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PATHOPHYSIOLOGICAL VARIATIONS AMONG ISOLATES OF *COLLETOTRICHUM TRUNCATUM* CAUSATIVE AGENT OF ANTHRACNOSE OF TOMATO

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ABSTRACT

Tomato is an important vegetable crop worldwide and one of the most important vegetables in India. Andhra Pradesh, Karnataka, Maharashtra, Orissa, West Bengal, Tamil Nadu, Madhya Pradesh, Gujarat, Assam, Rajasthan and Punjab are found to be important states growing Tomato in India. Anthracnose caused by *Colletotrichum truncatum* is a major problem in India and one of the more significant economic constraints of Tomato production worldwide, especially in tropical and subtropical regions. Three isolates of *C.truncatum* were collected from different Tomato growing areas of Sangli District and their pathogenicity was proved under laboratory conditions. Effect of different pH levels, temperature, light intensity and seed quality tested against the growth of C. truncatum under in vitro. Results indicated that the growth of *C. truncatum* was maximum at pH range of 6.50-7.00 and temperature range of 25-30°C. Exposure of the fungus to alternate cycles of 12 hr light and 12 hr darkness resulted in the maximum mycelia growth of *C. truncatum* compared to the 24 hr exposure to either continuous light or dark. Potato dextrose agar medium supported significantly the maximum growth of all the three isolates of *C. truncatum*. Further, the strains were found to vary morphologically between the isolates under the study.

KEYWORDS: Tomato, Anthracnose, pathogenicity test, seed quality test, temperature, pH and light intensity.

INTRODUCTION

Tomato is one of the most popular andwidely grown vegetables in the world and the most popular in India belongs to the family 'Solanaceae'.[6] There are more than 400 different

varieties of tomatoes found all over the world. The area cultivated with tomato worldwide is about 17,00,000 ha for producing fresh tomato and around 18,00,000 ha for producing dried tomato; a total area of 37,29,900 ha with a total production of 2,00,00,000 tonnes.[2]

The most important and exporters of tomato include China India, Mexico, Morocco,Pakistan, Thailand and Turkey. Tomato is an imperative cash crop and India is the largest grower, consumer and exporter of dry tomatoes and other products to over 90 countries around the world.[14] Andhra Pradesh, Karnataka, Maharashtra, Orissa, West Bengal, Tamil Nadu, Madhya Pradesh, Gujarat, Assam, Rajasthan and Punjab are found to be important states growing tomato in India. Tomato plants are been attacked by more than 100 different types of pathogens during their growth and development. Tomato is prone to number of fungi, bacteria, virus, nematode and mycoplasma like organisms, which significantly affect its production and quality. Fortunately, only a few of them cause economic losses. Three species of *Colletotrichum* including *C. truncatum*, *C. acutatum* and *C. gloeosporioides* have been identified as the most important pathogens causing anthracnose of tomato. However, *C. truncatum* is predominant and appeared to be virulent compared to *C. gloeosporioides*.[7] Anthracnose, derived from a Greek word meaning 'coal', is the common name for plant diseases characterized by very dark, sunken lesions, containing spores.[4] *Colletotrichum truncatum* is most adhesive that adhere to the plant surface and remain latent until such physiological changes occur in the fruit and cause economic losses to the farmers due to low fruit quality and is our marketability many post harvest diseases of fruit exhibit the phenomenon of quiescence in which symptoms do not develop until fruit ripen.[1] Anthracnose causes extensive pre and post harvest damage to tomato fruits causing anthracnose lesions. Tomato anthracnose usuallydevelops under high humid conditions when rain occurs after the fruits have started to ripen, losses of up to 84%.[15] In anthracnose, the ripe fruits turning red get affected by this disease, whereas green fruits are not generally attacked. Symptoms on the fruit first appear as sunken, water-soaked lesions that expand rapidly on the fruit.[16] Fully expanded lesions are soft, sunken and range in color from dark red to tan black, generally described as anthracnose disease.[17] A small, black, circular spot initially appears on the skin of fruits andit then spreads in the direction of the long axis of the fruit, thus becoming more or less elliptical. As the infection progresses, the spots get either diffused becoming black, greenish orgrey in color or they are markedly delimited by a thick and sharp black outline enclosing a lighter black or straw colored area. On this discolored area numerous black acervuli arefound scattered. When a diseased fruit is cut open

the lower surface of the skin is found covered with minute, spherical, black stromatic masses or sclerotia of the fungus. Seeds are also covered by a mat of fungal hyphae. Such seeds turn rusty in color. Affected fruits are deformed in shape and lose their pungency. Ultimately the diseased fruits shrivel and dry up. Once the lesion develops, the fungus produces the conidia within 3 to 5 days at 30 °C and at 90% relative humidity. A dark growth of the fungus may be visible in these lesions with tan to pink concentric circles of spores evident in some cases. Disease symptoms occur only on ripened fruits occasionally appears on leaf as leaf spot and also on mature green fruits. *Colletotrichum truncatum* is an air- borne, seed-borne, and also soil-borne pathogen. It can survive in moistsoil and plant debris for several years. Thefungus can spread by rain splash and irrigation water. It can also spread by infected soil, farm tools, and shoes. The fungus can be carried by tomato seeds intraembryonally. Enzymes produced by *C. truncatum* can disrupt seed tissues. Conidial masses generated from the acervuli can serve as primary inoculums source. The fungus can spread from the seed tothe placenta of the fruit, then penetrating the developing ovules or young seed. Infection of seeds can also occur directly from the mother plant. Conidia can remain dormant on the surface of the testa until seed germination. The disease has been observed to occur in three phases, which includes seedlings blight or damping off stage, leaf spot or die back and anthracnose or fruit rot. During die-back the fungus causes necrosis of tender twigs from the tip to backwards.[13] Infection usually begins during flowering stage and flowers dry up. This drying up spreads from the flower stalks to the stem and subsequently causes die-back of the branches and stem. The entire branch or the entire top portion of the plant may withers away and partially affected plants bear fruits which are few and are of low quality. The dead twigs look water soaked to brown, becoming grayish white or straw colored in advancedstage of the disease. A large number of black dots (acervuli) are seen scattered all over the necrotic surfaces of the affected twigs. Sometimes the necrotic areas get separated from the healthy area by a dark brown to black band. Die-back usually appears after the stoppage of rain and when there is prolonged deposition of dew on the plants.[9,12] Conidia from acervuli and microsclerotia get dispersed in water splash and thus spread to the foliage and fruit.[1]

MATERIALS AND METHODS

Healthy and diseased ripen tomato fruits were randomly collected from the fields of Sangli region. The collected samples were sun dried and then kept in brown paper bags with proper lable and stored in the refrigerator at 4^{o}c for subsequent studies The pathogens were isolated on potato dextrose agar (PDA) medium from the diseased specimen showing typical

symptoms. The infected portion of the fruit was cut in to small bits, surface sterilized in 0.01% sodium hypochlorite solution for 30 seconds, washed in repeated changes of sterile distilled water and plated on to PDA medium. The plates were incubated at temperature (28 ± 2°C) for five days and were observed for fungal growth.[10] The fungi were purified by single spore isolation technique. The purified isolates were maintained on PDA plates and preserved in refrigerator for subsequent studies.

Seed quality test

Collected untreated seed samples were subjected to initial seed health testing by Standard Blotter Method. Seeds of each variety obtained from different locations were tested by employing the standard blotter method with three replicates. Three pieces of blotting paper of 90 mm size were moistened with distilled water and placed in 90 mm sterilized peri plates after draining exess water. Untreated seeds were placed at the rate of 25 seeds per petri plate at equal distance in each petri plate. The plates were incubated at (28±2°c) under alternate cycles of 12 hours UV light and darkness. After 8 days of incubation, the seeds were examined for the associated fungi.

Pathogenicity test

The pathogenicity of each isolated fungi was tested by pin-prick (PP) inoculation method under in vitro conditions, on detached semi- ripe fruits (turning red) of tomato. The spore suspensions of different isolates were obtained by adding 10 ml of sterilized distilled water to 14 day old culture grown on PDA in 30 ml test tube, maintaining the spore concentration of 10^7 spores / ml. The tomato fruits collected from the field were surface sterilized with 0.01% of sodium hypochlorite solution for 30 seconds and washed thrice with sterile distilled water to remove any trace of sodium hypochlorite. These tomato fruits were air dried by placing on sterilized blotting paper and subsequently inoculated with spore suspension of isolated fungus. In pin prick (PP) method, the sterilized needle was used and two pricks were given on the fruit, prior to the inoculation through spray of spore suspension. Sterile distilled water was used as control instead of the conidial suspension. The inoculated fruits were placed in a plastic container lined with four layers of paper towel moistened with sterile distilled water to produce a humid environment and later sealed with plastic sheet and incubated at 28±2^{0}C. The disease development was recorded by measuring lesion length of the diseased portion after 8 days of inoculation. Disease severity was scored on a 0–5 scale.

Table 1: Disease severity.

Disease grade	Disease reaction
0.0-1.0	Resistant (R)
1.1-2.0	Moderately resistant (R^+)
2.1-3.0	Moderately susceotible (S')
3.1-4.0	Susceptible (S)
4.1-5.0	Highly susceptible (S^+)

Morphological characterization

Each *C. truncatum* isolate was cultured on PDA for eight days. The cultures were grown under dark 12/12 hr light cycles at 28 ºC in an incubator. Three replications were performed by taking three pieces of 5 mm diameter plugs from the culture edge of each isolate and placing each of them onto a new PDA plate (9 cm diameter). The new cultures were grown under the same conditions as described above. Morphological characteristics of colony, conidia and appressoria were investigated.

Colony growth rate and characteristics Colony diameter of each isolate was measuredat 8 days after inoculation (DAI), and mean colony growth rate was determined. Colony characteristics including surface mycelium,color and mass conidia color was recorded.

Conidia shape and size

Conidia suspension was made from 8-day-old colony of each isolate. To investigate its conidia shape and size, 15 conidia were randomly selected from each replicate to measure their length and width under compound microscope with 100x magnifier.

Appressoria shape and size

Appressoria shape and size were investigated using modified slide culture technique. A culture was prepared by placing a piece of 10x10 mm WA and dropping conidia suspension of each isolate at the edge of the WA. The slide culture was covered with a sterile cover slip and was held in a petri dish toserve as a moisture chamber for 24 hrs at roomtemperature. 15 randomly chosen appressoria length and width were measured from eachreplicate.

Temperature

The effect of temperature on growth of the pathogen was studied. Different temperatures maintained for the growth of pathogen on PDA were 5, 10, 15, 20, 25 and 30°C.

Mycelial disc of 8 mm was used to inoculate Petri plates. Three replications were maintained

for each treatment. Inoculated plates were kept in incubator and temperature was adjusted to required level. The mycelia growth was recorded on the seventh day after inoculation.

pH

The effect of pH on the growth of the pathogen was studied as per the method followed by Kiryu using PDA medium. Different pH levels *viz.*, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 were used. The pH levels of the medium were adjusted in a digital pH meter using 0.1 N Hydrochloric acid and 0.1 N Sodium hydroxide. The media with different pH levels were sterilized, cooled and poured in the sterilized Petri plates in 20 ml quantities and allowed to solidify. The eight mm disc of pathogen was placed on the centre of the Petri plates. The plates were incubated at room temperature (28 ± 2°C) for ten days. The diameter of the mycelia growth was recorded. Three replications maintained for each treatment.

Light Intensity

The effect of light on the growth of pathogen was studied by exposing the inoculated culture to alternate cycles of 24 h light, 24 h dark and 12 h light and 12 h dark in an environment chamber maintained at room temperature (28 ±2°C). Mycelial disc of eight mm was used to inoculate Petri plates. Three replications were maintained for each treatment. Inoculated plates were kept in environment chamber and light intensity was adjusted to required level. The mycelia growth was recorded on eigth day after inoculation.

EXPERIMENTAL RESULTS

The isolation of *C. truncatum* from the infected fruits (Plate-1) was made as described in material and methods. The pure culture obtained was again sub cultured on potato dextrose agar slants and kept in the refrigerator at 5°C for further studies.

Seed quality test

Of twenty tomato seed samples tested only five seeds get germinated under moist conditions provided. Seeds with black colored fungal mycelia were not germinated as a result of infection by pathogen showed in plate-2.

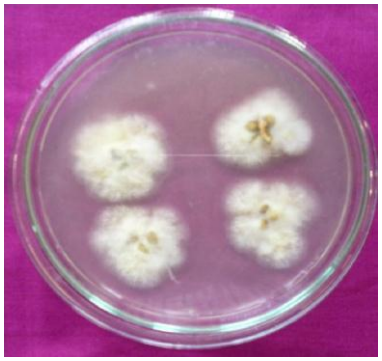

Fig 1: Isolation of pathogen from infected tomatoes.

Fig. 2: Tomato seeds on blotters colonized by Colletotrichum truncatum.

Table 2: Reaction of tomato fruits to *Colletotrichum truncatum* isolates.

Isolate	Lesion size on tomato (Cm±SE)
CT1	1.34±0.3
CT2	0.71±0.5
CT3	2.79±0.4

Values have been taken in triplicates

The isolates were grouped into resistant (R,R^+)and susceptible (S') categories to distinguish the isolates. Isolate SVUCC3 maximum size of lesion with an average length of 2.79±0.4 cm in fruits and in the remaining isolates it varied from 0.59±0.2 cm to 2.79 cm. The data on disease grade and disease reaction indicated differential interaction between host and isolates of pathogen. Isolate SVUCC3 was distinct from the remaining isolates as it gave susceptible reaction.

Table 3: Reaction of tomato fruits to pathogen.

Isolate	Disease grade and Reaction
CT1	(R^+)
CT2	(R)
CT3	(S')

(R) – Resistant, (R^+) - Moderately Resistant, (S') – Moderately suceptable.

Morphological characteristics of *C.truncatum* isolates

The morphological characteristics of three C.truncatum isolates were studied to characterize.

Colony growth rate and characteristics

Colony characteristics were classified basedon

1) colony growth rate 2) Surface mycelium description 3) Colony colour 4) Conidia mass colour.

Colony growth rate

Growth rate of three *C.truncatum* isolates on PDAwas ranged from 6.5 to 10.2 mm/day. The three isolates were divided into two groups according to colony growth rate, the growth rate less than 7.5 mm/day and the growth rate of 7.5 mm/day.

Surface mycelium

The *C.truncatum* isolates could be divided in to 2 groups according to the surface of mycelium: Uniform (U) and Concentric rings (C). Surface mycelium of 2 C.truncatum isolates were uniform, and 4 were concentric rings.

Colony and Conidia mass colour

Colour colour was divided in to three groups whitish to grey, whitish to brown. Conidia mass colour was divided into two groups 1) dark to grey and 2) brown to orange. Conidia are hyaline and fusiform.

Appressoria

Clavate forms were commonly observed.

Table 4: Cultural characteristics of isolates.

Isolate	Setae	Sclerotia	Conidia shape	Colony colour	Appressoria shape	Growth rate mm/day
CT1	Present	Absent	Falcate, fusiform	Greyish orange	Clavate	8.5
CT2	Present	Absent	Falcate, fusiform	Whitish Darkbrown	Clavate	6.6
CT3	Abundant	Absent	Falcate, fusiform	Whitish orange	Clavate	10.2

Effect of pH on the growth of *C. truncatum:* Effect of pH on the mycelial growth of different isolates of *C. truncatum* was studied. The results revealed that the maximum mean growth of 78.8±0.4 mm was observed at pH 6.5 in CT3 isolate followed by CT1 (75.5±0.5 mm) and CT5 (69.5±0.4mm). The lowest mean growth was recorded in SVUCC2 at pH-3 (19.2±0.6mm). The pH below six and above seven was detrimental tothe growth of pathogen (Table-5)

Table 5: Effect of pH on the mycelia growth of different isolates of *C.truncatum

Isolate	Colony diameter (mm ± SE) at different P^H									
	3.0	**3.5**	**4.0**	**4.5**	**5.0**	**5.5**	**6.0**	**6.5**	**7.0**	**7.5**
CT1	24.4±0.4	28.4±0.2	36.5±0.4	42.0±0.5	48.2±0.3	52.8±0.8	60.9±0.5	75.5±0.5	65.6±0.2	44.6±0.3
CT2	19.2±0.6	26.8±0.6	34.3±0.1	44.2±0.8	46.8±0.5	50.0±0.2	55.0±0.8	69.4±0.2	60.2±0.5	50.4±0.1
CT3	21.4±0.5	28.7±0.2	36.9±0.3	41.5±0.4	52.2±0.4	54.6±0.5	58.2±0.5	78.8±0.4	65.4±0.4	51.0±0.2

Effect of temperature on the growth of *C. truncatum*

Effect of different temperatures on the mycelial growth of different isolates of *C.truncatum* was studied. The results revealed that a highest mean growth of (72.4±0.2 mm) was observed at 25°C in SVUCC5 which was followed by 30°C (71.3±0.2 mm). Mean growth was found to be the lowest in SVUCC1at 5°C as it recorded 17.2±0.4 mm (Table-6).

Table 6: Effect of temperature on the growth of different isolates of *C. truncatum

Isolate	Colony diameter (mm ± SE) at different temperature						
	5°C	**10°C**	**15°C**	**20°C**	**25°C**	**30°C**	**35°C**
CT1	17.2±0.4	30.5±0.4	41.6±0.6	55.6±0.5	70.6±0.3	62.4±0.5	40.2±0.2
CT2	17.2±0.8	28.2±0.2	38.2±0.2	50.2±0.8	70.4±0.5	60.2±0.4	38.0±0.4
CT3	19.4±0.6	31.4±0.3	41.3±0.8	55.9±0.3	66.8±0.2	62.4±0.2	40.6±0.8

Values have been taken in triplicates

Effect of light intensity on the Growth of *C. truncatum*

Effect of light intensity on the growth of different isolates of *C. truncatum* was studied.The results showed that all the isolates grew well when they were exposed with alternate cycles of 12 h dark and 12 h light with mean growth of 72.6±0.2 mm followed by 24 h light exposure (48.3±0.4 mm) and the lowest growth of all isolates was found when exposedto 24 h dark (32.1±0.2 mm) (Table-7).

Table 7: Effect of light intensities on the growth of *Colletotrichum truncatum**

Isolate	Growth in (mm ± SE)		
	24 h light	24 h dark	12 h dark / light
CT1	54.3±0.6	39.6±0.8	70.6±0.2
CT2	42.4±0.2	36.2±0.5	69.5±0.8
CT3	43.1±0.8	34.4±0.6	72.5±0.4

Values have been taken in triplicates

DISCUSSION

Tomato (*Lycopersicon esculantum*L.) is one of the most important spices, used very widely throughout world. Anthracnose or fruit rot is the most important fungal disease of tomato in which cause tomato yield loss up to 84 per cent. Anthracnose disease is responsible for major economic losses in tomato production worldwide, especially in tropical and subtropical regions.[8] The assessment of plant diseases and their effects on yield normally involves five distinct process: developing a descriptive growth stage key for the particular crop species in question, developing methods to assess the incidence and severity of disease, developing statistically sound methods of sampling crop populations for assessment of the amount of disease, estimating the negative impact of particular levels of the disease on crop yield and quality and evaluating the economic benefit from various methods available for reducing the amount of disease. Hence, detailed studies were carried out on this pathogen on isolation, seed quality and proving pathogenicity. The pathogenicity study showed that the behaviour of *C. truncatum* isolates were homogeneous with regard to disease symptoms. However, variation in virulence or the level of disease (measured quantitatively) within the isolates was observed. The results of seed health tests confirmed the seed borne nature of this pathogen, a feature known in other *Colletotrichum* species[11], and its ability to infect internal seed tissues. Morphological characters including colony growth rate, surface mycelium, colony colours, mass conidia colours, condo size, and aspersoria size was studied in three isolates. Each character showed different phenotypes as follows: 1) colony growth rate: slow, medium, and fast 2) surface mycelium: uniform, concentric rings, sector, and irregular 3) colony colours: whitish to grey and whitish to brown 4) mass conidia colours dark grey to brown and orange. *C. truncatum* colonies appear as a dense white to dark grey mass that is dark brown on the reverse side, with abundant setae.[3] In studies on an optimum range of temperature, pH levels, light requirements for growth of fungus were 25°C to 30^{o}C, pH of 6.5 to 7.0, respectively. Temperature is most important physical, environmental factor in regulating the growth and reproduction of fungi. However, maximum growth and sporulation

of fungus was recorded at 25^{o}C temperature and 6.5 pH. At reduced pH, the cell membrane becomes saturated with the hydrogen ions which limit the passage of cations. The reverse could be obtained when medium are alkaline and accumulated hydroxyl ions preventing the passage of essential anions. An alternate cycles of 12 hour daylight under day light tubes and 12 hours darkness supported good growth and sporulation. Light has profound effect on the mycelial growth of pathogen Exposure of the colony to alternate cycles of light and darkness lead to maximum mycelial growth. The results agrees with findings of where in he found that alternate cycle of 12 h light and 12 h dark yielded maximum growth of fungus when compared to the continuous exposure of light or dark.[5]

CONCLUSION

This study revealed three fungal isolates were found associated with diseased tomato fruits, collected from Sangli region. By the morphological studies of the isolates growth rate was evaluated as slow, medium and fast ranging from 6.5 to 10.2 mm/ day. Abiotic factors such as temperature, pH and light intensities are also influenced the growth rate of *Colletotrichum truncatum*. Finally concluded that disease symptoms and morphological studies of the pathogen resembles as *colletotrichum truncatum* and are virulent as evident from pathogenecity test causes substantial reduction in fruit quality parameters of tomato.

REFERENCES

1. Bailey, J.A. and Jeger, M.J., *Colletotrichum*: Biology, Pathology and Control. Common Wealth Mycological Institute, Wallingford, 1992; 388.
2. FAO (Food and Agriculture Organization of the United Nations), FAO Production Yearbook 2001. FAO, Rome, 2003; 55(170): 333.
3. Holliday, P., Fungus Disease of Tropical crops. Cambridge University Press, 1980; 607.
4. Isaac, S., Fungal Plant Interaction. Chapman and Hall Press, London, 1992; p.115.
5. Kamanna, B.C., Epidemiology and control of anthracnose diseases of coffee incited by *Colletotrichum gloeosporioides*, 1996.
6. Makari, H.K., Ravikumer Patil, H.S., Abhilash, M. and Mohan Kumer, H.D., Genetic diversity in commercial varieties of chilli as revealed by RAPD method. *Indian Journal of Science and Technology*, 2009; 2: 91-94.
7. Montri, P., Identification of pathotypes of *Colletotrichum capsici* causing chilli anthracnose in Thailand. Thesis. Graduate school. Kasetsart University, 2008.

8. Pakdeevaraporn, P., Wasee, S., Taylor, P.W.J. and Mongkolporn, O., Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in *Capsicum*. *Plant Breeding*, 2005; 124(2): 206-208.
9. Rajeshwari, E., Chezhian, N., Saraswathy, S. and Subbiah, A., Dieback and fruit rot of chilli. Spice India, 2004; 17(4): 33.
10. Ratanacherdchai, K., Wang, H.K., Lin, F.C. and Soytong, K., RAPD analysis technique of *Colletotrichum species* causing chilli anthracnose disease in Thailand. *J. Agric. Technol*, 2007; 3(2): 211-219.
11. Richardson, M.J., An Annotated List of Seed-Borne Diseases. Phytopathological Papers. Kew, UK: Commonwealth Mycological Institute., 23 RN Okigbo; AO Emoghene, 2003. *J. Pl. Prot*, 1979; 20: 101-110.
12. Sharma, P.N., Kaur, M., Sharma, O.P., Sharma, P. and Pathania, A., Morphological, pathological and molecular variability in *Colletotrichum capsici*, the cause of fruit rot of chillies in the subtropical region of North-Western India. Journal of Phytopathology, 2005; 153(4): 232-237.
13. Siddique, M.R., Singh, D. and Gaur, A., Prevalence of chilli anthracnose fungus on seeds and its effective control. *Seed Research*, 1977; 5: 67-72.
14. Singhal, V., Chilli, spices and condiments. *Indian Agric*, 1999; 5: 420-420.
15. Thind, T.S. and Jhooty, J.S., Relative prevalence of fungal disease of chilli fruit in Punjab. *Indian Journal of Mycology and Plant pathology*, 1985; 15: 305-307.
16. Voorrips, R.E., Finkers, R., Sanjaya, L. and Groenwold, R., QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between *Capsicum annuum* and *C. chinense*. *Theoretical and Applied Genetics*, 2004; 109(6): 1275-1282.
17. Wharton, P.S. and Dieguez-Uribeondo, J., The biology of *Colletotrichum acutatum*. Anales del Jardin Botanico de Madrid, 2004; 61: 3-22.