

# LEAF TWISTER DISEASE OF ONION (*Allium cepa* L.)

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## ABSTRACT

Onion (*Allium cepa* L.) is one of the important cash crops grown in Sri Lanka. A disorder called "Leaf twister disease" has gained importance recently specially on onion crops grown during the Maha season. A severe outbreak of the disease was observed in both bulb crop and seed crop at Field Crops Research and Development Institute, Maha Illuppallama during the 1995/96 Maha season. The characteristic symptoms of the disease are leaf curling, twisting and chlorosis and abnormal elongation of the pseudostem followed by bulb rotting. Lesions with black, minute, slightly raised acervuli (fruiting bodies) with pink masses of conidia could be seen scattered on leaves and flower stalks. Isolations were made from infected leaves and flower stalks and the pathogen was identified as a fungus, *Colletotrichum gloeosporioides* (Penz.) Sacc. (the perfect stage of *Glomerella cingulata* (Stonem) Spauld. & Schrenck), and its pathogenicity was confirmed. High relative humidity (85 - 96 %), moderate temperature (20 - 31°C) and cloudy rainy weather are the factors that favour the development and spread of the disease. Spores of the fungus can spread through soil, water, seed and crop debris. Crop sanitation including the destruction of crop debris will reduce the infection; use of healthy seeds and planting material is important to control the disease.

**KEY WORDS :** Fungus, Leaf twister disease, Onion, Pathogen.

## INTRODUCTION

Onion is one of the important cash crops grown in Sri Lanka. The annual national requirement is about 55,000 - 60,000 t and only about 50 % of this requirement is produced locally during the Yala season (April - September). Due to unfavourable climatic conditions and high incidence of purple blotch disease, onion cultivation is difficult during the Maha season. In addition, a disorder called "Leaf twister disease" has gained importance recently on onions cultivated specially during the Maha season. The disease is locally named "disco" by the farmers. The characteristic symptoms of the disease are twisting and chlorosis of the leaves and abnormal elongation of the neck region of the bulb. This disease can cause considerable damage to onion production in Sri Lanka when it affects the crop at early stage of

growth. The disease was first reported in shallots (red onion) from the Trincomalee district in the 1970's and from the Puttalam district in 1987 (Anon, 1992). Similar symptoms had been reported on onion cultivated in the Matale district in 1994.

Research conducted on this problem during the past few years has been confined mainly to shallot onion. Initially, the possible cause of disease symptoms was suspected as nematodes. However, Nugaliyadda and Ekanayake (1986) reported that parasitic nematodes could not be found from diseased plants. Kuruppu (1992) isolated the fungus *Fusarium oxysporum* from the diseased shallot plants and reported that thrips, mites, nematodes and other fungi could also cause similar symptoms. Mithrasena (1994) was able to isolate the fungus *Colletotrichum* from infected samples collected from Ratnapura,

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and suggested that it could be due to a soil borne pathogen. Wijesinghe (1994) isolated both *Colletotrichum gloeosporioides*, and *Fusarium oxysporum* f. sp. *cepae* from the diseased plants collected at Kalpitiya (Personal communication). However, so far, no definite conclusion has been made on the cause of the disease.

During the Maha 1995/96 season, a severe outbreak of the disease was observed in onion research plots at the Field Crops Research and Development Institute, Maha Illuppallama. Both the seed and the bulb crops were affected. The disease incidence ranged from 20 - 30 % and 50 - 70 % in the seed crop and bulb crop respectively. These research plots were used to study symptomatology, causal organism of the leaf twister disease, environmental factors that favour disease development and the method of dispersal. The results of these studies are reported in this paper.

### MATERIALS AND METHODS

#### Pathogen isolation

Plant samples collected from affected fields were used on potato dextrose agar (PDA) for isolation of the pathogen. Small pieces of tissues from the periphery of the leaf lesions were excised, surface sterilized in 70 % ethyl alcohol for 2 - 3 minutes and placed on PDA containing 1.01 % w/v streptomycin and were incubated at room temperature. Within one to two days, a small piece of agar with mycelium from the edge of developing colonies on agar plates was carefully isolated and a series of sub-cultures was made to obtain a pure culture. Cultures were examined after 4 days of incubation.

#### Pathogenicity Tests

Inoculum for pathogenicity tests was prepared from single spore cultures grown on

PDA. Spores were suspended in sterile distilled water and the concentration of the collected spore suspension was adjusted by hemocytometer to about  $10^5$  conidia/ml.

One month-old seedlings and sprouted onion bulbs were raised separately in plastic pots containing autoclaved soil for the pathogenicity test. These plants were inoculated after one month of planting with a standard spore suspension ( $10^5$  conidia/ml) by pricking the neck of the plants. Plants inoculated with sterile distilled water was used as the treatment control. One set of pots were kept in the glasshouse without RH control and another set of inoculated pots was exposed to high humidity (100% RH). Disease symptoms were examined 4 days after inoculation.

#### Studies on the spread of disease

**Transmission through soil** - One month old onion seedlings were planted in pots with the following treatments:

1. Soil collected from an infected field.
2. Soil collected from an infected field and autoclaved at 121°C and 15 psi for 30 minutes.
3. Soil collected from a healthy field (control).

**Transmission through crop debris** - Infected crop debris was incorporated into pots containing autoclaved soil with one month old onion seedlings and seedlings raised in pots containing autoclaved soil were used as the control.

**Transmission through water** - A water suspension of infected soil was made using sterilized distilled water and filtered through a filter paper. This suspension was then added to one month old onion seedlings raised in

pots containing autoclaved soil. Distilled water was used for the control. All these pots were then kept inside a glasshouse with high humidity (RH nearly 100 %).

**Transmission through seeds** - Seeds collected from infected plants were surface sterilized by immersing them for 2 - 3 minutes in 5 % sodium hypochlorite solution, and were blotted and dried on sterilized paper, and were plated on PDA with 10 seeds per plate and incubated at room temperature. A series of sub-cultures was made to obtain a pure culture. They were examined after 4 days of incubation and further observation continued up to 8 days.

Seeds collected from infected plants were also sown on autoclaved soil and seeds collected from a healthy field were used as the control. These pots were then kept inside the glasshouse with high humidity (RH nearly 100 %).

#### Environmental factors

The weather data of January -March 1996 and June-September 1996 were recorded. Seedlings of onion were established in the first week of June, 1996 (Yala season) to find out whether the symptoms developed during the dry period. Infected crop debris collected from the Maha season crop was incorporated in to the soil.

### RESULTS AND DISCUSSION

#### Disease symptoms observed in the field

The symptoms appeared in the latter part of February, 1996 when the bulb crop (raised from seedlings) was one month old and the seedcrop (raised from bulbs) was at flowering to seed maturity stage (2 months old). Symptoms observed in the seed crop and seedling crop varied while the disease spread sporadically in the field.

In the seedling crop, appearance of leaf curling, twisting, chlorosis and abnormal elongation of the pseudostem (neck of the bulb) were observed (Fig. 1a). White sunken oval lesions appeared mainly in lower leaves. However few were also observed in some young leaves. These lesions enlarged with time and spread all over the leaf. Black, minute, slightly raised acervuli (fruiting bodies) with pink masses of conidia could be seen scattered on the surface of the lesions. Affected leaves withered and became detached (Fig. 1b). Bulbs produced from these plants were small and eventually rotted (Fig. 1c). Most of the affected bulbs rotted before harvest while the others rotted during storage.

None of the plants of the seed crop showed symptoms of leaf curling, twisting, chlorosis or abnormal elongation of the pseudo-stem. Lesions consisting of dense black acervuli with pink masses of conidia were observed on the flower stalks (Fig. 2a). These lesions increased in size and sometimes several lesions coalesced to form large infected areas on the flower stalk. When the lesions reached the base of the flower head, young flowers decayed prematurely (Fig. 2b). At early flowering stage, flower stalks collapsed at the lesions and as a result the upper portion of the flower stem drooped and dried up prematurely with no seeds produced in the flowers.

#### Isolation and inoculation studies

Visual and microscopic observations indicated that the colony on PDA was greyish white to dark grey in colour on the upper surface and grey to black on the lower surface. The conidia are hyaline, cylindrical with obtuse ends and aseptate. These conidia are taxonomically very similar to *Colletotrichum gloeosporioides* (Penz.) Sacc.

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Fig. 1a. Leaf twister symptoms in onion seedling



Fig. 1c. Acervuli forming on sheath of lower leaves and bulb rotting.



Fig. 1b. Withering of affected leaves



Fig. 2a. Lesions on flower stalks.



Fig. 2b. Lesions on base of the inflorescence head

The typical symptoms developed four days after inoculation with high humidity and the pathogen was reisolated from artificially infected plants. However, mild symptoms were observed from inoculated plants kept in the glasshouse without RH control. On the basis of microscopic examination and the pathogenicity test, the pathogen was identified as the fungus, *Colletotrichum gloeosporioides* (Penz.) Sacc. (the perfect stage of *Glomerella cinguiata* (Stonem) Spauld. & Schrenck). This identification was further confirmed by comparing it with CMI description chart No. 315 (Mordue, 1971).

#### Transmission studies

Typical disease symptoms were observed on seedlings raised on infected soil as well as on autoclaved soil incorporated with crop debris in about 7 - 10 days and at 4 - 5 days after planting respectively. But no symptoms were observed in seedlings raised on autoclaved and uninfected soil. This

suggests that the disease can be transmitted through soil and crop debris and the fungus could be inactivated by autoclaving. Appearance of the disease symptoms after 7-10 days of plantation on seedlings raised on autoclaved soil with the water suspension of infected soil indicated that the disease could also be transmitted through water. Disease symptoms also appeared in seedlings raised on infected seeds in about 2 - 3 weeks after sowing and also the fungus was isolated from infected seeds on PDA.

#### Environmental factors

The disease may appear in the field at any stage of growth depending on the weather conditions and inoculum. High humidity and moderate temperature (23 - 30°C) favour the disease development, but for spore germination and infection, very high relative humidity about 100% is required (Mordue, 1971). Moreover, rain splash has a direct influence on spore disposal. The spores are dispersed from soil to the lower leaves by splash-off and these spores are dispersed to the neck of the onion bulb and into the bulb by wash-off. Under field conditions water containing conidial inoculum can distribute the pathogen to the other areas of the field (Chawda and Rajasab, 1992). The weather data for January - March, 1996 and June - September, 1996 are presented in Fig. 3 and 4, respectively.

The weather conditions during late February, 1996 were very favourable for development and spread of the disease. The relative humidity in the morning and mid-day remained high (85 - 96 %) and mean temperature fluctuated from 30°C (maximum) to 20°C (minimum). During 25 - 27 February, 1996 Maha Illuppallama received (5 - 31 mm) of rainfall (Fig. 3). Usually February is a dry month at Maha Illuppallama. The high RH,

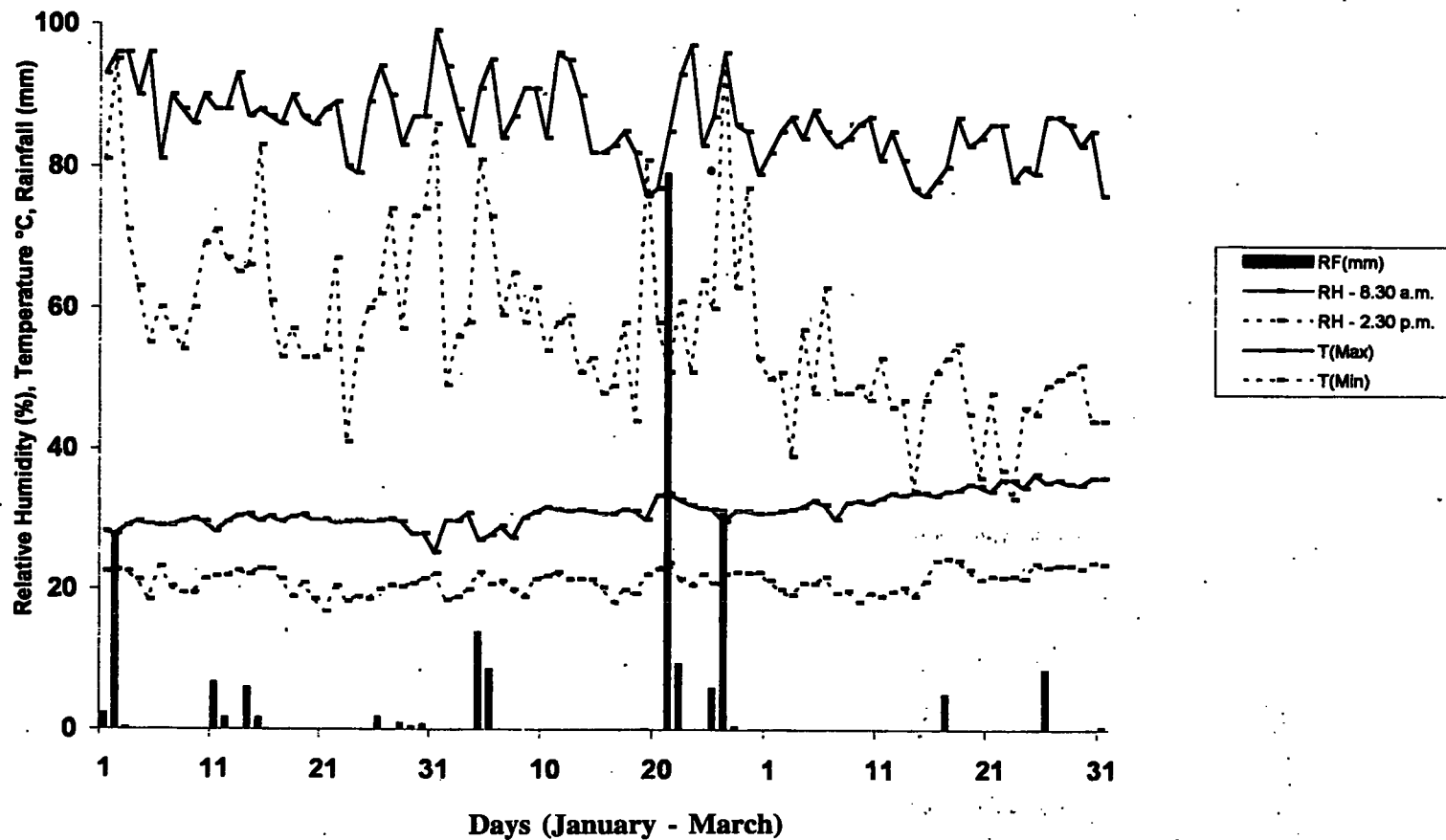


Fig. 3. Climatic data during January - March 1996 at Maha Illuppallama

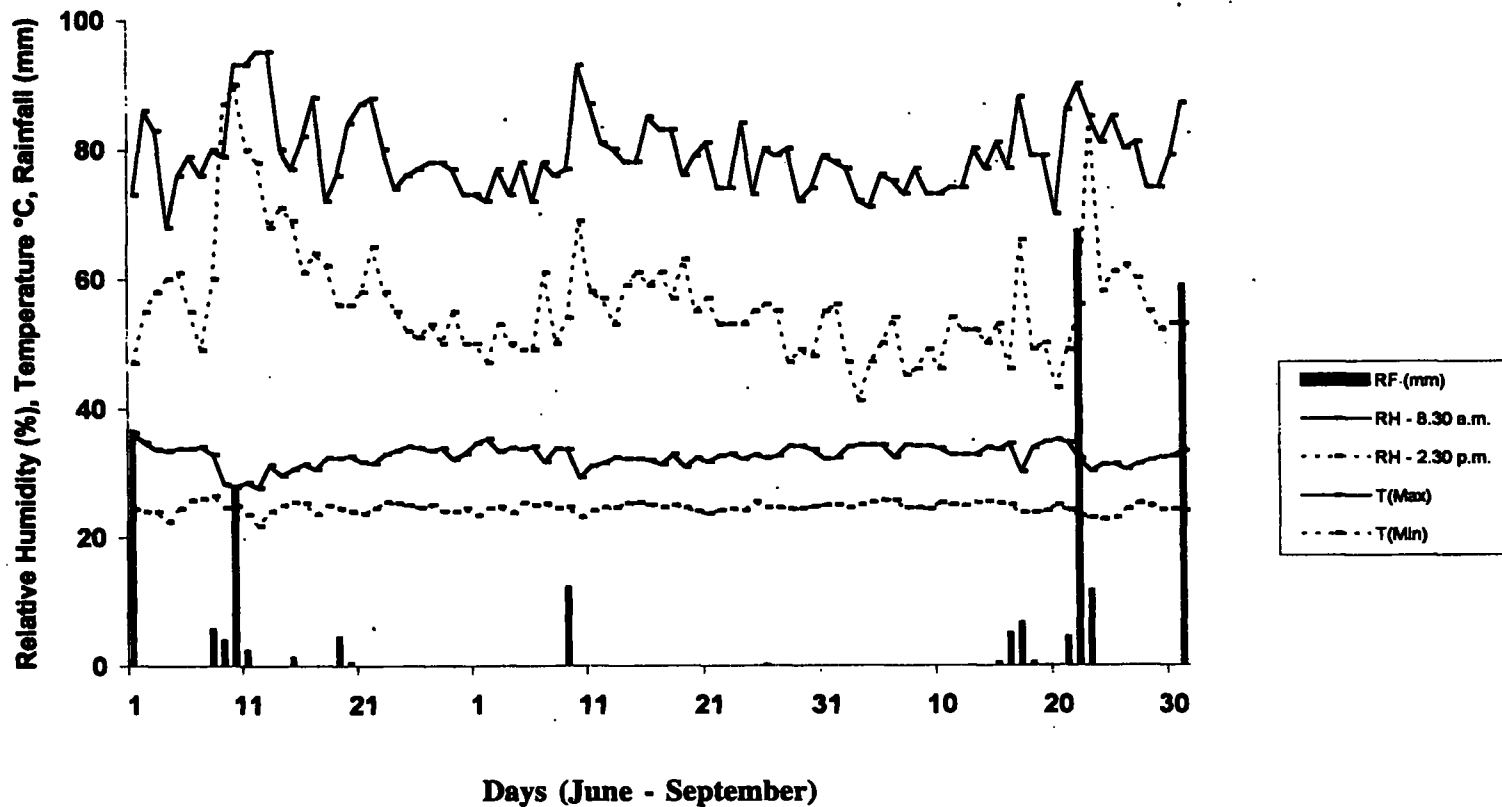


Fig. 4. Climatic data during June - September 1996 at Maha Illuppallama

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optimum temperature and unexpected rains all contributed to the development of the disease and its spread. Generally hot dry environmental conditions during the Yala season are not very favourable for the development of the disease. However, addition of crop debris and unexpected rains, high humidity and optimum temperature (Fig. 4) in late August may have created favourable conditions for disease development. The test location received 83 mm of rainfall during 20 - 30 August, 1996 and the RH was high (85 - 90%). The mean temperature varied from 30°C (maximum) to 22°C (minimum) (Fig. 4). However, disease incidence was very low (less than 3 %) in affected plots, and mild symptoms (few sunken lesions) were observed on the leaves and bulb scales but no leaf twisting or chlorosis occurred. Even though the weather conditions were favourable in August, the crop was at its later stages and therefore there was insufficient time for the disease to develop further.

This disease is reported to be widespread throughout the world but more common in the tropics and sub tropics (Anon, 1986). In other countries different names have been given to this disease such as 'seven curls' in Brazil, 'onion twister' in Nigeria, 'anthracnose' in Northern African countries and India (Chawda and Rajasab, 1996). This is not a widely spread disease in onion cultivations in Sri Lanka at present because onion production is mainly confined to the

Yala season. However attempts are being made to promote onion cultivation during the Maha season both as a bulb crop as well as a seed crop. Environmental conditions during Maha are favourable for the development of the disease. Hence, it has the potential to cause serious losses in the off-season (Maha) cultivation of onion in the future.

## CONCLUSIONS

Leaf twister disease on onion (*Allium cepa* L.) has been identified as a fungal disease caused by *Colletotrichum gloeosporioides* (Penz.) Sacc. (the perfect stage of *Glomerella cingulata* (Stonem) Spauld. & Schrenck).

High relative humidity (RH nearly 100 %) and a moderate temperature ranging from 20 to 31°C with rainfall (5 - 31 mm) appear to be very conducive for the infection and development of the leaf twister disease. Such meteorological conditions are found during latter part of Maha season (January - February). However, it occurs rarely during the Yala season (dry period).

Spores of the fungus can spread through soil, water, seed and crop debris. Crop sanitation including the destruction of crop debris will reduce the infection and also the use of healthy seeds and planting materials is important to prevent the disease. Further research on screening of fungicides and selection of suitable varieties to control and prevent the disease need to be carried out.

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