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Biological Control: A Promising Tool for Bulb-Rot and Leaf Twisting Fungal Diseases in Red Onion (*Allium Cepa* L.) In Jaffna District

Vigitha Naguleswaran, Kandiah Pakeerathan and Gunasingam Mikunthan

Department of Agricultural Biology Faculty of Agriculture,
University of Jaffna, 40000, Thirunelweli, Jaffna, Sri Lanka

Abstract: The queen of kitchen *Allium cepa* L. cultivation is catastrophic by devastating fungal pathogens in Jaffna, Sri Lanka. A field investigation was carried out to diagnose the fungal diseases of red onion and to provide possible alternative for hazardous fungicides. *Fusarium oxysporum* f. sp. *cepae* was isolated and characterized by cottony and irregular concentric shaped white colony, later turned to creamy non smooth and hyaline mycelium. Disease symptoms were identified as yellowing, curling and necrosis at the tip of leaf blades. With time, whole leaf blades showed symptoms and eventually wither and decay. *Colletotrichum gloeosporioides* produced greyish white to dark gray, circular cottony appearance and produce irregular concentric rings on PDA medium. Disease symptoms of leaf twister are identified as appearance of leaf curling, twisting, chlorosis and abnormal elongation of the pseudo stem. In the field experiment, *Trichoderma viride*, Neem leaf extract, Distillery spent wash were tested with untreated control. All the treatments were replicated four times under randomized complete block design The results of field trial revealed that, the bulb treatment together with foliar application of *Trichoderma viride*, performed very well in aspects, such as, bulb diameter (29.64 mm), circumference of bulb (76.06 mm), mean number of bulbs per bunch (6.95), yield (130.7 Mt/ha) with the negligible disease incidence (1.08 %) in relation to untreated control such as bulb diameter (26.24 mm), circumference (59.9 mm), mean number of bulbs per bunch (5.47), yield (79 Mt/ha) and percent of disease incidence (11.3 %). The results obtained in this study helps to manage the fungal diseases of red onion and best promising alternative to commercial sustainable agriculture

Key words: Bio-control · *Trichoderma viridae* · Red onion · Leaf twisting · Bulb-rot

INTRODUCTION

Onion (*Allium cepa* L.) is one of the oldest important cash crops grown in Sri Lanka; believed to be originated in central Asia and gives greatest income to the farmers. Queen of the kitchen (onion) is preferred mainly because of its flavoure and taste as well as colourful bulbs is often chosen for salads [1]. Moreover, Onion has many uses as folk medicine and recent reports suggests that onion plays an important role in preventing heart diseases and other ailments [2,3].

The global area under onion cultivation is 3.06 million ha with a production of 53.59 million Mt [4] as well as in Sri Lanka year around onion cultivation is more than 4840 ha with the production of 56,920 Mt; both in *maha* and *yala* season [5]. Among the onion varieties, Red onion cultivation is popular among farmers in Jaffna (Northern Sri Lanka) due to its higher demand and regional suitability. The total area of red onion cultivation in Jaffna district is 3919 ha and the average annual production is 58,695 Mt /ha [6].

In Jaffna, onion growers are losing their production hugely because of the fungal diseases. Among them, *Fusarium* basal rot and Leaf twister are the major constraints for successful red onion production. These diseases are causing severe yield loss in onion cultivation.

Corresponding Author: K. Pakeerathan, Department of Agricultural Biology,
Faculty of Agriculture, University of Jaffna, Jaffna, Sri Lanka.

Fusarium oxysporum f. sp. *cepae* can infect on onion at all stages of the plant's growth. It causes damping-off of seedlings, root rot in older plants that may result in the death of the plant and basal rot of bulbs, [7]. Disease symptoms of leaf twister consist of white, oval, sunken spots on the leaves at onset of infection followed by the appearance of concentric rings of orange acervuli in sunken necrotic spots [8]. Bulbs of affected plants are generally slender. Some bulbs rot before harvest; others decay rapidly when stored [9].

Complete control of aforesaid seed borne diseases in onion is yet to be developed. Management strategies for these diseases include use of recommended disease free bulbs, resistant cultivars, adaptation of proper nursery management and fungicidal sprays. Seed treatment is one of the best method but continuous and indiscriminate use of fungicides to manage the crop diseases results in accumulation of harmful chemical residues in the soil, water and bulbs. Recently, considerable success has been achieved by introducing antagonists to control seed-borne fungal pathogens [10].

Plant doctor fungi *Trichodema* spp. are considered most popular, an environmentally friendly bio-control agents against numerous phytopathogenic fungi and similar to soil pathogens, they successfully suppress leaf pathogens as well and limited chemical usage for an effective integrated pest management (IPM) system [11]. This intimate relationship between *Trichoderma* spp. and the host root cells are what induces localized and systemic resistance plant responses to pathogen attack but the development of formulation and delivery systems for antagonistic microorganisms is of great importance in the field of biological control [12,13]

This study was initiated with the objective of enhancement of bio-control as a promising tool for fungal diseases in red onion due to the banning of many agrochemicals with the report of booming cancer and kidney related incidences.

MATERIALS AND METHODS

Isolation of Pathogen: Pathogenic fungi were isolated by surface sterilization method described by Kinkel and Andrews, (1988) [14]. The fungal pathogens *F. oxysporum* f. sp. *cepae* and *Colletotrichum gleosporioides* were isolated from the diseased red onion bulbs and leaves. The samples were collected in the areas of *Idaikkadu* and *Kopay* in Jaffna, Sri Lanka during April 2013. Sample size of 10 mm bulbs and leaves were selected and subjected to surface sterilization by washing with 70 % ethanol for a minute and rinsed three times with distilled water.

Table 1: Details of treatments

Treatment	Details
T_1	Bulb treatment with *Trichoderma viride*
T_2	Bulb treatment and foliar application of *Trichoderma viride*
T_3	Bulb treatment with Captan (20 g/10 l of water)
T_4	Foliar application of neem leaf extracts (80 %)
T_5	Soil treatment with Distillery spent wash (DSW)
T_6	Control (Untreated)

Sterilized specimens were transferred to the Petri dishes containing PDA medium supplemented with 2-5 drops of Chloromphenicol (5 %) as an antibiotic to prevent the bacterial contamination. Then the Petri dishes were incubated in room temperature of 30 ± 3 °C for 6 days. After incubation, produced pure culture was subjected to sub-culture and stored in the refrigerator at 4 °C for further study.

Confirmation of *Fusarium oxysporum* f. sp. *cepae* and *Colletotrichum gloeosporioides*: Confirmation test was carried out by inoculating *F. oxysporum* f. sp. *cepae* and *C. gloeosporioides* mycelia in to healthy red onion plants in pots separately, using soil drench method. Symptoms and signs were confirmed, by using of Koch's postulate, culture morphology and growth pattern of mycelium on PDA.

Field Experiment to Manage Fungal Diseases of Red Onion: A field experiment was conducted during May-July 2013 at *Idaikkadu*, Jaffna Sri Lanka under basin irrigation system, in order to manage the fungal disease of red onion. Totally five treatments (Table 1) were tested with an untreated control was also maintained in the field. All the treatments were replicated four times under randomized complete block design. Plot size was 1.2 m x 1.2 m was planted with '*Vethalam'* cultivar.

Spore Concentration for Field Spray: Spore count of *Trichoderma viride* for bulb treatment and foliar application was $4x10^9$ spore/ml. All the general agronomic practices, except for the treatment, were followed.

Data Collection and Statistical Analysis: Randomly selected Plants height and the number of leaves per plant were recorded from 14 day after planting, in each plot. Disease incidence was calculated by visual rating method.

The crop was harvested by observing maturity indices. Plants were uprooted from the net area of each plot separately. Soil particles adhering to the bulbs were removed; yield parameters like weight, diameter and circumference of the bulb were measured.

Percent of disease incidence was calculated using the following formula,

$$\text{Disease incidence \%} = \frac{\text{Mean number of diseased plants}}{\text{Mean number of plants in plots}} \times 100$$

All the *In-Vitro* experiments were designed according to the complete randomized design (CRD) and field experiment was conducted under the randomized complete block design (RCBD) and the derived data were statistically analyzed using SAS package, significance among the treatments were determined according to the least significant difference (LSD) test at 95% of the confidence interval.

RESULTS AND DISCUSSION

Ecological approach is the viable mean of solving the pest and disease problem in the sustainable agriculture. Proper diagnosis is foremost important to apply solid management strategy where ever possible. This chapter describe the attributes of the fungi and results obtained in the field base management trails.

Character and Structure of *Fusarium Oxysporum* F. Sp. *Cepae*: Cottony and irregular concentric shaped white colony formed initially and turned to creamy non smooth and hyaline mycelium in the culture (Plate 1). The fungus formed one or two celled, oval to kidney shaped microconidia and three to four celled, curved macroconidia usually produced (Plate 2). Chlamidospores also formed in culture [15] reported that the Chlamidospores are the primary source of inocula in the field. Havey (2008) [16]also reported that the fungus produces curved three to four septa maroconidia.

Characters and structures of *Colletotrichum gloeosporioides*: *Colletotrichum gloeosporioides* fungus formed greyish white to dark gray, circular cottony appearance and produce irregular concentric rings on potato dextrose agar medium. Black colour concentric rings were observed on reverse of Petri plate (Plate 3). Orange colour spots were observed when the culture matured; these spots are the fruiting bodies, called acervuli. Conidia were hyaline (Plate 4), unicellular and cylindrical with obtuse ends or ellipsoidal. Black spikes, resembling short hairs, were observed among the fruiting bodies and similar observation recorded by Litz (1997)[17].

Symptoms of *Colletotrichum gloeosporioides*: Typically under field conditions, appearance of leaf curling, twisting, chlorosis and abnormal elongation of the pseudostem were observed (plate 5c). White sunken oval lesions appeared mainly in lower leaves. These lesions enlarged with time and spread all over the leaf. Black, minute, slightly raised acervuli with pinkish orange masses of conidia scattered on the surface of lesions. Roots tend to be stunted and caused the plants death. Affected bulbs produced from these plants were small and eventually rotted. Lowell (2012) [18] reported that the most characteristic field symptoms of onion twister disease are curling, twisting and chlorosis of the leaves. Ebenebe (1980)[9] reported that the bulbs of affected plants are generally slender. Some bulbs rot before harvest; others decay rapidly when stored.

A-Rotten onion plant, B- Mycelial growth of *Fusarium oxysporum* f. sp. *cepae* on root surface of onion, C-Leaf curling and chlorosis of onion at initial stage, infection by *Colletotrichum gloeosporioides*, D-Rotten onion due to the infection of *C.gloeosporioides*.

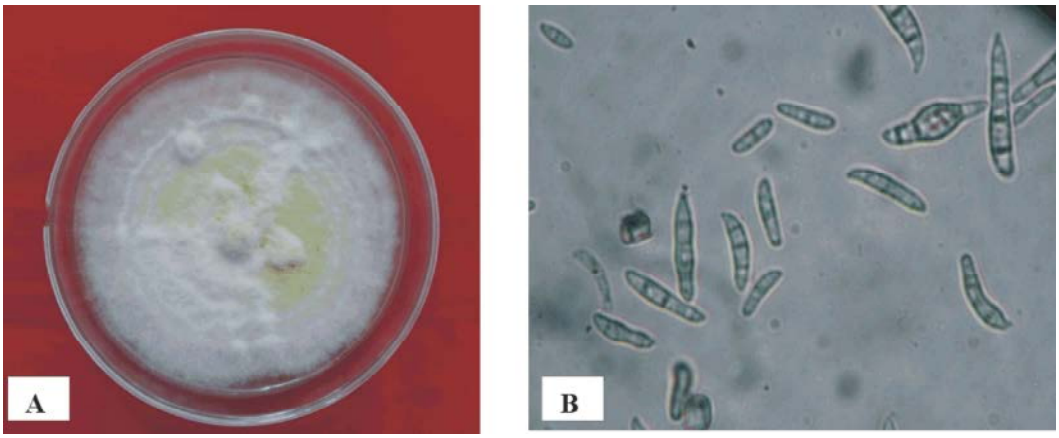

Plate 1: (A) Pure culture of *Fusarium oxysporum* f. sp. *cepae* on PDA and (B)Macroconidia (x 100)

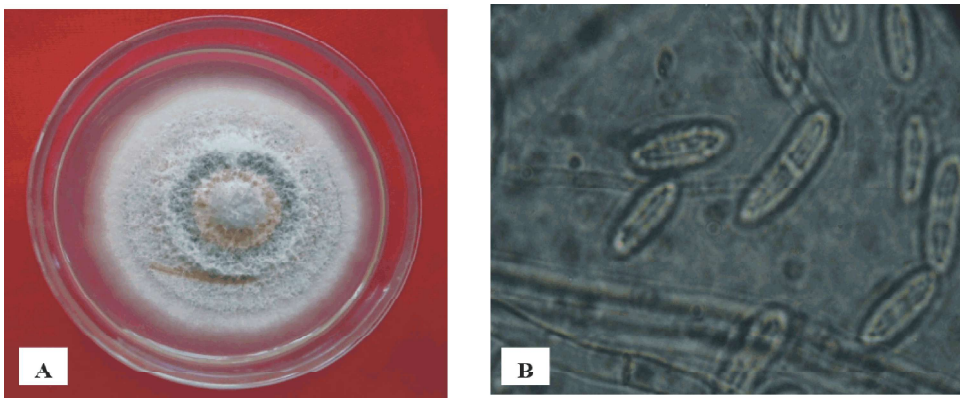

Plate 3: (A) Pure culture of *Colletotrichum gloeosporioides* grown in PDA media and (B) Conidia (x100)

Plate 5: Symptoms of *Fusarium* basal rot and Leaf twister of red onion

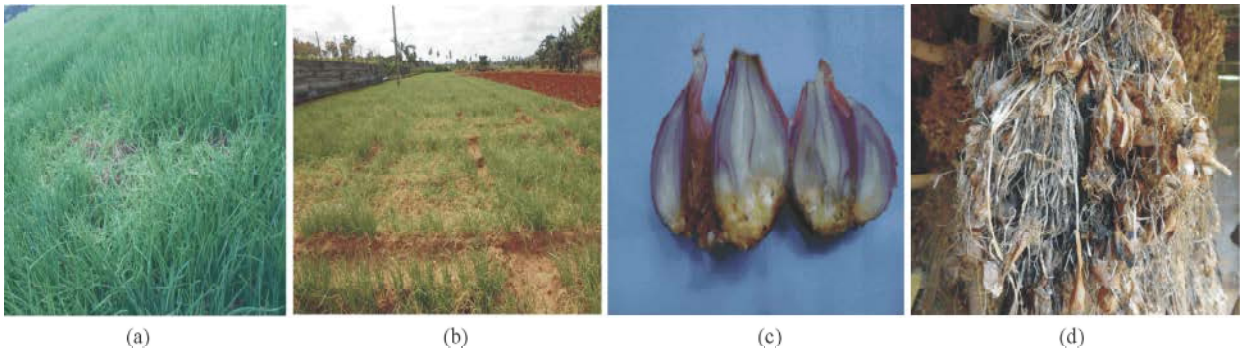

Plate 6: Various symptoms of fungal diseases on red onion

A-Patchy appearance of Leaf twister in field, B-completely destroyed field at Kopay in combination of fungal diseases, C- Watery, yellowish discoloration of stem plate due to infection of *F. oxysporum* f. sp. *cepae*. D- Rotting of onion during storage.

Field Experiment to Manage Buld-rot and Leaf Twisting Fungal Diseases in Red Onion: A field trial to manage the fungal diseases of red onion was conducted during May- July 2013 at *Idaikkadu*, Jaffna Sri Lanka under basin irrigation system.

The results indicated that there is a difference among the different treatments with respect to yield and yield related parameters. The results revealed that, the maximum yield of (130 Mt/Ha) and less disease incidence of 1.08% were obtained in T_2 (Table 2) but in T_1 yield, disease incidence, bulb diameter, circumference of bulb, mean number of bulbs per bunch were 109.78 Mt/Ha, 1.52 %, (29.69 mm), (76.06 mm) and (6.95) respectively with next best to T2. The yield of T_4, T_3 and T_5 were (100.9 Mt/Ha), (99.91Mt/Ha), (92 Mt/Ha), respectively and the disease incidences were 5.43 %, 6.08 %, 6.95 %, respectively.

Table 2: Field experiment to manage fungal diseases of red onion

Treatments circumference of (Mt/ha)	Percent of disease Mean diameter of incidence (%) bulb (mm)	Mean number of Mean bulb bulbs/bunch yield (Kg/plot)	Mean Yield bulb(mm)
T_1	1.52 [a]	6.4[a]	71.10[a]
	28.37[a]	6.56[a]	109.78
T_2	1.08 [a]	6.95[a]	76.06[a]
	29.64[a]	7.81[a]	130.70
T_3	6.08 [b]	6.22[a]	59.62[b]
	26.80[b]	5.97[a]	99.91
T_4	5.43 [b]	5.92[b]	62.06[b]
	26.84[b]	6.03[a]	100.90
T_5	6.95 [b]	5.72[b]	62.63[b]
	27.25[b]	5.50[a]	92.00
T_6	11.30[c]	5.47[b]	59.02[b]
	26.24[b]	4.75[b]	79.00

Means with same letters are not significantly different according to the dunnett at 0.05α

Trichoderma grows fast and compete with disease causing fungi for food, space as well as developing mycotoxin system for many soil or foliar pathogen [19], as well as stimulatory effect on growth of onion seedlings seed germination, vigour index and fresh weight of seedlings, [20]. It is well documented that the interaction of *Trichoderma* strains with the plant may promote growth, improves crop yield, increase nutrient availability and enhance disease resistance [21 and 13]. In addition, some species of *Trichoderma* are able to colonize root surfaces, interact with the plant and exchange compounds that can cause substantial changes in plant metabolism [22]. Several species of *Trichoderma* are reported that they develop exactly on other fungi's hyphae, coils around them and degrades the cell's walls. The action of parasitism restricts the development and activity of pathogenic fungi as well as also produces secondary metabolites like cellular exochitinases [13, 23 and 24] with antibiotic activity [21,25and 26].

The percent of disease incidence in T_4 was 5.43 % and it was next to T_2 (1.08%) and T_1 (1.52 %). Neem has insecticidal, antibacterial and antifungal activity due to the presence of chemicals such as Azadirectin, salannin, nimbin [27].

The mean circumference of bulb and mean bulb diameter of T_5 were 62.63mm, 27.75mm, respectively and it was next to T_2 and T_1, indicating that the diluted spent wash irrigation improved the physical and chemical properties of the soil and further increased soil micro flora [28, 29 and 30]. It contains high biological oxygen demand (BOD) and chemical oxygen demand (COD). The effluent contains high potassium (1.3 %), sulphur (0.4 %) and appreciable amount of nitrogen (0.2 %) and act as slow release fertilizer being mostly in the colloidal form [31]

CONCLUSION

Basal rot and leaf twister are the major fungal diseases of red onion detected in Jaffna. In the field experiment, bulb treatment together with foliar application of *T. viride* improved yield as well as yield related parameters such as, bulb diameter, circumference of bulb, mean number of bulbs per bunch. Moreover, percent of disease incidence was also negligible. These findings help to manage the fungal diseases in red onion effectively by using plant doctor fungi *Trichoderma* sp without dangerous fungicides.

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