light intensity over a month

To record the lunar light intensity, the observations were taken during night hours. These observations were taken from full moon night to new moon night for 15 days and vice-versa. The moonlight intensity data was collected for 1 month during the clear sky months at midnight around 2.00 am by holding the sensor of the lux meter towards the moon.

3. Selection of light hours duration for diurnal studies

Two conditions i.e. 6 hours of light + 18 hours of darkness, and 24 hours of light were taken for diurnal studies of light intensity on fungal growth of *A. solani*.

4. Isolation of *Alternaria solani,* from tomato plant, its pathogenicity test, and use in light intensity studies.

The tomato leaves showing typical symptoms of *Alternaria* leaf spot disease were collected from *Alternaria* leaf blight-infected tomato fields. The causative fungal pathogen was isolated from the disease leaf

by standard agar plate method (Thilagam et.al, 2017). To isolate the pathogenic fungus, the infected portion of leaves with characteristic symptoms was cut into small bits which were surface sterilized in 0.1 % mercuric chloride solution, followed by subsequent three washing with sterile distilled water, and then dried on sterile blotting paper. These infected bits were placed with slight pressure on fungal growth PDA media. These inoculated plates were then incubated at 28^0 C for 7 days. The fungal growth that emerged from the diseased area was separated and purified on the same media. The fungus was confirmed as *Alternaria solani* (Fig. 4) based on its morphological characteristics (Barnett and Hunter, 1972). The fungal culture was sub-culture once a month to maintain its viability. The pathogenicity of the culture was proved on the tomato plant leaves by spray inoculation of spore suspension of *Alternaria fungus* on leaves, followed by incubation in a humid chamber and observing the development of leaf spot symptoms.

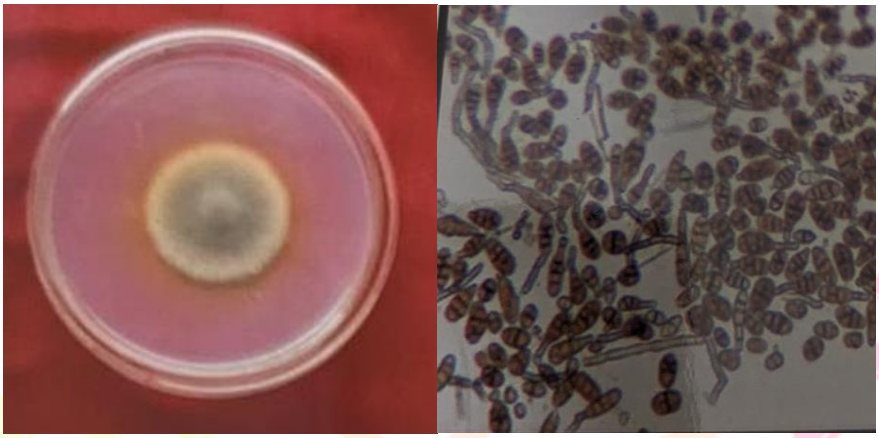

Fig.4. Pure fungal culture of *Alternaria solani* and microscopic *Alternaria* spores

5. Preparation of *Alternaria* fungal plates and studies on the effect of natural light intensities on fungal growth

As the sunlight from 6.00 am to 6.00 pm showed variation in light intensity, a period of 12 hours was taken for the experimentation (as before 6.00 am and after 6.00 pm, there was no considerable difference in light intensity). The 12-hour time duration i.e. from 6.00 am to 6.00 pm was divided into equal two hourly slots viz. 6.00-8.00 am, 8.00-10.00 am, 10.00 am – 12.00 pm, 12.00- 2.00 pm, 2.00-4.00 pm and 4.00-6.00 pm. to check the effect of different light hours slots having variation in light intensities on the growth of *Alternaria solani*.

The sterile petri plates were poured with 15-20 mL of sterilized PDA media under aseptic conditions in a laminar flow cabinet and kept overnight at room temperature to detect any contamination. The next day, the un-contaminated petri plates were inoculated with 8-day-old *A.solani* culture of a 1 cm diameter disc at the center of the plate (as shown in fig 3). The inoculated petri plates were sealed with sticky transparent tape to avoid contamination. These plates were exposed to different light intensities in the open field for respective 2-hour slots and incubated at 30 ± 1^0 C in the BOD incubator for 4 days and observation for fungal growth was recorded on the 5^{th} day.

6. Studies on the effect of lunar light intensity on *Alternaria* fungus growth

It was not possible to create the lunar light intensity under the *in vitro* condition as it has very little lux (0.1 Lx), and therefore, the experimentation with lunar light intensity was carried out under natural lunar light conditions during respective full moon night to new moon night and vice versa. The respective PDA plates inoculated with *A. solani* fungal disc (1 cm) were kept under natural lunar light on that particular moon night from 11.00 pm to 6.00 am, followed by incubation in the BOD chamber in the dark, and the observations on fungal growth were recorded after 5^{th} days.

7. Studies on the effect of different light intensities on conidial spore germination of *Alternaria solani*

A 7-day-old fungal culture of *A. solani* having sporulation was used to harvest conidial spores in distilled water and used for the studies. A 2 % sucrose solution was used to provide a temporary nourishing medium for the spore to germinate in the microscopic cavity slides. A drop of sucrose solution was placed in the cavity of a slide by using the sterile pipette and 0.1 mL of spore suspension was added in this sucrose solution in the cavity. A cover slip was placed on the cavity of the slide and sealed it. 5 slides so prepared for the experiment were exposed individually to different light intensities viz. of 2000 lx, 1000 lx, 600 lx, 200 lx, and 100 lx for 3 hours, under *in vitro* conditions, and observed for spore germination after 24 hours of incubation, under a microscope.

8. Preparation of tomato plant seedlings to study the effect of various light intensities on *Alternaria* infection in tomato plants.

8.1. Raising of Tomato Seedlings

The medium-sized plastic pots were filled with a mixture of soil+ FYM (2:1 ratio) to about 3/4th of the pot height, and water a day before sowing the seeds. The pure seeds of tomato variety JK-511 were used for sowing in the above pots at a shallow depth of about 2 cm. Three tomato seeds were sown in each pot. The pots were watered after sowing and frequent watering was given as per the requirement. These pots were kept in a place where sunlight and ventilation were available. These were maintained up to 45 days after sowing to attain the 5-6 leaf stage.

9.2. Inoculation of *A. solani* pathogenic culture on tomato seedlings and *in vitro* studies on the pathogenesis of inoculated seedlings under 5 light intensities

To obtain *A. solani* fungal conidial spore suspension for seedling inoculation, an aqueous suspension of fungal spores was prepared by pouring sterile distilled water onto agar plates containing sporulated *A. solani* cultures, and by gently scraping the fungal growth on the agar surface. This spore suspension was passed through four layers of sterile muslin cloth to remove mycelial fragments. The collected *Alternaria* spore suspension was poured into a small plastic sprayer, and the leaves of tomato seedlings (having eight leaf stage) were sprayed carefully and used to carry out the further experiment.

The *A. solani* inoculated seedlings pots were kept at a particular light intensity, in the light-intensity experimental setup room for *in vitro* studies. Five light intensities viz. 2000 Lx, 1000 Lx, 600 Lx, 200 Lx, and 100 Lx were used for the studies The plants inoculated with *A. solani* pathogen were placed under these lux intensities (fig 5) and were observed daily for the development of the leaf spot symptoms for up to 7 days.

Fig.5. Experimental setup to study the effect of light intensities on initiation of *Alternaria* leaf spot symptoms.

To calculate the percent disease index (PDI) on each infected plant a 1 to 5 disease grade scale was used.

Statistical analysis

The data obtained during different treatments were analyzed statistically by using standard statistical methods.

Results and Discussion

1. **Effect of natural visible light intensities on the growth of *Alternaria solani***

The results (table 1, fig 6) indicate that the fungal mycelial bit petri plates exposed to light intensities during a time slot of 12:00 – 2:00 pm and 2:00 to 4:00 pm could not develop the fungal growth (fig 6). At this time slot, the maximum light intensities were 1,05,200 Lx and 1,35,000 Lx respectively which was detrimental to the growth of the *Alternaria* fungus. In the morning hours up to 10:00 am and in the evening hours after 4 pm, the light intensities were favourable for the fungus growth. The more fungal growth was observed when the light intensities were in the range of 6,200 Lx to 8,100 Lx.

These results clearly indicate that natural sunlight intensities affect the growth of the *A. solani* fungus.

Table 1. Effect of visible light intensities on the growth of *Alternaria solani*

Sr.no.	*Alternaria* mycelial disc exposed to sunshine hours	Maximum light intensities (in Lx) during the exposure period	Minimum light intensities (in Lx) during the exposure period	Fungal growth (in cm) obtained upon incubation (on the 5th day) for the respective exposure period
1	*6:00 -8:00 am*	57,000	8,100	6.57
2	*8:00 – 10:00 am*	72,000	59,000	6.17
3	*10:00 -12:00 am*	1,08,100	72,000	5.97
4	*12:00 -2:00 pm*	1,35,000	1,10,100	0.0
5	*2:00 -4:00 pm*	1,05,200	45,000	0.0
6	*4;00 – 6:00 pm*	42,500	6,200	7.7
SEm (±)			0.053	
CD at 5 %			0.1835	

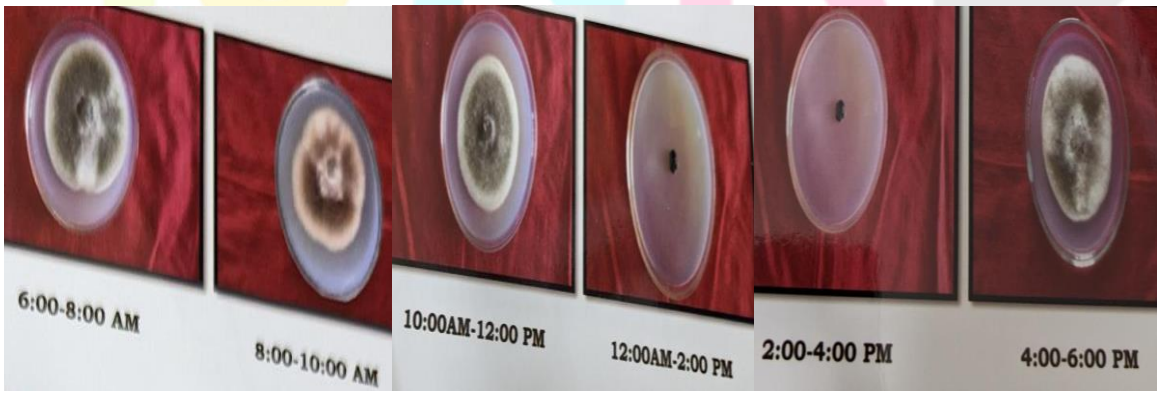

Fig.6. Growth of *Alternaria* as influenced by time slot/light intensity.

2. Variation in natural sunlight intensities over a period of a year

As light intensities played an important role in the growth of the *Alternaria* pathogen, the natural sunlight intensities were measured in different time slots during three agricultural crop seasons of a year (rainy season: June – September; winter season: October – January, and summer season: February – May). The light intensities were measured at a weekly interval and were recorded at 4 times a day i. e at 9:00 am, 12:00 pm, 3 pm, and 6:00 pm to record the variation in the light intensities during a day.

The results (table 2) indicate that the light intensity varied in all the months of the year on a given day and at a given time. In the month of June, the maximum light intensity was observed to be 1, 05, 000 Lx at 12:00 pm while 180 Lx at 6:00 pm. In the month of July, the maximum light intensity was 1, 50, 000 Lx at 3:00 pm whereas 280 Lx at 6:00 pm. In the month of August, the maximum light intensity was 1, 65, 000 Lx at 3:00 pm while 620 Lx at 6:00 pm. In the month of September, the maximum light intensity was 1, 40, 000 Lx at 3:00 pm while 1200 Lx at 6:00 pm. Thus, in some months the maximum light intensity was observed at 12:00 pm while in other months it was observed at 3:00 pm.

In all months of the year, the minimum light intensity was observed at 6:00 pm.

Table 2. Visible light intensities at different times during various months of the year

Month	Date	Light intensities (in Lux) at			
		9:00 am	12:00 pm	3:00 pm	6:00 pm
June	4	85,000	1,05,000	56,000	15,000
	10	9,100	12,500	6,500	**180**
	17	65,000	76,800	35,000	7,800
	25	7,800	11,500	6,700	900
July	1	89,400	1,05,000	1,30,000	19,500
	7	54,000	86,000	1,23,000	17,300
	15	6,700	18,900	45,700	280
	26	95,000	1,25,000	1,50,000	40,000
August	1	1,652	25,000	25,000	11,120
	4	8,050	52,000	75,000	30,000
	7	17,000	35,000	50,000	5,200
	14	4,800	**6,000**	8,860	620
	21	9,850	1,20,000	1,50,000	45,000
	28	90,000	**1,40,000**	1,65,000	**55,000**
September	4	4,600	15,000	9,200	1,200
	11	52,000	82,000	1,20,000	5,300
	18	18,000	45,000	7,000	4,300
	25	98,200	1,10,000	1,40,000	38,000
October	2	95,000	1,05,000	1,25,000	**20,000**
	9	98,000	1,20,000	1,00,000	10,200
	16	94,000	1,00,000	79,000	12,600
	23	48,000	**67,000**	94,200	2,800
	30	87,000	1,03,000	59,000	3,800
November	6	75,000	98,000	1,05,000	3,000
	13	92,000	1,22,000	1,40,000	2,000
	20	57,000	69,200	1,25,000	2,500
	27	98,000	1,10,000	78,000	1,500
December	2	67,000	79,000	59,000	5,000
	9	95,000	1,20,000	28,000	450
	16	88,000	1,02,000	39,500	1,500
	23	68,000	87,200	1,02,000	3,500
	30	91,000	1,03,000	54,200	2,700

January	6	72,000	1,02,000	35,200	3,600
	13	78,000	89,000	1,15,000	3,900
	20	98,000	**1,25,000**	1,61,000	**420**
	27	86,000	1,08,000	1,25,200	4,000
February	3	75,000	98,000	1,07,000	4,300
	10	1,05,000	**1,25,200**	1,35,000	15,000
	17	94,300	1,02,500	1,40,000	4,200
	24	98,000	1,15,000	78,000	**17,600**
March	2	25,000	82,000	65,000	**3,200**
	9	65,000	89,000	78,200	14,800
	16	89,000	1,02,000	1,15,000	7,200
	23	69,800	98,000	54,000	6,400
	30	38,900	55,000	78,000	3,500
April	7	8,900	**12,000**	45,000	9,000
	15	18,000	75,000	49,000	7,900
	21	55,000	68,000	47,000	8,100
	29	60,200	88,000	65,000	9,800
May	4	50,200	98,000	78,200	11,500
	15	64,800	91,000	73,000	12,500
	23	49,100	64,900	45,000	15,000
	31	35,600	70,200	32,900	7,500

The minimum light intensity of 180 Lx and 420 Lx was observed in the rainy and winter seasons respectively while the minimum light intensity of 3200 Lx was observed in the summer season. The varied light intensities in different months may be the reason for the prevalence of this fungus prominently during particular periods of the months of the year.

4.3. Measurement of Lunar light intensities and its effect on *A. solani* fungal growth.

The observations on lunar light intensities (table 3) indicate that the maximum lunar light intensity i.e. 0.7 Lx was observed on the full moon night, and on the subsequent nights the light intensity decreased. On the 6th night after a full moon night, the light intensity was 0.1 Lx and on the 7th day and onwards it was 0 Lx.

Table 3. Lunar light intensities over a month

Lunar Night	Lunar light intensity (in Lx unit)	Lunar Night	Lunar light intensity (in Lux unit)
Full moon night	0.7	New moon night	0.0
2nd night	0.65	2nd night	0.0
3rd night	0.60	3rd night	0.0
4th night	0.42	4th night	0.0
5th night	0.2	5th night	0.0
6th night	0.1	6th night	0.0
7th night	0.0	7th night	0.0
8th night	0.0	8th night	0.0
9th night	0.0	9th night	0.0
10th night	0.0	10th night	0.0
11th night	0.0	11th night	0.1
12th night	0.0	12th night	0.4
13th night	0.0	13th night	0.5
14th night	0.0	14th night	0.6
15th night	0.0	15th night	0.7

From the new moon night to the 10th night, the moonlight intensity was 0 Lx and after the 10th night, the moonlight intensity increased subsequently.

The results (table 4) indicate that the lunar light intensities did not have any significance in the proliferation of *A. solani* fungal growth, and the fungal growth obtained in total darkness was greater as compared to the growth obtained in the lunar light-exposed plates.

Table 4. Effect of lunar light intensity on A. solani mycelial growth

Lunar light intensity (0.1 to 0.7 lux)	*Alternaria solani* growth (in cm)
A. solani plates exposed to 12 hrs under lunar light followed by 12 hrs in darkness	8.2
Control (*A. solani* plates exposed totally in darkness for 24 hrs)	8.5

4.5. Effect of different light intensities on the conidial spore germination of *Alternaria solani*

The results indicate that the different light intensities of 2000 Lx, 1000 Lx, 600 Lx, 200 Lx, and 100 Lx under *in vitro* experimentation did not have any effect on the conidial spore germination of *Altenaria* solani as no conidial spore germination was observed under these light intensities.

4.6. Effect of different light intensities on the initiation of *Alternaria* leaf spot symptoms in tomato plants under *in vitro* studies

The results (Table 5) indicate that in the control treatment of 0 Lx light intensity, 6 leaves showed 100 percent leaf area covered with infection, while 1 leaf showed ¾ leaf area covered with infection of *Alternaria* leaf spot. At different lux intensities, the number of leaves infected varied in their area of infection and % disease index. The maximum leaf area infected was observed at 100 Lx with a PDI of 79.32 %. As the lux intensities increase, the PDI decreases proportionately.

The results indicate that for initiation of *Alternaria* leaf spot symptoms, the lower light intensities were much more helpful than the higher light intensities.

Table 5. Effect of light intensities on initiation of *Alternaria* leaf spot symptoms on tomato plant.

Sr.no.	Light intensity (in Lx)	No. of. Leaves On plant	Infected Leaves/ plant	Healthy Leaves/ plant	Leaf area covered with leaf spot symptoms with no.of leaves				PDI (%)
					100 %	75 %	50 %	25 %	
1	0 (Control)	8	7	1	6	1	0	0	96.22 (78)
2	2000	8	5	3	2	2	0	1	53.54 (47)
3	1000	8	6	2	2	1	1	2	54.98 (47)
4	600	8	5	3	2	2	0	1	57.39 (49)
5	200	8	7	1	2	2	1	2	64.27

									(53)
6	100	8	6	2	3	3	0	0	79.32 (62)
SEm (±)					1.527				
CD at 5 %					5.285				

4.7. Measurement of visible light intensities at different plant heights in the tomato plant canopy.

As different light intensities were observed to affect the growth of *Alternaria solani* pathogen, and the initiation of leaf spot symptoms in tomato plants, under *in vitro* studies, these were measured in tomato plant canopies (at different plant heights canopies i. e upper, middle, and lower plant canopies). These light intensities in tomato crops were measured at 9:00 am, 12:00 pm, 3:00 pm, and 6:00 pm respectively. The data on the light intensities in these canopies were recorded for the whole year i.e. during the rainy season, winter season, and summer season grown tomato crop, and is presented in Table 6.

The results (table 6) indicate that in general, the minimum light intensities were present in the lower canopy of tomato plants and were followed by the middle and upper plant canopy. The maximum light intensities were recorded at noon followed by 9:00 am and 3:00 pm. The lowest light intensities were obtained at 6:00 pm. These light intensities varied from week to week in the respective season and the month of the year. In general, the lowest light intensities of 100 Lx to 800 Lx were observed in the plant canopy during the months of June and July which are the months, in which more leaf spot/blight disease symptoms in tomato plants are observed under the field-grown tomato plants.

These results indicate that since the lower light intensities are available in the lower canopies of tomato plants, the disease initiation first takes place in the leaves at lower canopies of the plant. This statement is substantiated by the results that light intensities of 100 Lx to 600 Lx under laboratory conditions produced more growth of *Alternaria solani* and also caused the initiation of infection with more leaf area covered due to the infection.

Table 6. Status of visible light intensities (in Lx units) at different plant heights in field tomato plant canopy (in 3-month-old plant)

Month	Date	Light intensities observed (in lux) at different canopy level in tomato crop during a day period around the year											
		9:00 am			12:00 pm			3:00 pm			6:00pm		
		Upper	Middle	Lower	Upper	Middle	Lower	Upper	middle	lower	upper	middle	lower
June	04	85000	45000	25700	105000	71000	38000	56000	28000	98000	15000	9800	2700
	10	9100	7500	2300	12500	6500	2500	6500	2500	1400	180	150	<100
	17	65000	55000	28900	76800	45000	18300	35000	18900	6300	7800	4700	2100
	25	7800	5700	1500	11500	5200	1200	6700	1200	<100	900	350	<100
July	01	89400	53800	23600	105000	81000	42000	130000	86700	35000	19500	11100	3200
	07	54000	28200	12000	86000	35200	18200	123000	76500	31800	17300	9700	2500
	15	6700	4800	980	18900	9100	3000	45700	18700	7600	280	<100	<100
	26	95000	65800	48000	125000	88200	40200	150000	102000	30100	40000	21900	9000
August	01	1652	750	<100	25000	11200	4400	25000	14200	5300	11120	5200	850
	04	8050	4900	1370	52000	25000	12000	75000	37200	10900	30000	12800	4300
	07	17000	8000	2100	35000	27900	14000	50000	19900	6500	5200	1100	430
	14	4800	1200	<100	6000	2000	<100	8860	3200	9800	620	280	120
	21	9850	5100	3100	120000	81000	39000	150000	90000	25100	45000	20100	7800
	28	90000	62500	35700	140000	98000	42500	165000	101000	58900	55000	25100	6800
Sept	04	4600	1500	520	15000	7200	3000	9200	4100	1200	1200	750	210
	11	52000	25400	10800	82000	41000	18900	120000	87900	25800	5300	3100	1150
	18	18000	12300	3800	45000	21800	9000	7000	2900	500	4300	2300	890
	25	98200	68000	35000	110000	78200	28900	140000	105000	61000	38000	22300	11000
Oct	02	95000	56000	21900	105000	81900	37800	125000	92000	51000	20000	9800	3200
	09	98000	71000	39000	120000	87400	41000	100000	76800	18900	10200	4500	1100
	16	94000	62500	32800	100000	68900	29100	79000	32800	7600	12600	5600	1200
	23	48000	29000	15200	67000	39900	17400	94200	55400	14000	2800	1000	<100
	30	87000	41800	25100	103000	85000	38200	59000	21000	8700	3800	1300	490
Nov	06	75000	40200	22000	98000	58900	24000	105000	74900	14500	3000	1100	200
	13	92000	67000	31800	122000	91000	60100	140000	88200	29800	2000	990	<100
	20	57000	38500	12900	69200	35800	14200	125000	71200	22000	2500	1150	670

	27	98000	69800	41000	110000	79000	283000	78000	31900	8000	1500	740	220
Dec	02	67000	35100	15200	79000	51100	22000	59000	28900	12000	5000	3100	1100
	09	95000	59900	31300	120000	81000	38900	28000	15200	4200	450	190	<100
	16	88000	45300	22000	102000	79200	51000	39500	21500	6100	1500	760	210
	23	68000	21800	10400	87200	65500	42000	102000	78100	19800	3500	1200	560
	30	91000	65700	44000	103000	72200	49000	54200	27800	2100	2700	1000	350
January	06	72000	53700	29200	102000	68900	41000	35200	12900	1600	3600	1300	610
	13	78000	41000	21900	89000	53000	37600	115000	80000	64800	3900	1700	990
	20	98000	71000	47000	125000	91000	65000	161000	102000	39900	420	<100	<100
	27	68000	35900	18000	108000	77000	45200	125200	98000	75000	4000	2100	1100
Feb	03	75000	41600	21100	98000	62200	36900	107000	83000	21000	4300	2200	1300
	10	105000	86900	41000	125200	85000	54100	135000	91200	26700	15000	5100	2700
	17	94300	64200	39000	102500	69900	31400	140000	100000	41000	4200	1900	1200
	24	98000	68000	34000	115000	72500	37600	78000	51800	10900	17600	12500	4200
March	02	25000	12400	40200	82000	59200	25800	65000	46900	9800	3200	1290	720
	09	65000	31800	13200	89000	63500	31200	78200	54000	14200	14800	6300	1600
	16	89000	71100	37100	102000	69200	33000	115000	82000	27800	7200	3200	890
	23	69800	45000	13700	98000	61000	28900	54000	31000	10400	6400	3000	1200
	30	38900	25900	9900	55000	21800	10800	78000	32300	14900	3500	1400	450
April	07	8900	4700	250	12000	4300	280	45000	23100	7900	9000	6500	2200
	15	18000	8300	1800	75000	38000	14500	49000	25100	9600	7900	3500	1000
	21	55000	21900	8900	68000	32800	11600	47000	21900	11900	8100	5200	2800
	29	60200	38700	11000	88000	51000	34200	65000	36000	5400	9800	7100	3300
May	04	50200	32900	15000	98000	58900	36900	78200	41000	17600	11500	6200	960
	15	64800	30000	17800	91000	50400	13900	73000	32900	11000	12500	7500	1200
	22	49100	25000	14300	64900	36500	14500	45000	12800	5900	15000	64000	3100
	31	35600	12900	7500	70200	37200	19000	32900	11700	3700	7500	2800	980

These results on the effect of light intensities on the fungal growth and initiation of disease symptoms clearly indicate that light intensities play an important role in the growth of the *Alternaria* leaf blight pathogen and the initiation of diseased symptoms in tomato plants.

Though this is the first report on the role of light intensities on the growth of fungal pathogen, and the initiation of leaf spot disease symptoms by this pathogen in tomato plants, some workers studied the effect of illumination on the fungal plant pathogen. Aragaki (1963) reported that the culture of *A. tomato* grown under continuous illumination at several constant temperatures sporulated readily at 20^0 C but produced conidiophores without spores at 25^0 C or 30^0 C and reported the phenomenon of high-temperature x light on inhibition of sporulation. Stevenson and Pennypacker (1988) studied the conidial germination of *A. solani* irradiated with simulated sunlight (> 300 nm) and reported that the inhibition of conidial germination increased as the light intensity increased. Light intensity between 300 – 500 nm appeared to be responsible for the inhibition of spore germination. A wavelength of > 750 nm did not inhibit conidial spore germination indicating that spore inhibition was a true light-responsive phenomenon and not a response to increased temperature. At the same time, the effect of the diurnal cycle of light and darkness on fungal growth was reported by others. Hubballi et.al (2010) reported that the exposure of the fungus *A. alternata* to alternate cycles of 12 hours of light and 12 hours of darkness resulted in the maximum mycelial growth of the fungus as compared to continuous light and dark. Shabana et.al (2015) reported that continuous darkness was the most suitable light regime for the mycelial growth and sporulation of the CP01 and CP02 isolates of *Curvularia prasadii* while contentious or diurnal light was best for CP03 growth. However, all degrees of light regimes were suitable for the spore production of the isolate CP03, with no significant differences among them. Douglas (1972) observed that the sporulation of *A. solani* was significantly affected by both fluorescent illumination and temperature. However, cultures of *A. solani* grown at 20^0 C with 18 hours of light + 8 hours of darkness produced the greatest number of spores. Ray et.al (2009) studied the effect of light on the growth, sporulation, and toxin production of *A. alternata* in three light regimes i.e. 24 hours of continuous darkness where maximum growth was obtained, followed by 12 hours of alternate light and darkness and 24 hours of continuous light. Maximum phytotoxicity was caused by the toxin produced at 12 hours of alternate dark and light periods. Leach (1961) observed that near ultraviolet stimulated sporulation in the fungus *Helminthosporium oryzae* while visible light did not. Conidiophore development was initiated under irradiation but not in darkness. Both continuous irradiation and intermittent irradiation (2 hours ultraviolet, 2 hours dark cycle) caused the formation of conidiophores. Conidia developed

only when a period of irradiation was followed by a period of darkness. No conidia developed under continuous irradiation or in continuous darkness. Cohen and Rotem (1970) reported that extending the photoperiod from 6 to 48 hours before exposing *Pseudoperenospora cubensis* on cucumber to dark and moist conditions increased subsequent sporulation. The effect of light on subsequent sporulation depended on whether it had been applied before or after the exposure of plants to a light period. Singh et.al (2001) studied the effect of light/darkness on the percent germination and length of germ tubes in *Alternaria tenuissinia* and observed the maximum germination and germ tube length in total darkness followed by 8 h light/ 16 h darkness, and 16 h light/ 8 h darkness. 8 h light/16 h darkness cycle showed the minimum time (17.66 h) for initiation of the first spore formation. Maximum spores in a chain (7.66 h) were also formed at total darkness and 16 h light/8 h darkness (7.33 h) period. In 24 h light, sporulation was completely inhibited. Dube (2014) studied morphological and epidemiological characteristics of *A. alternata* and found that different light regimes influence the radial growth, sporulation, and pathogenicity of the pathogen. The combined (isolate-to-light effect) interaction has a significant influence on radial growth and disease severity. Koley et.al (2019) reported that the culturing of the fungus *Rhizoctonia solani* in different light intensities of 120, 20, and 5 lux and darkness shows that low light intensity is best for its growth and neither darkness nor high light intensities were preferred.

Conclusion

The natural sunlight intensities vary in the day and months during the three agricultural crop seasons (rainy season: June – September; winter season: October – January, and summer season: February – May) of a year. The sunlight intensities of 72,000 – 1, 08, 100 Lx (received from 12.00-2.00 pm) and 45, 000 – 1, 05, 200 Lx (received from 2.00- 4.00 pm) have a detrimental effect on the growth of a tomato leaf spot fungal pathogen *Alternaria solani*. The light intensities lower than 72, 000 Lx were favourable for the growth of this fungal pathogen. The lunar light intensities which were 0.1 to 0.7 Lx during the moon nights, have a negligible effect on the proliferation of fungal growth as compared to total darkness which produced more fungal growth. The diurnal treatment of 6 hours of light + 18 hours of darkness produced more fungal growth of *A. solani* as compared to continuous light without any dark period. To initiate *Alternaria* leaf spot disease symptoms in tomato plant leaves, the lower light intensities up to 2000 Lx were much more helpful than the higher light intensities under *in vitro* studies. Such lower light intensities were available in the lower-leaf canopies of the tomato plants under field conditions, which may be one of the reasons for the first initiation and development of *Alternaria* leaf spot and blight symptoms in lower leaves of tomato plants. The minimum light intensity of 180 Lx and 420 Lx was observed in the rainy and winter seasons respectively while the minimum light intensity of 3200 Lx was observed in the summer season. The varied light intensities in different months may be the reason for the prevalence of this fungus prominently during particular periods of the months of the year. These results of light intensities are useful to determine the structure of the shade-net or poly-houses, which can regulate the light intensities in the protected cultivations to hamper fungal growth and disease development by this pathogen.

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Conflict of Interest

There is no conflict of interest in this research manuscript