Fungi associated with diseases of big onion (*Allium cepa* L.) prevalent in Matale District, Sri Lanka.

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Abstract

Big onion (*Allium cepa* L.) is used as a condiment in many countries of the world including Sri Lanka. However, onion yield is reduced due to a number of diseases some of which are of fungal origin that occur in different parts of the big onion plants. It is important that the presence of the more prevalent diseases are surveyed and the causative fungi characterized as it will provide the background essential to carryout disease management practices. Therefore, the present study was aimed at surveying the diseases prevalent at different growth stages of onion crops in the Matale district and isolating and identifying the causative fungi associated with these diseases. The more common symptoms observed in the fields were lesions at the collar region of seedlings, yellowing of leaves and leaf die back, formation of 2-3 mm wide oval shaped patches on leaves and flower stalks and discoloration and softening of bulbs. A *Fusarium* sp. was isolated and identified from infected bulbs. *Colletotrichum gloeosporioides* was isolated from the infected leaves and flower stalks. *Fusarium, Curvularia, Alternaria,* and *Sclerotium* spp were isolated and identified from seedlings showing

damping off symptoms. Koch's postulates were carried out to confirm the pathogenicity of *Fusarium* sp., two *Curvularia* sp. and *Alternaria* sp. isolated from seedlings and it was confirmed that the *Fusarium* sp. isolated was the causative agent of damping off disease of big onion seedlings in the Matale district.

Keywords: *Allium cepa* L., fungal pathogens, *Fusarium* sp., damping off disease, Pathogenicity

Introduction

Big onion (*Allium cepa* L.), belonging to the family Liliaceae, is a condiment grown for its pungent and flavorful bulbs. It plays an essential part of daily diet creating a constant year round consumer demand. Its production is limited to a specific period (Rajapakse & Edirimanna, 2002). According to the Field Crops Research and Development Institute, Mahailluppallama national annual requirement of big onion is about 120,000 mt. But the local production is about 40,000 mt with an average yield of 13 mt/ hectare. Agriculture and Environment Statistics Division, Department of Census and Statistics, Sri Lanka reported that extent of big onion cultivation in Matale district was about 1889 hectares which is about 23.5 % of onion growing areas of Sri Lanka.

Most parts of the big onion plants are prone to different diseases and most of these are of fungal origin. As these diseases cause heavy losses to the yield, it is important that the presence of the more prevalent diseases is evaluated and the causative fungi characterized. This will provide the background essential to carryout appropriate disease management practices. Therefore, the present study aims at surveying the prevalent diseases of onion at different stages of growth and isolating and identifying the causative fungi. Matale district was selected for the first stage of the study.

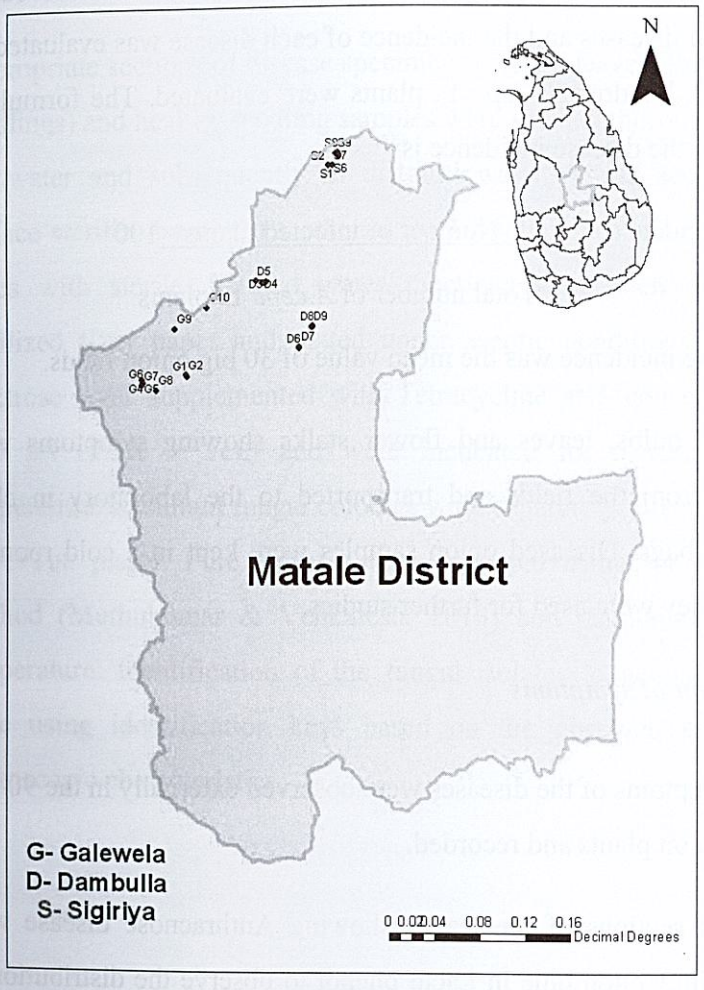
Methodology

Collection of specimens

Collection of seedlings

Diseased and healthy big onion seedlings belonging to different stages (7-30 days after cultivation during *yala* season) were collected from 30 big onion fields shown in Fig 1 in the Matale district. One Field from each location was sampled and 5 samples were collected from each field. The locations were recorded through a Global Positioning System (GPS) receiver and a map of sites was constructed using Arc Map Version 9.2 (Figure 1). These seedlings were transported to the laboratory in clean polythene bags.

Figure 1: Map showing the sampling sites



Collection of disease specimens

The big onion fields at the described locations (2 ½ - 3 months after transplanting) and fields cultivated to obtain seeds were observed for presence of diseases and the incidence of each disease was evaluated. In every field, hundred *A. cepa* L. plants were evaluated. The formula in calculating the disease incidence is:

$$\text{Disease incidence} = \frac{\text{Number of infected plants} \times 100}{\text{Total number of } A.cepa \text{ L. plants}}$$

The disease incidence was the mean value of 30 big onion fields.

Big onion bulbs, leaves and flower stalks showing symptoms were collected from the fields and transported to the laboratory in clean polythene bags. Diseased onion samples were kept in a cold room (9 ^{o}C) until they were used for further studies.

Observation of symptoms

Macro symptoms of the diseases were observed externally in the 90-120 day old onion plants and recorded.

Free hand sections of the leaves showing Anthracnose disease were stained with Cotton blue in Lacto phenol to observe the distribution of the pathogen within the host tissue.

Isolation of fungi from seedlings and diseased plant parts

Appropriate sections of disease specimens (scales, leaves, flower stalks, seedlings) and healthy seedling samples were washed thoroughly first in tap water and subsequently in distilled water. These sections were surface sterilized with 70% ethanol for 1 minute and then rinsed three times with sterile distilled water. Sections were then dried on a sterilized filter paper and plated under aseptic conditions on Potato Dextrose Agar supplemented with Tetracycline at a concentration of 0.05 g/l (PDA + Tet.) and were incubated for 5 days at room temperature. Resultant fungal colonies were separately subcultured onto PDA+Tet. plates. Pure cultures were prepared using the hyphal tip method (Muthukumar & Venkatesh, 2013) and maintained at room temperature. Identification of the fungal isolates to genus level was done using identification keys based on the observed colony and microscopic characteristics.

Confirmation of pathogenicity of fungal isolates using Koch's postulates

Preparation of inocula

Fusarium sp., two *Curvularia* spp., *Alternaria* sp., isolated were used to confirm their pathogenicity on *Allium cepa* L. seedling.

A spore suspension (1×10^5 spores/ml) of each fungus was prepared by adding 5 ml of sterile distilled water to a 7 day cultures maintained in PDA and stirring with a sterile glass rod. The suspensions were quantified using a haemocytometer.

Pathogen Inoculation

Nursery trays were prepared by mixing compost and soil in a 1:10 ratio. Compost was heated in an oven at 60°C for 8 hours while soil was sterilized at 100°C in an oven for 3 hours. *Allium cepa* L. Lanka seeds were placed in the trays at the rate of about 5 seeds per compartment.

Ten day old *Allium cepa* L. seedlings grown in this manner were inoculated by adding 750 µl of spore suspension of each fungus per compartment of the tray while the controls were treated with 750 µl of sterilized distilled water per compartment. Each compartment contained 5 seedlings and there were 24 replicates for each treatment. All compartments bearing seedlings i.e. treated and controls were sprayed

with sterilized distilled water twice a day and Albert solution was applied on both treated and control seedlings as a growth enhancer. A hand atomizer was used for spraying. After inoculation, seedlings were incubated at room temperature.

After ten days, the number of seedlings showing damping off symptoms was recorded and disease incidence (%) was assessed according to Tarr (1981):

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total No. of inoculated plants}} \times 100$$

Re-isolation and identification of pathogens

Inoculated seedlings showing damping off symptoms were surface sterilized by 70% ethanol for 1 minute and then rinsed three times with sterile distilled water. They were then dried on a sterilized filter paper and plated under aseptic conditions on PDA+Tet. These were incubated for 5 days at room temperature. The identity of resultant fungal colonies was confirmed microscopically.

Results and Discussion

The preliminary survey of big onion diseases in the Matale District revealed that several fungal diseases were present at different stages of plant growth and with different levels of disease incidence. The diseases, associated symptoms and their incidence are as follows:

Table 1: Growth Stage, common symptoms and disease incidence associated with Allium cepa L.

Growth Stage	Common Symptoms	Disease Incidence associated with *Allium cepa* L.[1]
Seedling stage (nursery stage)	Lesions at the collar region	22 %
60 day old plants in the field	Yellowing of leaves and leaf die back	70 %
	Formation of 2-3 mm wide oval shaped patches on	5 %

	leaves	
	Formation of 2-3 mm wide oval shaped patches on flower stalks	1 %
	Discoloration and softening of bulbs	12%

[1]Incidence of disease in each field was calculated using:

$$\text{Disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants assessed}} \times 100$$

Results of the survey demonstrated that damping off disease affects 22 % seedlings in a field. The symptoms appear as circular to irregular shaped areas within fields or on individual plants between healthy appearing ones. According to the Department of Agriculture, Sri Lanka, moderate to high soil moisture and soil temperature present in Matale district favor onion damping off disease development.

Colony characters on PDA and microscopic observations of causative fungi isolated from disease specimens are reported in Table 2. The fungal genera isolated were identified by comparison of their colony characters and microscopic features with identification keys.

Table 2: Characteristics of fungal genera isolated from diseased specimens.

Diseased plant part	Isolated fungal sp	Colony Color	Reverse Colony Color	Shape, type and size of Conidia
Bulbs	*Fusarium* sp.	White at early stage purple when colony gets older	Purple	Conidia two types Macro conidia- Sickle shape, larger size Micro conidia- smaller size
Leaves	*Colletotrichum*	Grey at early stage, Black	Black	Straight rounded ends, unicellular

	gloeospori oides	when colony gets older		conidia
Flower stalks	*Colletotric hum gloeospori oides*	Grey at early stage, Black when colony gets older	Black	Straight rounded ends, unicellular conidia
Seedlings	*Fusarium* sp.	White	Yellow	Conidia two types Macro conidia- Sickle shape, larger size Micro conidia- smaller size
	Curvularia sp. 1	Dark brown	Black	Pale brown conidia, septate, curved

	Curvularia sp. 2	Dark brown	Black	Brown conidia, curved, septate, central cell larger and darker
	Alternaria sp.	Greyish white at the beginning, darkens and black when colony gets older	Brown to black	Large conidia with transverse and longitudinal septations, the end of the conidiuum nearest the conidiophores is round while it tapers towards the apex.
	Sclerotium sp.	White mycelium	Yellowish white	White sclerotia at the beginning

				transformed in to light brown and then dark brown

Both intercellular and intracellular fungal mycelium dispersal within epidermal and mesophyll cells could be observed from a lesion by free hand cross sections and staining with cotton blue in lacto phenol (Plate 1).

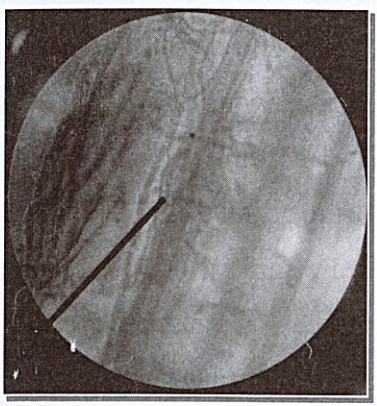


Plate 1: Distribution of the *Colletotrichum gloesporoides* mycelium within the *A. cepa* L. leaf tissue.

Koch's postulates were carried out to confirm the pathogenicity of four different plant pathogenic fungi that were isolated from seedlings showing damping off symptoms **i.e**. *Fusarium* sp., two *Curvularia* sp. and *Alternaria* sp. using a conidial suspension of 1×10^5 spores/ml.

Various concentrations of conidial suspensions have been used effectively as inocula during artificial inoculation in a number of studies on pathogenicity of different organisms. In one such study, *Medicago truncatula* seedlings were inoculated by immersing in a 10^6 conidia/ ml conidial suspension of *Fusarium oxysporum.* Fourteen days after inoculation seedlings showed first symptoms i.e. leaf yellowing and wilting (Lichtenzveig *et.al.,* 2006). In another experiment, tomato growth substrate consisting of soil and cattle manure inoculated with 10^6 and 10^5 conidia/ml with *Fusarium oxysporum* f.sp. *lycopersici* were found to be pathogenic to tomato, causing reduction of plant height (Silva & Bettiol, 2005). In the present study, the *Allium cepa* L seedlings inoculated with 1×10^5 spores / ml of *Fusarium* sp. showed a high disease incidence and caused the death of inoculated seedlings. Therefore, the level of inoculum in the form used can be utilized as a means of artificial inoculation in subsequent studies.

The seedlings were inoculated by adding the conidial suspension to a mixture of sterilized soil and heated and cooled compost. Although collapse of seedlings and lesions at the collar region associated with

damping off was observed in seedlings inoculated with *Fusarium* sp., the two *Curvularia* spp. and *Alternaria* sp. tested did not cause damping off symptoms when inoculated artificially into the soil. A 60 % disease incidence was shown in seedlings inoculated with *Fusarium* sp., whilst only slight weakening of seedlings and slight yellowing of foliage was shown by seedlings inoculated with two *Curvularia* and *Alternaria* spp.

Various *in vitro* inoculation methods have been described to evaluate the pathogenicity of fungi. These include the spot technique, the spraying technique, injection inoculation and soil infestation. (Posada *et.al*. 2007: Gargouri *et.al*, 2009). In this study, soil inoculation was carried out as the fungi isolated are reported to be predominantly soil borne.

Fusarium, Phytophthora, Phythium and *Rhizoctonia* have been reported to cause damping off on seedlings of a number of plant species i.e. *Glycine max* L., *Capsicum annum* L., *Solanum lycopersicum* and *Solanum melongena* (Rahman & Bhattiprolu, 2005).

Major fungal pathogens that have been reported as causal agents of damping off disease in *Allium cepa* L. are *Fusarium* spp., *Pythium* spp. *Rhizoctonia* spp. (Srivastava, 1993). However, in the present study, *Fusarium* sp., two *Curvularia* sp. and *Alternaria* sp. were isolated from diseased onion seedlings and only *Fusarium* sp was confirmed to be the

causative agent of damping off disease of big onions in the Matale district.

Conclusion

Fusarium spp., *Colletotrichum gloeosporioides*, *Curvularia* spp., *Alternaria* sp., *Sclerotium* sp.

are associated with fungal diseases of different plant parts of big onion in the field.

Damping off is the only observed disease in the *Allium cepa* L. at seedling stage.

The *Fusarium* sp. that was isolated from seedlings was the causative agent of damping off disease of big onion seedlings in Matale district.

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