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RESEARCH ARTICLE :

Survey, morphological identification and effect of culture media on the growth of *Lasiodiplodia theobromae* causing die back disease in acid lime

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SUMMARY : The die back disease of acid lime caused by *Lasiodiplodia theobromae* is a serious disease that causes severe yield loss. Hence, attempts were made to assess the incidence of the disease by survey in Tirunelveli and Thoothukudi districts. The survey report revealed that the die back of acid lime disease incidence ranged from 46.55 to 64.42 per cent. The mycelium of *L. theobromae* were hyaline, fast spreading, immersed, branched and septate and black coloured pycnidia were produced on the PDA medium. Conidia were initially hyaline, unicellular and ellipsoidal with granular content. Matured conidia were bi celled, thick walled dark brown in colour and it possessed longitudinal striations. Among the growth media tested, potato dextrose agar medium (90.00 mm) supported the highest mycelial growth followed by beetroot dextrose agar and nutrient agar medium (79.00 mm). The colony spreading rate was also found to be higher with the potato dextrose agar medium. While the King's B agar medium recorded the minimum mycelial growth (47.00 mm).

KEY WORDS :
Acid lime, Die back, *Lasiodiplodia theobromae*, Morphology, Culture media

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BACKGROUND AND OBJECTIVES

Citrus species are important among the most widely cultivated crops in different parts of the world (Wali *et al.,* 2013). Citrus ranks the third place among the fruits grown in India and thus assumes considerable importance. It grows much better in tropical and subtropical conditions and there is an increase in area under citrus cultivation from 90.7 thousand hectares in 1961 to 279.4 thousand hectares in 1988 to 1042 thousand hectares in 2012 (Anonymous, 2014).

Acid lime is cultivated largely in Andhra Pradesh, Maharashtra, Tamil Nadu, Gujarat, Rajasthan and Bihar and to a limited extent in other states. *Citrus* spp. are prone to attack by more than 150 diseases and disorders caused by fungal, viral and few bacterial pathogens right from nursery level to bearing stage resulting in considerable yield losses.In acid lime, bacterial canker (100%), bark eruption (10.67-20.86%), citrus greening (13.7-21.18%), root rot (6.13-18.18%), twig blight (10.25-21.88%) and longitudinal bark

and wood disease (3.8-6.88%) were the major diseases. Iron deficiency (5.98-10.67%) was also observed in most of the young orchards as documented by Nagalakshmi *et al.* (2014).

Pathogen: *Lasiodiplodia theobromae* :

The fungus is distributed ubiquitous; it is mainly confined in an area between 40°N to 40°S (Punithalingam, 1976). The fungus *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl is the synonymous of *Botryodiplodia theobromae,* a member of the Coelomycetes having pycnidia that are immersed to erumpent or superficial, pilose or glabrous, simple or often aggregated reaching upto 5 mm wide, with or without a stroma. Conidiogenous cells are holoblastic, annelidic and the conidia are initially hyaline, unicellular and ellipsoidal to oblong, thick walled with granular contents. Mature conidia are two celled, cinnamon to fawn or dark brown, usually 20-30 x 10-15 ìm in size, with differently pigmented longitudinal bands resembling striations (Punithalingam, 1979).

Uduebo (1975) reported that the hyaline non-septatepycnidiospores were highly vesiculated in light microscope while the pigmented septate one exhibited longitudinal hyaline striations. Phipps and Porter (1998) reported that *L. theobromae* produced two types of pycnidiospore, single celled hyaline (immature pycnidiospore) and two celled dark brown spores (mature pycnidiospore). Khanzada *et al.* (2004) reported that conidia of *L. theobromae* isolated from mango twigs were initially hyaline, unicellular and sub ovoid to ellipsoidal with a granular content. Mature conidia were two celled, cinnamon to dark brown, thick walled, ellipsoidal, often with longitudinal striations of 18 to 30 x 10-15µm in size.

B. theobromae causes shoot blight, die back, twig blight, cankers, etc., mainly in woody plants including fruit and tree crops such as pear, apple, *Albiziafalcataria*, peach, mango, avocado, *Citrus* spp., *Eucalyptus* spp., *Azadirachta indica*, *Pinus* spp. had documented by Sharma *et al.* (1984); Verma and Cheema (1984); Mattos and Ames (1986); Sharma and Sankaran (1987); Darvas and Kotze (1987); Britton *et al.*(1990); Sangchote (1991); Cedeno and Palacios-Pru (1992); Cedeno *et al.*(1995) and Mohali *et al.* (2005).

Effect of culture media on the growth of *L. theobromae* :

Qureshi and Meah (1991) observed fastest liner growth of *B. theobromae* on Richard's agar, mango leaf extract agar and on potato dextrose agar medium (PDA). They recorded the highest number of pycnidia on mango leaf extract followed by potato dextrose agar. Phipps and Porter (1998) reported that morphological features of the fungus on diseased plants and potato dextrose agar were consistent. Several other workers also stated that PDA was the best media for the mycelial growth of *L. theobromae*as per the findings of Xu*et al.* (1984) and Maheswari *et al.* (1999). The fungus grew and sporulated at 10-40°C, the optimum being 25-30°C and the highest mycelial growth (78-90mm) and sporulation (27-38 conidia /0.01 ml) were observed on PDA. The maximum pigment formation occurred on potato dextrose agar medium (Black 75% and white 25%) and minimum on potato carrot agar medium (Black 10% and white 90%). Maximum pycnidia formed on Czapek's (35/plate) medium and minimum on potato carrot agar medium (6/plate) medium as reported by Alam *et al.* (2001).

Khanzada *et al.* (2004) observed that potato sucrose agar (PSA), corn meal dextrose agar (CMDA) and yeast extract mannitol agar (YEMA) were most suitable for the mycelial growth of the *L. theobromae* whereas potato carrot agar (PCA) was not suitable for either mycelial growth or pycnidia production. Khanzada *et al.* (2004) observed that on potato sucrose agar colonies were initially white, soon becoming black and fast spreading with immersed and superficial, branched, septate mycelium of *L. theobromae*. Ko *et al.* (2004) reported *Lasiodiplodia theobromae* as a causal agent of kumquat die back in Taiwan and provided that the fungus produced greyish black colonies on V8 agar and black ostiolatepycnidia (125 to 650 µm in diameter) with ovoid to elongate conidia (20 to 32 × 12 to 16 µm) on autoclaved whole wheat grains that were placed on V8 agar. Young conidia were hyaline and non-septate whereas mature conidia were brown, one septate and striate. Yildiz *et al.* (2014) documented that the fungus produced white colonies and later turned olivaceous black with dense aerial mycelium after four to five days incubation at 27°C. Dark brown to black pycnidia that formed on 20-30 day old pure cultures under day light conditions produced abundant conidia that were two celled, thick walled, and oval shape with longitudinal striations on potato dextrose agar medium.

Objectives:

– To study the die back disease in acid lime growing

areas of Tirunelveli and Thoothukudi districts

– To study the morphological characters of a pathogen

– To study the effect of culture media on the growth of *L. theobromae.*

RESOURCES AND METHODS

Collection of die back diseased samples :

A survey was conducted in acid lime growing areas of Tirunelveli and Thoothukudi districts during the year 2014 - 15. Acid lime trees showing die back symptoms were collected from different places of Tirunelveli and Thoothukudi districts. The samples were brought to the laboratory for microscopic examination, isolation, purification and the effect of media on pathogen is studied.

Occurrence of die back disease in acid lime growing areas of Tirunelveli and Thoothukudi districts :

An intensive field survey was conducted during 2014 - 15 to assess the occurrence of die back disease in acid lime growing areas of Tirunelveli and Thoothukudi districts in Tamil Nadu. The die back incidence was assessed by counting the number of affected plants out of total number of plants in each garden. In each area three fields were assessed and the mean disease incidence was calculated by using the following formula (Latha, 2009).

$$\text{Per cent infection (\%)} = \frac{\text{Number of plants affected by die back}}{\text{Total number of plants observed}} \text{x100}$$

Isolation of the pathogen *Lasiodiplodia theobromae*:

Isolation of pathogen from samples was done on potato dextrose agar (PDA) medium. Sections of 3 - 5 mm diameter were cut using sterilized scalpel from the die back showing twigs. Pieces of diseased twigs were surface sterilized in 2 per cent sodium hypochlorite solution for three minutes and washed in three changes of sterile distilled water. Sterilized pieces were blotted dry in between sterile filter paper and inoculated on PDA in Petri dishes. Petri dishes were incubated at 28±2°C. Colony growth was observed for seven days. Hyphal tip of cultures were transferred to obtain pure cultures. Identification of cultures was done by microscopic examination and by pycnidial production.The pathogen was purified by single hyphal tip method as described by Riker and Riker (1933) and maintained in PDA slants at 4ºC throughout the study.

Effect of solid culture media on the growth of *L. theobromae* under *in vitro*:

The effect of culture media on the growth and sporulation of *L. theobromae* were evaluated. Agar media *viz.*, beetroot dextrose agar, carrot dextrose agar, Czapok'sDox agar, King's B, modified Czapok'sDox agar, nutrient agar, oats meal dextrose agar, potato dextrose agar and rose bengal agar were poured in 90 mm diameter Petri plates. The culture was grown as per the procedure described by Luo *et al.* (2011). Five mm diameter agar plugs were removed with a sterile cork borer from the edges of colonies and one such plug was placed in the center of each 90 mm Petri plate containing this media. Plates were then wrapped with parafilm and incubated at 28ºC. There were three replicate plates of each medium. The colony diameter in each plate was measured at 24h interval and daily radial growth rates were calculated. After 15 days of inoculation, the number of pycnidia produced per plate was recorded

Effect of liquid culture media on the mycelial dry weight of *L. theobromae* under *in vitro* :

The effect of liquid culture broth *viz.*, beetroot dextrose, carrot dextrose, Czapok'sDox, King's B, modified Czapok'sDox, nutrient medium, oats meal dextrose, potato dextrose and rose bengal were prepared separately without adding agar. Luo *et al.* (2011) procedure was adopted for culturing of fungus.One hundred ml of each broth was distributed uniformly into 250 ml Erlenmeyer conical flasks separately and autoclaved at 15 lbs for 20 min and cooled. Then each flask was inoculated with a five mm disc pathogen culture separately. Three replications were maintained for each treatment. Ten days after incubation the mycelial mat of the pathogen was filtered through a preweighedWhatman No.1 filter paper separately and dried in hot air oven at 100º C for 24 hours. The mycelial dry weight of the pathogen in each treatment was obtained by subtracting the weight of the filter paper.

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads:

Collection of die back diseased samples:

The acid lime trees showing typical die back

symptoms were collected from 16 villages of Tirunelveli and Thoothukudi districts of Tamil Nadu. The pathogen was isolated from the die back disease affected acid lime twigs and sub cultured. The cultures were maintained in the slants containing potato dextrose agar medium at room temperature (28±2ºC) for further studies.

Occurrence of die back disease in acid lime growing areas in Tirunelveli and Thoothukudi districts :

A field survey was conducted during August-January, 2014-15 in major acid lime growing areas of Tirunelveli and Thoothukudi districts in Tamil Nadu. The survey report revealed that the die back of acid lime disease incidence ranged from 46.55 to 64.42 per cent. The die back disease was maximum in Chinnthamani (64.42%) followed by Rayagiri (61.56%) and Vannikonenthal (59.82%) of Tirunelveli district. The minimum incidence of 46.55 per cent was recorded in Ariyakulam of Tirunelveli district (Table 1).

Table 1: Occurrence of die back disease in acid lime growing areas in Tirunelveli and Thoothukudi districts

Sr. No.	Location	District	**Per cent disease incidence (PDI)
1.	Puliyankudi	Tirunelveli	58.17 d (49.70)
2.	Chinnthamani	Tirunelveli	64.42 a (53.38)
3.	Vasudevanallur	Tirunelveli	51.85 k (46.06)
4.	Rayagiri	Tirunelveli	61.56 b (51.68)
5.	Sankarankovil	Tirunelveli	52.43 i (46.39)
6.	Sivagiri	Tirunelveli	57.29 e (49.19)
7.	Panniyur	Tirunelveli	54.57 g (47.62)
8.	Kadayam	Tirunelveli	55.28 f (48.03)
9.	Vannikonenthal	Tirunelveli	59.82 c (50.66)
10.	Killikulam	Thoothukudi	58.39 d (49.83)
11.	Ariyakulam	Tirunelveli	46.55 l (43.02)
12.	Irumangalam	Tirunelveli	52.11 kj (46.21)
13.	Subramaniyapuram	Tirunelveli	53.45 h (46.97)
14.	Punniyapuram	Tirunelveli	52.18 kj (46.25)
15.	North pudur	Tirunelveli	55.17 f (47.96)
16.	Ramanathapuram	Tirunelveli	51.85 kj (46.06)

**Mean of three replications
Values in parentheses are arcsine transformed
The treatment means are compared using Duncan multiple range test (DMRT). In a column, means followed by a common letter (s) are not significantly different (P=0.05)

Morphology of the pathogen :

The mycelium of *L. theobromae* were hyaline, fast spreading, immersed, branched and septate and black colouredpycnidia were produced on the PDA medium. Conidia were initially hyaline, unicellular and ellipsoidal with granular content. Matured conidia were bi celled, thick walled dark brown in colour and it possessed longitudinal striations. Based on the symptoms and morphological characters of the fungus, it was identified as *Lasiodiplodia theobromae* (Punithalingam, 1976).

Effect of culture media on the growth of *L. theobromae* under *in vitro*:

The various culture media *viz.*,beetroot dextrose agar, carrot dextrose agar, Czapok'sDox agar, King's B agar, modified Czapok'sDox agar, nutrient agar, oats meal dextrose agar, potato dextrose agar, rose bengal agar medium were tested for the growth of *L. theobromae*. Among the solid growth media tested, potato dextrose agar medium (90.00 mm) supported the highestmycelial growth followed by beetroot dextrose agar and nutrient agar medium (79.00 mm). The colony spreading rate was also found to be higher with the potato dextrose agar medium. While the King's B agar medium recorded the minimum mycelial growth (47.00 mm) (Table 2). Among the liquid growth media tested, potato dextrose broth (1.101 g) recorded the highestmycelial dry weight followed by beetroot dextrose broth (1.070 g) and nutrient broth (1.052 g). The maximum pycnidial production per plate was recorded in potato dextrose agar (51 numbers) (Table 3).

The occurrence of die back disease in acid lime by *Lasiodiplodia theobromae* was reported by Rashid *et al.* (2009) and per cent disease incidence recorded was

Table 2: Effect of solid culture media on the growth of *L. theobromae* under *in vitro*

Tr. No.	Medium	**Mycelial growth (mm)	No. of pycnidia/ plate
T_1	Beetroot dextrose agar	79.00 b	46
T_2	Carrot dextrose agar	77.50 b	41
T_3	Czapok's dox agar	77.50 b	6
T_4	King's B agar	47.00 e	0
T_5	Modified Czapok'sDox agar	52.00 d	4
T_6	Nutrient agar	79.00 b	27
T_7	Oats meal dextrose agar	71.50 c	35
T_8	Potato dextrose agar	87.00 a	51
T_9	Rose bengal agar	72.00 c	19

**Mean of three replications
The treatment means are compared using Duncan multiple range test (DMRT)
In a column, means followed by a common letter (s) are not significantly different (P=0.05)

Table 3: Effect of liquid culture media on the mycelial dry weight of *L. theobromae* under *in vitro*

Tr. No.	Medium	**Mycelial dry weight (g)	No. of pycnidia/ plate
T_1	Beetroot dextrose broth	1.070 b	44
T_2	Carrot dextrose broth	1.045 b	39
T_3	Czapok's dox broth	1.037 b	4
T_4	King's B broth	0.900 e	0
T_5	Modified Czapok's dox broth	0.920 d	2
T_6	Nutrient broth	1.052 b	21
T_7	Oats meal dextrose broth	0.962 c	30
T_8	Potato dextrose broth	1.101 a	48
T_9	Rose bengal broth	0.970 c	14

**Mean of three replications
The treatment means are compared using Duncan multiple range test (DMRT). In a column, means followed by a common letter (s) are not significantly different (P=0.05)

22.58 per cent. Latha (2009) reported that the occurrence of collar and root rot incidence was more in Coimbatore district (75.32%) as compared to Tanjore district (39.44 %). *Lasiodiplodia theobromae* produces hyaline non-septatepycnidiospores, single celled hyaline (immature) and two celled matured, dark brown spores with longitudinal striations (Uduebo, 1975; Phipps and Porter, 1998 and Khanzada *et al.*, 2004). The mycelial growth of *B. theobromae* isolates were classified as fluffy or depressed, uniform to irregular and cottony white turning to black. Pycnidia were produced either on the edge, centered or scattered on Petri dishes after 20 to 34 days of incubation as described by Shah *et al.* (2010).

The result from the present investigation showed that potato dextrose agar encouraged the maximum mycelial growth and mycelial dry weight as well as pycnidial production followed by beetroot dextrose agar and nutrient agar medium.Qureshi and Meah (1991) reported mycelium has fastest linear growth on Richards's agar, mango leaf extract agar and PDA. They recorded the highest number of pycnidia on mango leaf extract followed by PDA. Alam *et al.* (2001) recorded the highest mycelium growth of *B. theobromae* on PDA and the maximum pycnidia on Czapek'sDox agar. In contrast, Alasoadura (1970) observed the maximum stromata of *L. theobromae* on malt agar and oatmeal agar. The size and number of pycnidia varied greatly within the substrate and biggest pycnidia was produced in nutrient rich medium as reported by Sabalpara *et al.* (1991). Khanzada *et al*. (2006) reported that the potato sucrose agar and yeast extract mannitol agar (YEMA) were the most favourable for fast radial growth of mycelium of *L. theobromae*. The highest number of pycnidia per plate was formed on yeast mannitol agar medium. The radial mycelial growth and pycnidial production of *L. theobromae* was medium dependent. Luo *et al.* (2011) reported that the potato dextrose agar supported the mycelial growth and pycnidial production.

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REFERENCES

Alam, M.S., Begum, M.F., Sarkar, M.A., Islam, M.R. and Alam, M.S. (2001). Effect of temperature, light and media on growth, sporulation, formation of pigments and pycnidia of *Botryodiplodia theobromae* Pat. *Pak. J. Bio. Sci.*,**4**(10):1224-1227.

Alasoadura, S.O. (1970). Culture studies on *Botryodiplodia theobromae* Pat. *Mycopathologiaet Mycologiaapplicata.*, **42**:153-160.

Anonymous (2014). *National Research Center for Citrus*, Nagpur (M.S.) INDIA.

Britton, K.O., Hendrix, F.F., Pusey, P.L. and Okie,W.R.(1990). Evaluating the reaction of peach cultivars to infection by three *Botryosphaeria* species. *Hort. Sci.*, **25** : 468-470.

Cedeno, L. and Palacios-Pru, E. (1992). Identification of *Botryodiplodia theobromae* as the cause of lesions and gummosis on citrus. *Fitopatol. Venez.*, **5**:10-13.

Cedeno, L., Carrero, C., Mohali, S. and Palacios-Pru, E.(1995). Identification of regressive death in perchita caused by *Lasiodiplodia theobromae* in Venezuela. *Fitopatol. Venez.*, **8** : 11-14.

Darvas, J.M. and Kotze,J.M. (1987). Fungi associated with pre- and postharvest diseases of avocado fruit at West falia Estate, South Africa. *Phytophylactica*, **19**:83-85.

Khanzada, M.A., Lodhi, A.M. and Shahzad, S. (2004). Decline and gummosis diseases of mango in Sindh caused by *Lasiodiplodia theobromae. Online. Plant Health Progress*, doi:10.1094/PHP- 204-0302-01-DG.

Khanzada, M.A., Rajput, A.Q. and Shahzad,S. (2006).Effect of medium, temperature, light and inorganic fertilizers on *in vitro* growth and sporulation of *Lasiodiplodia theobromae* isolated from mango. *Pak. J. Bot.*, **38**(3):885-889.

Ko, W.H., Wang, I.T. and Ann,P.J.(2004). *Lasiodiplodia theobromae* as a causal agent of kumquat dieback in Taiwan. *Plant Dis.*, **88** : 1383.

Latha, P. (2009).Ecologically sustainable management of collar and root rot [*Lasiodiplodia theobromae* (pat.)] Griffon & Maubl.) disease in Physic nut (*Jatrophacurcas* Linn.*)*,Ph. D (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, T.N. (INDIA).

Luo, M., Dong, Z.Y., Bin, S.Y. and Lin,J.T. (2011). First report of fruit rot disease on pomelo caused by *Lasiodiplodia theobromae* in China. *Plant Dis.,* **95**(9):1190

Maheswari, S.K., Singh, D.V. and Sahu,A.K. (1999). Effect of several nutrient media, pH and carbon sources on growth and sporulation of *Alternariaalternata*. *J. Mycopathol. Res.,***37:**21-23.

Mattos, L. and Ames,T. (1986). *Botryodiplodia theobromae*, pathogenic on apple. *Fitopatología,* **12**:26-32.

Mohali, S., Burgess, T. and Wingfield, M.J. (2005). Diversity and host association of the tropical tree endophyte *Lasiodiplodia theobromae* revealed using simple sequence repeat markers. *Forest Path.,* **35**:385-396.

Nagalakshmi, T., Gopi, V., GouriSankar, T., Sarada, G., Mukunda Lakshmi, L., Ramana, K.T.V. and Gopal, K.(2014). Status of diseases in sweet orange and acid lime orchards in Andhra Pradesh, India. *Int. J. Curr. Microbiol. App. Sci.,***3**(5):513-518.

Phipps, P.M. and Porter, D.M. (1998). Collar rot of peanut caused by *Lasiodiplodia theobromae*. *Plant Dis.*, **82**:1205-1209.

Punithalingam, E. (1976). *Botryodiplodia theobromae*. CMI description of pathogenic fungi and bacteria no. 519: *Commonwealth Mycological Institute*, Kew Surrey, England.

Punithalingam, E. (1979). Plant diseases attributed to *Botryodiplodia theobromae.* In: *Biblioteca Mycologica*. *J. Cramer*, 123.

Qureshi, S.U. and Meah, M.B. (1991). Studies on physiological aspects of *Botryodiplodia theobromae* Pat., causing stem end rot of mango. *Bangladesh J. Bot.,* **20** (1) : 49-54.

Rashid, M.H., Hossain, M.T., Abdullah, M.R., Hossain, S.M.M. and Hasan,M.S. (2009). Survey on diseases of commercial Agroforest nurseries. *J. Agrofor. Environ.,***3**(1):133-136.

Riker, A.J. and Riker,R.S. (1933). Introduction of Research on Plant Disease. *John Swift Co.*, St. Louis, USA.

Sabalpara, A.N., Vala, D.G. and Solanky,K.U. (1991). Morphological variation in *Botryodiplodia theobromae* Pat. causing twig blight and die back of mango. *Acta Hort.,* **291**:312-316.

Sangchote, S. (1991). *Botryodiplodia* stem end rot of mango and its control. *Acta Hort.,* **291** : 296-303.

Shah, M.D., Verma, K.S., Singh, K. and Kaur, R.(2010). Morphological, pathological and molecular variability in *Botryodiplodia theobromae* (*Botryosphaeriaceae*) isolates associated with die back and bark canker of pear tree in Punjab. *India. Gent. Mol. Res.,***9**(2):1217-1228.

Sharma, J.K., Mohanan, C. and Florence, E.J.M. (1984). A new stem canker disease of *Eucalyptus* caused by *Botryodiplodia theobromae* in India. *Trans. Br. Mycol. Soc.,* **83**:162-163.

Sharma, J.K. and Sankaran, K.V. (1987). Diseases of *Albiziafalcataria*in Kerala and their possible control measures. *Kerala Forest Res. Inst. Res. Rep.,* **47**: 50.

Uduebo, A.F. (1975). Fine structural studies on the pycnidiospores of *Botryodiplodia theobromae* Pat. *Ann. Bot.,* **39** : 605-610.

Verma, K.S. and Cheema, S.S.(1984). *Botryodiplodia theobromae* the causal agent of die back and bark canker of pear in Punjab. *Indian Phytopathol.,***37**: 325-327.

Wali, S., Munir, F. and Mahmood,T. (2013). Phylogenetic studies of selected citrus species based on chloroplast gene, rps14. *Int. J. Agric. Biol.,***15**:357-361.

Xu, S.O., Yuan, S.Z. and Chen, X.C. (1984). Studies on pathogenic fungus (*Alternariatennuis Nees*) of poplar leaf blight. *J. North East Forestry Inst.,***12**:56-64.

Yildiz, A., Benlioglu, K. and Benlioglu, S.(2014). First report of strawberry dieback caused by *Lasiodiplodia theobromae*. *Plant Dis.,* **10**:1094.