Morphological and Molecular Identification of Fungi Causing Canker Disease on Melia Azidarh Trees in Some Regions of Mosul Provinces, Iraq

Anwer Noori Alkhero\(^1\) and Zainab Waadallah Rassem\(^2\)

\(^1\)College of Agriculture and Forestry, University of Mosul, Mosul, Iraq.
\(^2\)Email: Zainab.agp73@student.uomosul.edu.iq

Abstract

The aim of this study was to identify the fungi associated with canker disease on *Melia azidarh* trees inside Mosul University campus and the presidential palaces regions in Mosul Province, Iraq. Results of isolation showed the presenting of the fungi (*Nattrassia mangiferae*, *Neoscylitidium dimidiatum* Penz., *Fusarium graminearium* Schw., *Alternaria brassicicola* Schw., *Aspergillus* sp. and *Penecillium* sp.), which accompanied with the samples displayed canker symptoms during the period from April to December / 2020, the maximum of dominance was 85% for the fungus *Neoscylitidium dimidiatum* in August, while the lowest was 49% in April for the same year, followed by *Fusarium graminearium* with 38% in December, while the lowest percentage was 4% in October, then *Alternaria brassicicola* Schw. was 25% in April and the lowest value was 0% in August, followed by *Aspergillus* sp. and *Penecillium* sp. with low isolation percentages the maximum of which 25% and the lowest is 0% in August. When studying the pathogenicity of the isolated fungi, the results showed a high pathogenic effect in terms the length, diameter and the area of cankers symptom. Based on the results of the molecular diagnosis, the morphological identification was confirmed and it was clear that *Fusarium austroamericanum*, detection is considered the first record of this fungus in Iraq *Melia azidarh* trees.

Keywords: Melia azedarach, Canker disease, Morphological, Molecular.

1. Introduction

*Melia azedarach* L. trees belong to Meliaceae family, and its habitat is in Bloshestan, Kashmeer and India. They are also spreading in West Asia countries, Africa (Nigeria) and Latin America. They grow in the dry areas with rainwater of 150-300 mm annually and water should be secured for them when needed. They are caducous trees with violet aromatic flowers and their fruits are green that turn to yellow when ripen [1]. The adult trees are about 25 m high [2] and they need fertile soil with good drainage and should be low acidity (3.5 – 6.5) [3]. The crown of this tree is umbrella-like shape, and its leaves are like feather appearance that could be 90 cm in length, the leaflets are spear-shaped and egg-like with a pointed top and a cogged edge. Furthermore, they contain a high portion of nutrients and proteins that was estimated as 12% [4]. This tree is planted in the north of Iraq such as in Nineveh governorate and the South regions [5]. Cankers symptoms are dead parts of the bark on the main trunk or branches of the trees that were caused by the mechanical injury or plant pathogens especially the fungi and bacteria. Most of these pathogens are unable to penetrate the bark directly but they can colonize the wounded tissues and then may cause diseases that inflict to serious damages to the trees when affecting the bark in a certain area and this eventually leads to the death of the branch or the main trunk [6]. Pathogens are considered one of the most prominent factors in terms of planting the trees in the world, especially fungi in addition to that they cause deterioration of the wood produced from these infected trees, which eventually decrease their touristic importance as the landscape of the forest becomes distorted as well as causing human and material damages by the fall of their huge trunks [7]. This disease is one of the most serious diseases that affect these trees in Mosul and other provinces of Iraq [8-12]. Thus, the aim of this study was identifying morphologically and molecularly of the causal agents of this disease in some regions of Mosul Province, Iraq. The research aims:

- Recording the fungi species that accompany *Melia azidarh* trees cankers.
- Isolating and morphological diagnosis of the pathogens and then conducting the molecular identification using the polymerase chains replication technique.
- Testing the pathogenicity of the fungi associated with cankers.
2. Materials and Methods

2.1 Isolation and diagnosis of pathogens

Fungi were isolated from the trees of *Melia azedarach* that showed cankers in the gardens of Mosul University within a period averaged by two months per a survey. These months included April, June, August, October and December 2020, to monitor the development of the disease during the period of the study. Random samples were taken on which the symptoms of canker were evident to the laboratory to conduct the isolation using a method [13]. Fungal growths around the selected pieces were examined and they were purified in Petri plates that contain PDA (Potato dextrose agar), and Chloramphenicol antibiotic (250 mg/l). After that the plates were incubated at temperature of 25 ± 2 ºC. Also, purification was conducted in test tubes that contain the PDA (Potato dextrose agar) as a slant agar and kept in the refrigerator at 5ºC to be used in the subsequent experiments. Fungi were examined by the microscope to be morphologically diagnosed based on the international classification standards keys to the genus level [14], [15] as well as to the species rank [16-19]. In addition to these, the percentage of isolates was determined for each fungus using the Polymerase Chain Replication (PCR) technology at the central laboratory of the College of Agriculture and Forestry.

2.2 The Molecular diagnosis of fungi using Polymerase chain reaction.

2.2.1 primer design

The Primers were designed depending on the Gen Bank gene location and for each of the following fungi:

- The *Neoscytadium dimidiatum* fungus, the Gene which had been tested, it contained two sequences, the first sequence (MN447201) and the sequence second (LC474120).
- The *Fusarium graminearium* fungus was selected by gene (M86819).
- The *Alternaria brassicicola* fungus, the selected gene had contained two sequences, the first codon (18s) and the second gene codon (MT635275)
- Requesting Polymerases.

**Table 1.** The Primers were ordered from the Canadian Promege company to obtain the following Polymerases.

| Fungi                    | Forward and Reverse prefixes                  |
|--------------------------|-----------------------------------------------|
| Neoscytidad dimidiatum   | 1-Forward CAGATAGGCTGGCGG 1- Reverse CGTTGGGCGCCATAGA |
|                         | 2-Forward CGGCCCGATCCTCCCC 2- Reverse CGGCCCGATCCTCCCC |
| Fusarium austroamericanum| Forward CAGATAGGCTGGCGG Reverse CGTTGGGCGCCATAGA |
| Alternaria brassicicola  | 1-Forward GACTGTGAAACTGCG 1- Reverse TAAGTTCAGCCTTG |
|                          | 2-Forward GTTAGGTCTCTCGTAG 2- Reverse TTCCCTCCCCGCTAT |

2.2.2 Extraction of deoxygenated DNA

Experiments were carried out for the molecular analysis of the isolates of the fungi, Neoscytidad dimidiatum, Fusarium austroamericanum, Alternaria brassicicola, in the central laboratory of the College of Agriculture and Forestry / University of Mosul. To extract DNA, follow these steps:

For optimal performance, add beta-mercaptoethanol (user supplied) to the Genomic Lysis Buffer to final dilution of 0.5 % (v/v) i.e., 500 µl per 100 ml.

- Add 50 – 100 mg (wt weight) fungal or bacterial cells that have been resuspended in up to 200 µl of water or isotonic buffer (e.g, PBS) to a ZR BashingBead™ Lysis Tube (0.1 mm & 0.5 mm). Add 750 µl BashingBead™ Buffer to the tube.
- Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for ≥ 5 minutes.
- Centrifuge the ZR Bashing Bead™ Lysis Tube (0.1 & 0.5 mm) in a microcentrifuge at 10000 × g for 1 minute.
- Transfer up to 400 µl supernatant to a Zymo-Spin™ III-F Filter in a Collection Tube and centrifuge at 8000 × g for 1 minute.
- Discard the flow through from the Collection Tube and repeat Step 6.
• Add 200 μl DAN Pre-Wash Buffer to the Zymo-Spin™ ICR Column in a new Collection Tube and centrifuge at 10000 × g for 1 minute.
• Add 500 μl DAN Wash Buffer to the Zymo-Spin™ ICR Column and centrifuge at 10000 × g for 1 minute.
• Transfer the Zymo-Spin™ ICR Column to a clean 1.5 ml microcentrifuge tube and add 100 μl (35 μl minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10000 × g for 30 seconds to elute the DNA.

2.2.3 PCR amplification of nucleic acids

For the amplification procedure, the main mixture was used, and produced by the Canadian company Promega, which contained 5 ml of the following ingredients in each 50ml Eppendorf tube:

(1) Taq DNA polymerase , (250 mM) Dntp , (10 mM) (PH = 9) Tis – HCL , ( 1.5 mM) MgCl₂, (30 mM) KCL and Stabilizer and tracking ]5 ml (10 gn) of DNA of each fungal isolate and 1 ml (0.5 mM) of each of the primer (forward and reverse) were added separately to each of the five primers used in the study and according to the optimal conditions mentioned in the table to apply the PCR technique [20,21].

Standard PCR reactions of 25 cycles of 99°C denaturing stage for 30 seconds, a 65°C annealing stage for 30 seconds, and a 72°C elongating step for 90 seconds were carried out on E. coli K12 using 0.5 μL Phusion enzyme or Taq enzyme. PCR reaction products were monitored by 1% agarose gel.

| Table 2. PCR reaction products were monitored by 1% agarose gel. |
|---------------|---|---|---|
| The process   | °C | Time | No. cycle |
| Primary denaturizing | 98 | 2 minutes | 1 |
| Denaturizing   | 98 | 20 s   | 45 |
| Annealing      | 50 | 30 s   | 45 |
| Elongations    | 72 | 90 s   | 45 |
| Final elongation | 72 | 10 minutes | 1 |

2.2.4 Technique of electric relay for PCR product electrophoresis technology Technical of electric relay

The technique of electric relay PCR product was used on agarose gel, where a DNA marker (bp10000) was placed in the first hole by mixing 3 μm of the molecular weight with 10 μl of methyl blue dye, while the rest of the pits put the PCR product in the amount of 7 μl. On the agarose gel at a concentration of 1.5%, i.e., dissolving 0.5 g of agarose in 25 ml of TBE x 0.5 solution with the addition of 0.5 of Diamond TM Nucleic dye, then fixing the electric current at 100 volts for an hour. After the migration is completed, the gel is examined with a UV device to know the quality of the packages in the gel.

3.3 Testing the pathogenicity of cankering pathogens

The experiment was conducted at the nursery of the College of Agriculture and Forestry, Mosul University as the pathogenicity of the fungi isolated from infected stems in intact trees after growing the fungi on potato agar and dextrose PDA in sterilized Petri plates with diameter of (8.5) cm and incubated in a temperature of (25 ± 2) for (5-7) days. Intact branches of Melia azidarch trees with similar size (1-2) cm were wounded with a length of 13 mm at campium position using a sterilized scalpel. The injury was inoculated with a disk obtained from the edge of the pure growing each fungus colony with a diameter of 4 mm [22]. An average of three branches was repeated each fungus. The wounds were then covered with a sterilized and wet cotton and left for 60 days. The control treatment was by making a wound using a sterilized scalpel and was not inoculated with the isolated fungi.

After two months and four months from the beginning of fungi inoculation, the mean of cankering length, width, and area for each period in each type of the trees were examined. Also, the difference between the two areas of cankering was calculated during the two periods. The experiment was conducted using the random complete sectors design and statistically analyzed using SAS package and tested using Duncan method.

Koch’s hypotheses were applied by repeating the isolation from the parts artificially inoculated to ensure of the same fungi from the injury’s tissues. were artificially inoculated and they were identical to the same fungi that were first isolated from the cankerings.

3. Results and Discussion

3.1 Isolation

Fungi causing cankering on the Melia azidarch trees were isolated. These fungi were Neosylatidium dimidiatum, Fusarium graminearium (F.austroamericanum), Alternaria brassicicola, Aspergillus sp. and Penecillium sp., during the months April,
June, August, October and January in 2020. As can be seen from figure (1) the increase of *Neoscylitidium dimidiatum* isolate value as its maximum value was 85% in August, while the lowest value was 49% in April for the same year, followed by *F.austroamericanum*(*Fusarium graminearium*) with maximum infection percentage of 39% and minimum of 4% in October, followed by *Melia azidarch*, with maximum infection percentage of 25% in April and minimum value (0%) in August and finally the two fungi *Aspergillus* sp. and *Penecillium* sp. with low isolation percentages.

![Figure 1](image)

**Figure 1.** Percentage of *Melia azidarch* canker fungi during the months of isolation.

### 3.2 Diagnosis of the isolated fungi

#### 3.2.1 Morphological diagnosis

##### 3.2.1.1 Alternaria brassicicola Schw

The morphological test of *Alternaria brassicicola* on the potato agar and dextrose shows the growth of the new colony of the fungus with white color, which changes into dark olive color with time at the age of (7-10) days and in the ends with black color on the upper surface of the agar medium. The nature of the growth was filament cotton with low growth. The growth in the bottom of the plate appeared as black too. When the fungus colony examined the mycelium of the fungus appeared as a dark color and its spores were characterized with containing multiple cells with lateral and longitudinal walls (figure 2) divided into (5-9) cell per spore, with a short peak that is almost hidden inside it. The properties of the type mentioned are identical to the fungus characteristics [18] and were identical to the characteristics of the fungus mentioned previously [23].

##### 3.2.2 The fungus *F.austroamericanum* (*Fusarium graminearium* Schw.).

When conducting the morphological examination of the fungus colony grown on, and during (7-10) days after purifying the fungus, the mycelium growth appeared in pale white-cottony color with low growth high on the PDA and with wavy edges. The color in the center of the colony turned, with age, to a color mixed with rosy-red due to the secretion of dyes (Figure 3). On the other hand, these dyes disappeared at the edges of the colony. The characteristics of the fungus are in conformity with the classifications of previous description [10] as the Macroconidia (Figure 4) are divided into three transversal walls with dimensions of (3.5-4 × 30-50) micron and the second type includes (5-7) transversal walls with dimensions of (36× 3.5-5) micron. The Chlamydospores is rarely observed in the sexual stage *Gibberella Zeae*.
Figure 2. Cultural appearance and spores of the fungus, *Alternaria brassicicola*.

Figure 3. The mycelium growth of *F.austroamericanum* (*Fusarium graminearum*) fungus on the potato dextrose agar.
3.2.3 *Neoscyllitidium dimidiatum* Penz. (*Nattrassia mangiferae*) fungus

The results of the morphological examination of the fungus colony demonstrated the formation of white mycelium growth on PDA in the early days of the colony age and it changed after several days into black-olive color and then to black on the upper face of the colony, while the fungus showed a dark black color in the bottom of the plate (Figure 5). The diameter of the colony was 8.5 cm after 5 days of incubation at 25 ± 2 °C. When the colony was microscopically examined, it was shown that the fungus formed arthrospores due to the segmentation of the fungal hypha. The arthrospores were transparent in the beginning and then the color changed with age to dark and it occasionally included more than one cell and all these characteristics were identical to the fungus characteristics in the international classification to the genus rank by [8] and [11] the genus rank by [21].

![Image 1](image1.jpg)

**Figure 4.** Spores of *F.austroamericanum* (*Fusarium graminearum*) fungus as shown by the magnification power of X40

![Image 2](image2.jpg)

The upper face of the fungus colony

![Image 3](image3.jpg)

The lower face of the fungus colony

**Figure 5.** Arrows point to *N. dimidiatum* fungus Arthrospores as shown using X40 microscope magnification power.

By using the DNA sequencing technique, it was shown clearly from the diagnostic results that the molecular weights matched, which was shown a genetic match of the phenotypically diagnosed and isolated fungi with the sequence of nitrogenous bases of the genes belonging to the fungi and targeted to confirm the gender and of type each of the following fungi:

| DNA Band Size (pb) |
|-------------------|
| 1500              |
| 1250              |
| 1000              |
| 750               |
Where the first fungus, *Neoscylitidium dimidiatum*, showed that no match occurred with the gene after designing the Primer bundle the third fungus, *Fusarium austroamericanum*, showed a genetic match with the bundle 250 pb. While the fourth fungus, *Alternaria brassicicola*, showed a genetic match with the bundle 1000 pb, but for the previous repeated of the fungus, which has the same sequence with a different genetic code, no genetic match occurred with the bundle. Therefore, this similarity to the genera or species of each of these fungi has been genetically diagnosed instead of the phenotypic diagnosis of each of these fungi based on the genes selected for each of these types, as shown in the figure below:

![Figure 6](image_url)

**Figure 6.** Percentages the electrical conductivity of agarose gel with a percentage of 1.5%.

This figure involves the following:
M : represents the various molecular weights imported from PROMEGA (1Kb DNA Ladder company).
- Represents DNA isolate of *N. Dimidiatum* fungus using the forward primer CAGATAGGCTGGCGG and the reverse primer CGTTGGCGCCATAGA.
- Represents DNA isolate of *N. Dimidiatum* fungus using the forward primer CGGCCCGATCCTCCC and the reverse primer ATGATATGCTTAAGT.
- Represents DNA isolate of *F. austroamericanum* fungus as it belongs to the main fungus *F. graminearium* using the forward primer CAGATAGGCTGGCGG and the reverse primer CGTTGGCGCCATAGA.
- Represents an isolate of *A. brassicicola* DNA using the forward primer GACTGTGAAACTGCG and the reverse primer TAA
- Represents an isolate of *A. brassicicola* DNA using the forward primer GGTTAGGTCTCGTAG and the reverse primer TTTCCTCCCGGCTAT.

Based on the morphological and molecular diagnosis results, it was clear that the fungus *F. austroamericanum* (*Fusarium graminearium*) is considered the first record by the researcher in Iraq on *Melia azidarch* trees.

### 3.2.4. Pathogenicity capability of Melia azidarch trees fungi

The results of the pathogenicity capability of fungi isolated (Table 3) showed the efficiency of these fungi in causing the canker disease on the intact branches. As the fungus *F. graminearium* caused the maximum impact of canker length during the first period of time of the test which was two months with a value of 16 cm, followed by *N. Dimidiatum*, while *A. brassicicola* showed the lowest value of canker length which was not different from the value of the control treatment.
Table 3. The effect of canker fungi on the dimensions and areas of the cankers in the Melia azizarch trees.

| Fungi          | Period from 22/9/2020 to 22/11/2020 | Period from 22/9/2020 to 22/1/2021 | Canker length (cm) | Canker width (cm) | Areas (cm²) | Canker length (cm) | Canker width (cm) | Areas (cm²) | Difference between the two areas (cm²) |
|----------------|----------------------------------|----------------------------------|-------------------|---------------|-----------|-------------------|---------------|-----------|---------------------------------|
| A. brassicicola |                                  |                                  | 4.3333 c          | 4.0000 b      | 17.3333 b  | 27.3333 ab        | 12.3333 a     | 340.6667 a | 323.33 a                         |
| F. graminearium |                                  |                                  | 16.0000 a         | 6.6667 ab     | 113.3333 a | 23.3333 b         | 10.6667 a     | 246.6667 a | 133.33 ab                        |
| N. dimidiatum   |                                  |                                  | 9.0000 b          | 8.0000 a      | 73.0000 ab | 30.0000 a         | 13.0000 a     | 398.3333 a | 325.33 a                         |
| Control        |                                  |                                  | .0000 c           | .0000 c       | .0000 b    | .0000 c           | .0000 b       | .0000 b    | .0000 b                         |

The numbers which have some letters have no significant differences between them at level 0.05.

In terms of the canker width, N. Dimidiatum showed the highest effect with a value of 8 cm and it was not significantly different from F. Graminearium. However, A. brassicicola had the lowest significant effect with a value of 4 cm.

For the second period of testing the pathogenicity potential, which lasted for two months after the results of the first period, there was an increase in the dimensions of canker due to the period fungi existing and the highest value of canker dimensions was caused by N. Dimidiatum which showed the highest significant difference in terms of the canker width (30 cm), followed by A. brassicicola, which was not significantly different from it. F. graminearium demonstrated the lowest significant effect on the canker length (27.33 cm) although it was significantly different from the control treatment.

These results are in agreement with what [4] reached about the pathogenicity of the fungus as the effect of canker length was 47 and 29 cm in the branches of Eucalyptus and Sycamora after two months of fungus inoculation, also the pathogenicity of the aforementioned fungus is terms of causing the cankers [13].

In addition to that, the fungi tested showed a significant effect on the canker width with a highest value of 13 cm for N. Dimidiatum and a lowest value 10.67 cm by F. graminearium. Although the fungi tested was not significantly different from each other, but they were significantly different from the control treatment.

As for the effect of fungi tested on canker area only, F. graminearium showed the maximum effect on the cankered area (106.33 cm²) after two months of testing the pathogenic potential followed by N. dimidiatum, which was not significantly different from it, while A. brassicicola showed the lowest effect in terms of the cantering area (17.33 cm²). Although all the fungi were significantly different from the control treatment, results are in agreement with what was mentioned previously [2] about the capability of fungi to increase the canker area with the increase of the infection period.

In addition to that, the canker area increased due to the effect of the period of time of testing the pathogenicity after two months from the first reading of the canker area. The fungus N. Dimidiatum showed the highest significant effect (390 cm²), followed by F. graminearium and A. brassicicola, which demonstrated a significant effect on the cankered area (248.89 cm²) and (340.67 cm²) respectively, though they were not significantly different from the first fungus. Moreover, all the tested fungi differed significantly from the control treatment. As for the effect of these fungi in terms of the difference between the cankered area, A. brassicicola exhibited the highest significant difference for the two periods; with a value of 325 cm², followed by N. dimidiatum with a value of (317 cm²), while F. graminearium demonstrated the lowest value (142.6 cm²). All the fungi showed a significant difference in terms of the effect on the difference in the two areas of cankered and for periods compared with the control treatment.

References

[1] Albeeri, B. H. (2004). Cankering of stems as a deterioration factor for Populous trees, A master thesis, College of Agriculture, Duhok University, Iraq.
[2] Aldabbagh, M. N. and N. M. Younis (2012). Biological compact of cypress trees cankered caused by Nattrassiae mangiferae fungus, AlRafidain Agriculture Journal, Vol. 41, No. 4.
[3] Aldawoodi, D. M. (1979). Classification of forest trees, Books House for Printing and Publishing, Mosul University, Mosul, p. 347.
[4] Alkhera, A. N., (2009). Diagnosis of some poisons of Phoma exigua and Nattrassiae mangiferae fungi and the induced defenses by them in Eucalyptus and Sycamora, Ph.D. thesis, College of Agriculture and Forestry, Mosul University, Iraq.
[5] Alnahal, I. and Adeeb R. and M. N. Shalabi (1996). Some feed values Project, International: 72, Duhok University, Iraq.
[6] Bajracharya, D. T, T. B, Bhattarai, M. R Dhakal, M. R. Mandal, S. Sharma, Sitaula and B. K. Vimal, (1985). Some feed values and biological compact of cypress trees cankering caused by Nattrassiae mangiferae fungus, AlRafidain Agriculture Journal, Vol. 41, No. 4.
[7] Blankert and Tatter (1992), translated by: Salih AlMath, Abdulqader Abdulraouf, Badullah Mohammed AlTarhawi and Fawzi Adam Tarbha. Manual of laboratory and field of trees diseases, University of Omar AlMukhtar, AlBaidhaa, Libya, p. 354.
[10] Summerell, B. A., Salleh and J. F. Leslie (2003). Aultilitarian approach to Fusarium identification. Plant Des. 87: 117 - 128
[11] Crous and Slippers (2006). "Mycobank Database". www.mycobank.org. Retrieved 9 October 2016.
[12] Davidson J. (1985). Species and Sites. UNDP/FAO Project JBD/791017, Field Document No. 5, 50pp.
[13] Ghasem-Sardareh R. and H. Mohammadi (2020). Characterization and pathogenicity of fungal trunk pathogens associated with declining of neem (Azadirachta indica A. Juss) trees in Iran. Journal of Plant Pathology. https://doi.org/10.1007/s42161-020-00598-z/ Accepted : 23 June 2020. Published online
[14] Hudler; G.W.(2015). Canker on Trees Various. Plant pathology and plant-Microbe Biology Section 334 Plant Science Building Ithaca, NY 14853-5904 Cornell University College of Agriculture and Life Sciences.
[15] Kazem, S. K. and J. J. Kazem (2013). Study of the phenotypic and microscopic characteristics of Fusarium species and the effect of environmental conditions on its growth and reproduction. Journal of Babylon University / Pure and Applied Sciences / Issue 3 / Volume 31: 871-891.
[16] Slomy AK, Jasman AK, Kadhim FJ, AL-Taey DKA and Sahib MR. 2019. STUDY IMPACT OF SOME BIOFACTORS ON THE EGGPLANT SOLANUM MELONGÉNA L. VEGETATIVE CHARACTERISTICS UNDER GLASS HOUSES CONDITIONS. Int. J. Agricult. Stat. Sci. Vol. 15 (1) :371-374
[17] Mohammed, N. Y. (2005). Recording the first withering of wilting branches Hendersonula on the Sycamora trees in Iraq. AlRafidain Agriculture Journal Vol. 33, No. 4, 33:106-133.
[18] Morton, F. J. (1964) : Species of Alternaria Brassica on hosts in New Zealand. N.Z. Jl Bot. 2: 19-33.
[19] Sharma, J. K. ; C. Mohanan and E.J. Maria Florence (1984). A new stem canker by Botryodiplodia theobromae in India. Mycol. Soc. 83:162-163.
[20] Sutton, B.C. and B.J. Dyko (1989). Revision of Hendersonula . Mycol. Research, 93:466-488
[21] Yangrae (2015). How the Necrotrophic Fungus Alternaria brassicicola Kills plant cells, Remains an Enigmal Eukaryotic cell . Vol 14 No (4) :335-344.
[22] Hirano, Y. and Arie, T. (2006). PCR-based differentiation of Fusarium f. sp. lycopersici and radicis lycopersici and races of F.