



# Plant Pathology Journal

ISSN 1812-5387

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## Studies on Coloured Leaf Spot Disease of *Alchornea cordifolia* Caused by *Taphrina deformans*

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**Abstract:** The coloured leaf spot disease of *Alchornea cordifolia* leaves was investigated and the fungal causal agent was isolated on yeast malt extract agar. The epidemiology of the disease on the university of Lagos, Akoka campus was also studied. *Taphrina deformans* was isolated from the diseased leaves of *Alchornea cordifolia*. The coloured leaf spot disease is characterised by an initial appearance of pink spots on the leaves, as the disease progresses, an intermediate stage occurs which is brownish-red in colour and eventually turned brown at the old stage. The disease epidemic is rampant between June and November of the year. The average leaf area of the pink spot was  $1.61 \pm 0.22 \text{ cm}^2$  and brown spot was  $1.74 \pm 0.13 \text{ cm}^2$ . The mean leaf area of the healthy leaf is  $8.64 \pm 0.15 \text{ cm}^2$ . Free hand sections of the diseased leaves revealed the presence of *T. deformans* ascus on the epidermal layer of *A. cordifolia* leaf, rupturing the epidermis to reveal the ascus, as well as disjointed intercellular presence of the fungal hyphae. The chlorophyll content of the healthy leaves of *A. cordifolia* was  $0.24 \pm 0.059 \mu\text{g g}^{-1}$ , while the diseased leaves had  $0.22 \pm 0.099$ ,  $0.22 \pm 0.065$  and  $0.16 \pm 0.070 \mu\text{g g}^{-1}$  chlorophyll content for the pink, brown-red and brown spot stages. *T. deformans* grew best on *A. cordifolia* leaf extract broth than on potato dextrose broth and yeast malt extract broth.

**Key words:** Leaf spot, *A. cordifolia*, *T. deformans*, histopathology, epidemiology, chlorophyll

### INTRODUCTION

Leaf spots are the most prevalent of plant diseases, so common we seldom notice them and rightly so, for if we should attempt to control all the miscellaneous leaf spots that appear in a small suburban garden in a single season, we'd quickly go mad<sup>[1]</sup>. Leaf spots on plants may be caused by toxic gases, insects, bacteria and fungi<sup>[2]</sup>. The life history of leaf spot fungi is generally similar. First infection in the spring which comes either from ascospores that have developed on dead fallen leaves or from spores produced by fructifications on the bark, if the fungus is one that also attacks the bark tissue of twigs. Then during the remainder of spring and summer the rapid spread of these fungi by means of spores produced on the killed tissue of the leaves. Economically, leaf spots reduces the yield of plants by bringing a reduction in the available area necessary for photosynthesis to occur<sup>[3]</sup>. Leaf spots also bring about the production of toxins which cause injury to the host cells<sup>[4]</sup>.

*Alchornea cordifolia* (Schum and Thonn) commonly called christmas bush is in the family Euphobiaceae. *A. cordifolia* is a multistemmed, almost climbing shrub or small spreading tree up to 15 m high and 1 m girth<sup>[5]</sup>. *A. cordifolia* has a variety of uses including commercial

purpose and medicinal usage. Medicinally, the roots are used for Jaundice, leprosy and snakebites. The pith of the roots, made into a lotion or chewed is said to cure thrush and buccal ulceration<sup>[6]</sup>. Infusions of bark shavings are used for diarrhea. A decoction of the leaf is a wash for feverish chills, rheumatic pains and sores. In Ghana, the decoction of the leaf with lime juice is commonly used to cure venereal disease. The leaf decoction is also used to relieve menstrual pain<sup>[7]</sup>.

The chemical constituents of *A. cordifolia* include alkaloids, tannins and other polyphenols, as well as phytochemicals such as alcorneione, anthranilic acid, gentisinic acid, isoalchorneine and yohimbine<sup>[8]</sup>.

Recently, the sudden appearance of the coloured leaf spots on *A. cordifolia* leaves in some parts of Lagos state, Nigeria have generated interest on the pathology of the plant. The fungus associated with the leaf spot on *Alchornea cordifolia* have not been reported. The aim of the present paper was to document the isolation of the fungal causal agent of the coloured leaf spot of *Alchornea cordifolia* and determine the effect of the disease on the anatomy of the leaf. Growth studies of the fungal isolate on different broth media as well as some physiological effect (chlorophyll content) on the diseased leaves would be investigated.

**MATERIALS AND METHODS**

**Field assessment:** The isolation of the diseased and healthy plants of *Alchornea cordifolia* were identified within the University of Lagos, Akoka campus (Fig. 1). The number of diseased leaves per tree was counted for 50 trees in each location. The leaf areas of the diseased (pink, brown-red and brown) leaves, 1000 spots each and the leaf area of healthy leaves were determined according to the methods of Jackson<sup>[9]</sup>.

**Isolation, identification and pathogenicity test:** *Taphrina deformans* was isolated from the pink and brown leaf spot of *Alchornea cordifolia* following the methods of Booths<sup>[10]</sup> using Yeast Malt Extract Agar (YMEA). The fungal isolate was identified using conventional taxonomic techniques. The morphology of the fungus were studied and compared with the descriptions of Talbot<sup>[11]</sup>, Deacon<sup>[12]</sup> and Bryce<sup>[13]</sup>. Pathogenicity test of *T. deformans* of *A. cordifolia* leaves was carried out. Spore suspension of the fungus was prepared by adding 10 ml of sterilized distilled water to a 14 day old YMEA

petri dish culture, dislodging the spores with a sterile glass rod and filtering through two layers of sterile muslin cloth. The spore suspension was made up to 200 ml by adding sterilized distilled water and with the aid of haemocytometer the number of spores was estimated to be  $6.38 \times 10^3$ . The spore suspension was used to spray, with an atomizer, twenty, 6-months old visually healthy *A. cordifolia* trees grown in an experimental plot. The test plant leaves were covered with sterile polythene bags for 24 h, to allow the spores to germinate. The pathogen was re-isolated from artificially infected leaves after 40 days post spore inoculation and its morphology compared with that of the original fungal isolate to proof Kochs postulate.

**Histopathological studies:** Naturally infected leaves of *A. cordifolia* were observed for disease symptoms. There were 3 observed stages of infection, characterized by the colour changes of the symptoms on the leaves as a result of age. A diseased leaf from each of the stage, as well as a health leaf (of similar age) were embedded transversely in he cork and free-hand sections were meticulous cut, with a sterilized razor blade. The cut sections were placed

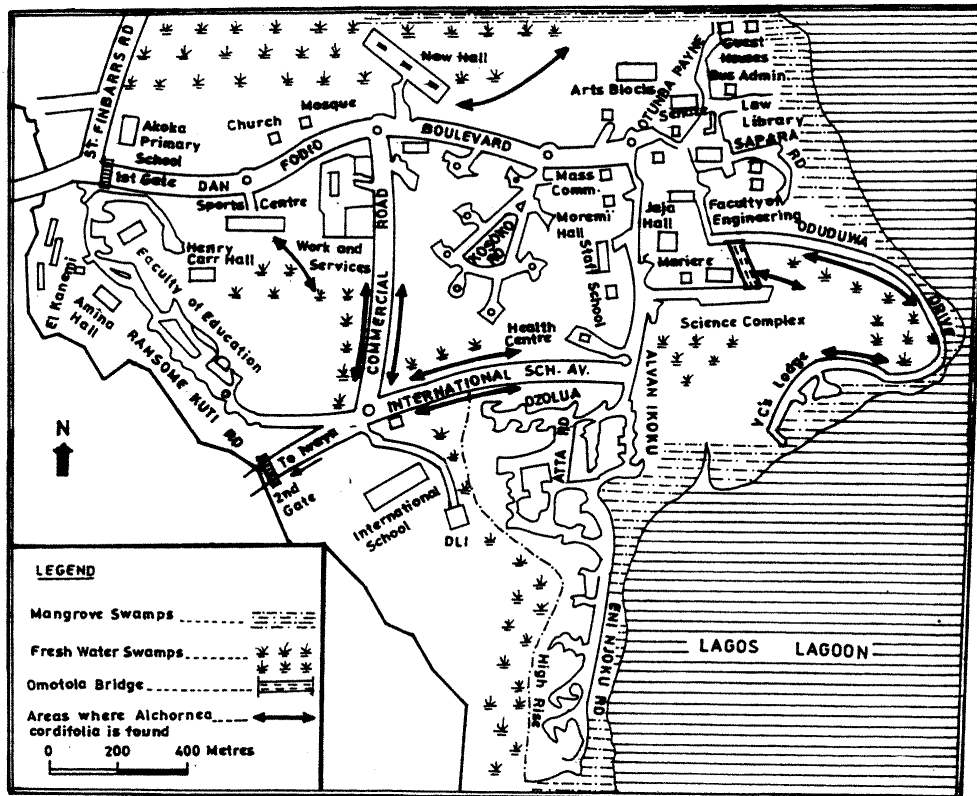


Fig. 1: Map of University of Lagos

in 5% commercial bleach solution for about 15 min. After which they were transferred into distilled water for another 10 min and finally rinsed in three changes of distilled water. The cut pieces from each disease stage and healthy leaf were placed on a microscopic slide and stained with Lactophenol cotton blue before counter staining with safranin. They were then observed under the Zeiss light microscope and traced with camera lucida<sup>[14]</sup>.

A spore solution *Taphrina deformans* was made from the pure culture on YMEA. This solution was kept in a closed petri dish, incubated at room temperature and observed daily up till the 10th day of incubation. Spore slides were made each day and stained with lactophenol and observed with the microscope and traced with camera lucida.

**Chlorophyll determination:** The method of Wintemans Demots (1965) adapted by Edwards<sup>[15]</sup> was used. The healthy and diseased (pink and brown) stages of the leaf spot disease of *Alchornea cordifolia* were investigated for chlorophyll content. Single leaves in 3 sets from each disease stage and the healthy leaf were cut into pieces (5 mm<sup>2</sup>) and each type of leaf placed in a 30 ml test tube. The test tubes were then filled with 20 ml of 80% ethanol and boiled in a water bath for 15 min. Thereafter, they were allowed to cool for 5 min. Then 5 ml of the extract in the test tube was pipetted into a curvette and placed in a photocalorimeter for chlorophyll determination and read at 620 and 660 nm, which were the respective wave lengths of chlorophyll A and B. This was carried out for all the tubes and repeated twice.

**Growth studies on different broth media:** The method of Booths<sup>[10]</sup> was used for the growth studies. Three media were used, Leaf Dextrose Broth (LDB); Yeast Malt Extract Broth (YMEB) and Potato Dextrose Broth (PDB). LDB was prepared with 25 ml of healthy *A. cordifolia* leaf extract (made from 100 g of leaves, blended, mixed with 400 ml of distilled water and filtered with mushin cloth), 10 g of dextrose and 10 g of agar (oxid) made up of 500 ml with distilled water and sterilized; YMEB was prepared from 25 ml of malt, 5 g of yeast and 10 g of agar, made up of 500 ml with distilled water and sterilized, while the PDB was prepared by boiling, meshing, sieving of 100 g of peeled potato, 10 g of dextrose and 10 g of agar, made up to 500 ml with distilled water and sterilized. Ten milliliters of the broth was poured into a sterilized 20 ml test tube, ten replicates of each media was made. Spore suspension of *Taphrina deformans* was made from a pure culture plate and 0.5 ml of it was pipetted into each test-tubes.

The tubes were then incubated for 10 days at 28°C. The growth of *T. deformans* was monitored daily by

reading the Optical Density (O.D) on the photocalorimeter at 470 nm. The average OD was plotted against time (days).

## RESULTS

A yeast-like pathogenic fungi, *Taphrina deformans* was isolated from the coloured (Pink, Brown) leaf spot of *Alchornea cordifolia*. Pure cultures of *Taphrina deformans* could only be isolated from diseased leaf on yeast malt extract agar. The leaf spot symptoms are shown in Fig. 2-4. The numerous stages of the colour leaf spot



Fig. 2: Healthy *Alchornea cordifolia* leaves



Fig. 3: Coloured leaf spot of *Alchornea cordifolia* showing the pink stage of the disease



Fig. 4: Coloured leaf spot of *Alchornea cordifolia* showing the brown stage of the disease

Table 1: Average leaf area of infected and uninfected portions of healthy pink and brown diseased stages of *Alchornea cordifolia* leaf spot

Type of <i>A. cordifolia</i> leaf	Average leaf area of portions (cm <sup>2</sup> )		Total leaf area (cm <sup>2</sup> )
	Infected	Uninfected	
Healthy leaf	0.00±0.00	8.64±0.15	8.64±0.15
Pink diseased stage	1.61±0.22	7.11±0.21	8.72±0.43
Brown diseased stage	1.74±0.13	7.35±0.61	9.09±0.74

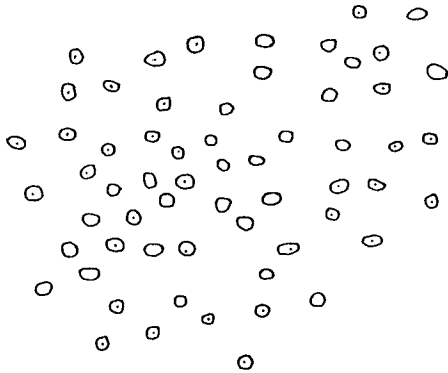


Fig. 5: *Taphrina deformans* spore in solution from the pink diseased stage on the first day it was made

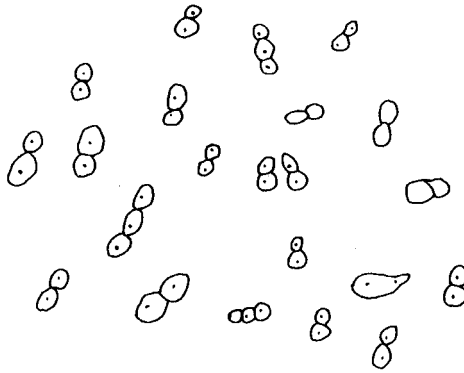


Fig. 6: *Taphrina deformans* spore in solution from the pink diseased stage on the 3rd day it was made, showing budding

symptom suggest that there are 3 stages in the infection of *Alchornea cordifolia* which are, the young stage (Pink) which develops 8-12 days after spore inoculation; the intermediate stage (Brown-red) develops 15-20 days after inoculation and the old stage characterised by the brown colour and it develops 25-30 days post spore inoculation. The coloured symptoms are found on the upper surface of the *A. cordifolia* leaf. The pink colour symptoms transformed to brown-red then to the brown colour symptoms as the leaves aged. Averagely about 45% of the leaves per tree were diseased in infected areas. Table 1 shows the average leaf area of infected and

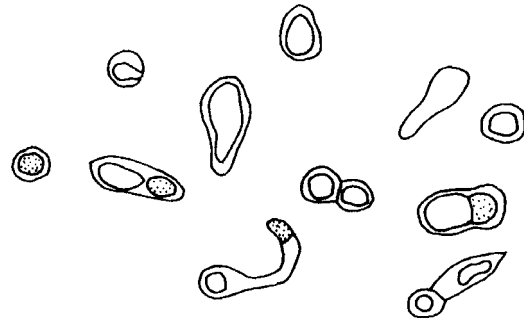


Fig. 7: *Taphrina deformans* spore in solution from the pink diseased stage on the 6th day it was made

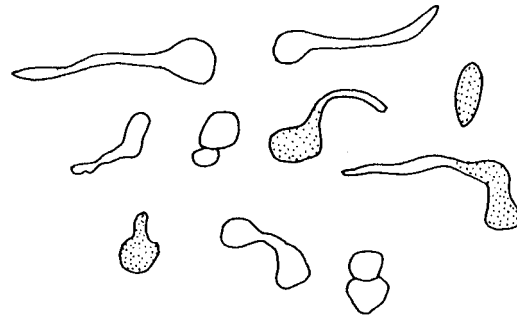


Fig. 8: *Taphrina deformans* spore in solution from the pink diseased stage on the 8th day it was made, showing 'germtube'(a)

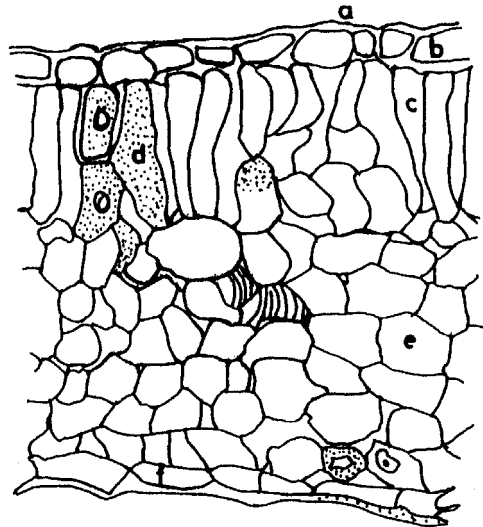


Fig. 9: Transverse sections of the healthy stage of *Alchornea cordifolia* leaf, showing (a) cuticle (b) upper epidermis (c) palisade mesophyll (d) chloroplast (e) spongy mesophyll (f) lower epidermis

uninfected portions of healthy, pink and brown diseased parts of *Alchornea cordifolia* leaves. The average leaf area of the pink stage was  $1.61 \pm 0.22 \text{ cm}^2$  and brown disease stage was  $1.74 \pm 0.13 \text{ cm}^2$ . The mean leaf area of healthy leaf is  $8.64 \pm 0.15 \text{ cm}^2$ . The disease epidemic is rampant between June-November of the year. There was faster defoliation of the diseased leaf than the healthy leaves.

*T. deformans* spores as they are discharged from the ascus on the infected leaves cling together in a little ball and the tiny spore balls disintegrate immediately when brought into contact with a drop of water and the spores float apart from one another. The spores after 24 h of incubation at  $28^\circ\text{C}$  germinated by budding, these buds elongated into germ tube by the 8th day (Fig. 5-8). The colour of the spores was white originally, which changed to pink and later to brown as the spores aged in solution.

Transverse section of the healthy leaves (Fig. 9) shows intact cuticle, upper and lower epidermal cells, palisade mesophyll, had cells. The spongy mesophyll had fewer chloroplast than the palisade mesophyll. Figure 10 shows the TS of the young stage (pink) of the infected leaf. The development of asci is obvious, rupturing the upper epidermal cells and forcing themselves out through the cuticle. The asci are globe like in shape with a thick wall. These asci showed different number of ascospores between 2-4. The fungal hyphae (picked up the blue colour of the lactophenol) are found at the bottom of the ascus, between the cuticle and the upper epidermis and between the cells of the palisade. At the intermediate stage (Brownish-red) shown on Fig. 11, the size of the asci has increased tremendously, it is almost twice the size as observed in the young stage.

The spores are 8 in number with thick wall. The hyphae are disjointed within the intercellular space. At the old stage (Brown) shown on Fig. 12, the fungus is no longer encroaching upon the healthy tissue but remains strictly localized in the already invaded regions. The asci are much larger and matured, ascospores are probably discharged at an opening.

The chlorophyll content of the health leaves of *A. cordifolia* was  $0.24 \pm 0.059 \mu\text{g g}^{-1}$ ; pink stage was  $0.22 \pm 0.099 \mu\text{g g}^{-1}$ ; in the brown-red diseased leaves it was  $0.22 \pm 0.065 \mu\text{g g}^{-1}$  while the brown diseased leaves had  $0.16 \pm 0.070 \mu\text{g g}^{-1}$  chlorophyll content.

The fungus *T. deformans* grew best on the *A. cordifolia* leaf dextrose broth followed by the potato dextrose broth and then the yeast malt extract broth at the 10th day of incubation (Fig. 13). Turbidity of the broth increased every 24 h from the first day to the sixth day of incubation after which there was a steady growth on

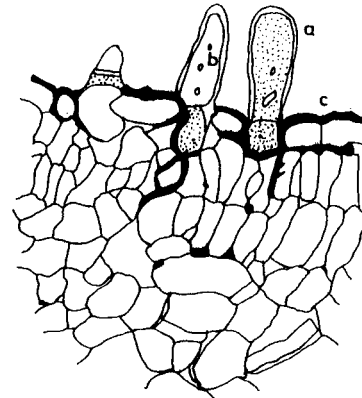


Fig. 10: Transverse sections of the young stage (pink) of the infected *A. cordifolia* leaf, showing (a) ascus (b) ascospores (c) fungal hyphae

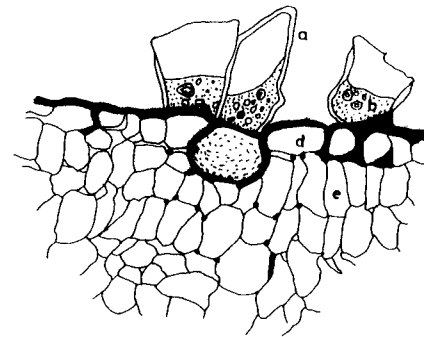


Fig. 11: Transverse sections of the Intermediate stage (Brown-red) of the infected *A. cordifolia* leaf, showing (a) ascus (b) ascospores (c) fungal hyphae (d) epidermal cell (e) palisade cell (f) resting cell

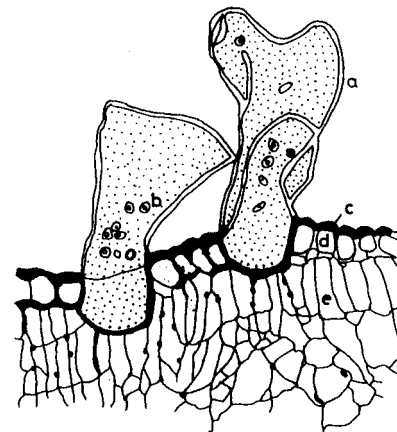


Fig. 12: Transverse sections of the old stage (Brown) of the infected *A. cordifolia* leaf, showing (a) ascus (b) ascospores (c) fungal hyphae (d) epidermal cell (e) palisade cell



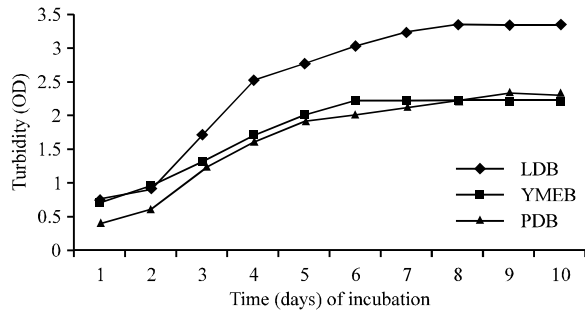


Fig. 13: The growth of *Taphrina deformans* on the different broth media

YMEB while the LDB recorded steady growth from the 8th day and PDB from the 9th day of incubation.

### DISCUSSION

In this study, *Taphrina deformans* was isolated from the leaf spot disease of *Alchornea cordifolia* and found to be pathogenic. Although, *T. deformans* have been found to be the causal agent of many disease in plants such as leaf curl of peach, almond and nectarine<sup>[16-18]</sup>, this report is probably the first to have implicated *T. deformans* as a causal agent on *A. cordifolia* leaf spot disease. The colonies of *T. deformans* produced are slightly ovoid in shape, varying in size and had colour change as the colonies aged. This was similar to the observation made by Deverall<sup>[19]</sup> on other plants. Deverall<sup>[19]</sup> attributed the colour change to the production of carotenoid. This carotenoid production might have been responsible for the colour change from pink to brown-red and then brown of the diseased leaves. A true mycelium was never observed in culture asserting the fact that *Taphrina* species lack true mycelium as reported by Mix<sup>[20]</sup>, Trione and Johnston<sup>[21]</sup> and Babadoost<sup>[18]</sup>. Instead of the *T. deformans* producing a typical sprout conidia, it gave rise to a very elongated out growth resembling germtube. These elongated tubes were longer than the parent cell and they were never septate. In old culture, where budding was less frequent, the cells are more varied in appearance and typical forms, the most striking are those which produce thick wall and it was suggested by Fitzpatrick<sup>[16]</sup> that these are resting cells which play important role, including the survival of the fungus over favourable periods, probably explaining how the fungus survived the unfavourable month of December to April of the year (when the disease incidence does not occur). Mix<sup>[22]</sup> described the so called resting cells as ascogenous cells. Young leaves of *A. cordifolia* are susceptible to the leaf spot disease, which was equally observed in the leaf

curl disease of peach and almond as reported by Syrop and Beckett<sup>[18]</sup> and peanut leaf spot<sup>[9]</sup>.

Analysis of the chlorophyll content showed reduction of the chlorophyll content of the diseased leaf when compared with that of the healthy leaves of similar age and equal exposure. This shows that the photosynthetic activity of the diseased leaf is adversely affected. The decrease in the chlorophyll content was a gradual process as revealed by the continual decrease in the chlorophyll content of the 3 stages of the infected leaves which is similar to investigations on fungal infections of other plants<sup>[9]</sup>. Babadoost<sup>[19]</sup> reported a severe early defoliation and crop loss on nearly all peach and nectarine cultivars, with the leaf curl disease caused by *T. deformans*, which is similar to observations here on the *Alchornea cordifolia* leaf spot.

A knowledge of the mode of infection, various anatomical effects of the *Taphrina deformans* on *A. cordifolia* leaves would help design appropriate method of disease control for the disease. Results of this investigation also help to understand the etiology of the *A. cordifolia* colour leaf spot caused by *T. deformans*.

### REFERENCES

1. Agrois, G., 1997. Plant Pathology. 4th Edn., Academic Press, New York, pp: 849.
2. Boyce, J.S., 1961. Forest Pathology. 3rd Edition. McGraw-Hill Book Company Inc. New York, pp: 385.
3. Theberge, R.L., 1985. Common African Pests and Diseases of Cassava Yam, Sweet potato and Cocoyam. IITA Publication, Ibadan, pp: 168.
4. English, P.D., J.D. Jurale and E. Albersheim, 1971. Host-pathogen interactions. Plant Physiol., 47: 1-6.
5. Sary, F., 1992. The Natural Guide to Medicinal Herbs and Plants. Dorest Press, New York, pp: 150.
6. Ayensu, E.S., 1978. Medicinal Plants of West Africa. Publications Incorporated Michigan, pp: 281.
7. Burhill, H.M., 1997. The Useful Plants of West Tropical Africa. (Vol. 4). Royal Botanic gardens, Kew, London, pp: 1067.
8. Bruneton, J., 1995. Pharmacognosy, Phytochemistry and Medicinal Plants. Intercept Ltd andover, pp: 522.
9. Jackson, L.E., 1981. Distribution and severity of peanut leaf spot in Florida. Phytopathology, 71: 324-327.
10. Booths, C., 1971. Introduction to General Methods. In: Methods in Microbiology. Vol. 4 (Morris, J.R. and D. Ribbons, Eds.). Academic Press, London, pp: 1-47.
11. Talbot, O.H.K., 1971. An introduction to mycology. Leonard Hill, London, pp: 525.

12. Deacon, J.W., 1980. Introduction to Modern Mycology. Blackwell Scientific publications, London, pp: 197.
13. Boyce, K., 1992. The Fifth Kingdom. Mycologue publications, Ontario, pp: 412.
14. Bruzzese, E. and S. Hassan, 1983. A whole leaf clearing and techniques for host specificity studies of rust fungi. *Phytopathology*, 73: 335-338.
15. Edwards, G.E., 1977. The measurement of ozone injury by determination of Lead and chlorophyll concentration. *Plant Physiol.*, 60: 606-608.
16. Fitzpatrick, R.E., 1934. The life history and parasitism of *Taphrina deformans*. *Sci. Agric.*, 14: 305-306.
17. Syrop, M. and A. Beckett, 1975. Leaf Curl disease of almond caused by *Taphrina deformans*. *Canadian J. Bot.*, 54: 293-305.
18. Babadoost, M., 1999. Peach Leaf Curl and Plum Pockets. Report on Plant Disease, 895: 1-3.
19. Deverall, J.B., 1981. Fungal parasitism. 2nd edition. Camelot Press Ltd, South Hampton, pp: 546.
20. Mix, A.J. 1936. The genus *Taphrina*. University of Kansas Science Bulletin, 24: 113-149.
21. Trinone, E.J. and J.C. Johnston, 1974. Cytokinin production by the fungi *Taphrina cerasi* and *T. deformans*. *Can. J. Bot.*, 2: 59-64.
22. Mix, A.J., 1939. New species of *Taphrina* and new records from Western North America. *Am. J. Bot.*, 26: 44-48.