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Allium cepa L. (Onion) Storage Diseases and Effect of *Trichoderma asperellum* and *Trichoderma virens* Pre-harvest Treatments on Postharvest Quality

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ABSTRACT

Substantial yield losses have been reported during the storage of *Allium cepa* L., and effective methods to minimize postharvest losses are currently lacking. Therefore the objectives of this study were to investigate the impact of pre-treatment with *Trichoderma asperellum* and *Trichoderma virens* on post-harvest losses during storage and to evaluate factors associated with these losses. The storage behaviors of *Allium cepa* L. bulbs after different field treatments with *Trichoderma* spp. were examined using a complete randomized design. The common onion bulb diseases encountered during storage were basal rot and black mould diseases, with *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Aspergillus niger* and *Aspergillus flavus* being associated with diseased bulbs. The results regarding the percentage of diseased bulbs showed that pre-harvest treatments with *Trichoderma asperellum* and *Trichoderma virens* did not have a significant effect on disease control during storage. Furthermore, storage losses of onions were attributed to sprouting, rooting and wilting, which were exacerbated by the prevailing temperature (~30-32°C) and relative humidity (~77%-79%) during the storage period.

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INTRODUCTION

Allium cepa L., commonly known as big onion, is utilized as a condiment in many countries worldwide and holds significant economic value as a cash crop in Sri Lanka. Onions thrive in moderate climates, avoiding excessive rainfall and extreme temperatures. Optimal conditions for early growth involve cool temperatures ranging from 20°C to 25°C, alongside sufficient moisture supply. As the plants mature and approach harvest, warm and drier conditions are preferable. The pH requirement falls between 5.8 and 8.0, with the optimum range being 6.0 to 7.0 (Maankotte, 2004; Rathnayaka, 1992). The Agriculture and Environmental Statistics Division of the Department of Census and Statistics in Sri Lanka (2020) reports that big onion cultivation is primarily concentrated in four districts: Matale, Anuradhapura, Mahaweli H and to a lesser extent in Polonnaruwa.

Big onion in Sri Lanka experiences a consistent consumer demand of approximately 200,000 MT throughout the year. However, the annual national production falls significantly short at only about 68,643 MT (Department of Census and Statistics, Sri Lanka, 2021). Consequently, it becomes crucial to increase production and minimize losses caused by various factors.

The objective of storing onion bulbs is to preserve their quality and extend the marketing period. Successful storage of onion bulbs is vital to ensure a year-round supply for consumption and seed production. As storage organs by nature, bulbs are well-suited for this purpose. Onion dormancy initiates through a decline in respiration and abscisic acid levels within the bulbs. Nonetheless, during storage, challenges arise from issues such as sprouting, drying and rotting, resulting in losses (Coolong, 2007).

Big onions are susceptible to various diseases that can occur at any stage of their growth in the field or during storage. These diseases can be classified into three categories: seedling diseases, foliage diseases and bulb diseases, primarily caused by fungi and bacteria, resulting in significant losses to the harvest.

Seedling diseases encompass damping off and seedling blight, which are caused by *Fusarium* spp., *Pythium* spp., *Rhizoctania* spp. and *Sclerotium* spp. Major foliage diseases include purple blotch, caused by *Alternaria porri*; anthracnose disease, caused by *Colletotrichum gloeosporioides*; and downy mildew, caused by *Peronospora destructor*. Profuse bulb diseases include *Fusarium* basal rot, caused by *Fusarium oxysporum* f. sp. *cepae*; white rot, caused by *Sclerotium cepivorum*; and bacterial rots, caused by *Pseudomonas* spp., *Xanthomonas* sp. and *Erwinia* sp. Diseases associated with storage include black mold, caused by *Aspergillus niger*; blue mold rot, caused by *Penicillium* spp.; and neck rot, caused by *Botrytis* spp.

Substantial losses in crop yield result from plant diseases caused by phytopathogenic agents. In recent times, there has been an increasing focus on biological control methods for fungal pathogens as alternatives to the use of chemicals. Synthetic pesticides are expensive, contribute to environmental pollution, and pose potential risks to animals and humans. Furthermore, their repeated application leads to the development of chemically resistant strains of pathogens (Vinale et al., 2014).

One of the most effective strategies for pest management in agriculture is the utilization of microbes in biological control. Biological control involves harnessing the benefits of beneficial organisms, their genes, and/or their products, such as metabolites, to mitigate the negative impacts of harmful organisms, including plant pathogens, while promoting positive interactions with desirable organisms like crops, beneficial insects, and microorganisms (Pal and Gardner, 2006).

Biological control emerges as the most favorable alternative, particularly for combating soil-borne pathogens. *Trichoderma* spp. have proven highly effective as biological control agents against various soil-borne phytopathogenic fungi, including *Fusarium* spp. and *Pythium* spp. (Akrami et al., 2011; Amal et al., 2005; Gveroska and Ziberoski, 2011).

While commercial formulations of *Trichoderma* are available, it is advisable to utilize locally isolated *Trichoderma* spp. as biocontrol agents, as they cause minimal disruption to the soil's microflora. Previous efforts have been made to develop an efficient formulation of locally isolated *Trichoderma* spp. for biocontrol against damping off disease and basal rot of onions (Gunaratna et al., 2020 (a), Gunaratna et al., 2020 (b)). Based on the literature, two methods, seed coating and soil inoculation, were tested for introducing the prepared *Trichoderma* inoculum under greenhouse conditions. The results indicated that seed priming and soil treatment with *Trichoderma* spp. were suitable for field trials. Previous studies demonstrated the efficacy of inocula prepared from two locally isolated *Trichoderma* spp. in significantly reducing the incidence and severity of damping off and basal rot diseases in *A. cepa* L., both under greenhouse and field conditions, when applied individually or in combination. Therefore, the prepared inoculum could effectively control damping off and basal rot diseases of *A. cepa* L. in the field. Furthermore, treatment with *Trichoderma* spp. enhanced the plant height, dry weight, and fresh weight of tested *A. cepa* L. seedlings and transplants (Gunaratna et al., 2020 (b)). Based on the above findings, the objectives of this study were formulated to evaluate the effect of pre-treatment with *Trichoderma* spp. on disease control during the storage of *Allium cepa* L., identify factors contributing to storage losses of *Allium cepa* L., identify fungi associated with storage deterioration of *Allium cepa* L., and identify fungi associated with stored bulb tissue samples of *Allium cepa* L. after different seed pre-treatments in Sri Lanka.

METHODOLOGY

Effect of pre-harvest treatments to control diseases during storage

Seeds of the onion cultivar "Galewela light red" were sown into seed beds in May 2015 and grown for four weeks. In the nursery trial, twelve treatments were tested, which included Talc-based formulations of *Trichoderma asperellum* and *T. virens* (Gunaratna et al., 2020 (a)). In June 2015, the seedlings were uprooted and transplanted into raised beds. During the transplanted stage, eleven treatments were tested, including the aforementioned Talc-based *Trichoderma* spp. formulations (Gunaratna et al., 2020 (b)). In September 2015, the plants were manually harvested, stubbed, and had their leaves mowed, while stones and clods were removed. The bulbs were then cured at 32°C for 3 days under ambient drying conditions, with careful attention given to avoiding physical injury to the bulbs during these operations.

Storage

Seventy-five (75) randomly selected healthy bulbs harvested from different pre-treated fields were stored according to a complete randomized design with three replications. The onions were stored in open-mesh packages at a temperature and relative humidity of approximately 30-32°C and 77-79%, respectively. During storage, there were four categories: bulbs neither pre-treated with *Trichoderma* spp. nor fungicides, bulbs pre-treated with *Trichoderma asperellum*, bulbs pre-treated with *Trichoderma virens* and *Trichoderma asperellum*, and bulbs pre-treated with fungicides (Mancozeb, Homai, Captan).

At two-week intervals, the bulbs were turned, and the number of diseased bulbs was recorded. After removing the bulbs from storage bags, they were weighed, cut longitudinally, and visually assessed for disease symptoms. Diseased bulbs were carefully separated and recorded to avoid contaminating the healthy bulbs. The final number of bulbs on the last data collection date was subtracted from the initial number of bulbs, and the difference was calculated as the percentage lost due to diseases. The weights of healthy bulbs were measured at the end of the 14th week period to determine the storage losses during this period. The disease status in each category was calculated using the following formula.

$$\text{Percent diseased bulbs} = \frac{\text{number of infected bulbs}}{\text{total number of bulbs}} \times 100\%$$

Determination of weight loss during storage

The weight loss during storage was calculated, after removing the dry scales at the end of the storage period using the following formula.

$$\text{Weight loss (\%)} = \frac{(\text{Initial weight of onion bulbs} - \text{Final weight of onion bulbs})}{\text{Initial weight of stored onion bulbs}} \times \text{x100\%}$$

Isolation and identification of fungi associated with diseased big onion bulbs during storage

The diseased onions during storage were sorted out based on macro symptoms of infection, and these samples were pooled together according to their macro-symptoms. Infected scales of the onion bulbs measuring 1 cm x 1 cm were removed using a sterile scalpel blade and surface sterilized with 70% ethanol for one minute. The pieces were then rinsed three times with sterilized distilled water and dried on a sterile filter paper. The edges of the sections were trimmed, and pieces measuring 0.5 mm x 0.5 mm were plated under aseptic conditions on Potato Dextrose Agar medium (PDA) supplemented with Tetracycline (PDA+Tet.). The plates were incubated at room temperature for seven days. Similarly, the infected outer dry scales of onion bulbs showing black mold were removed with a sterile scalpel and directly plated on PDA+Tetracycline plates, which were then incubated at room temperature for seven days. The fungal isolates were identified based on their macro and micro-morphological characteristics using the sticky tape method. The frequency of occurrence of each fungal species was calculated using the following formula:

$$\text{Frequency of occurrence} = \frac{\text{number of times a fungus was encountered}}{\text{total fungal isolations}} \times 100\%$$

Isolation of associated fungi from roots / leaves of sprouted onion bulbs during the storage

Fungal isolation was conducted using the tissue plating method described above. Root pieces measuring 1 cm in length and leaf pieces measuring 5 mm x 5 mm (from roots and leaves of onions pre-treated with *Trichoderma* spp., onions neither pre-treated with *Trichoderma* spp. nor fungicides, and onions pre-treated with fungicides) were placed on PDA+Tet. medium and incubated at room temperature for seven days. The fungal isolates were identified based on their macro and micro-morphological characteristics, using the sticky tape method.

Determination of seed quality

Seed infestation test (Agar Plate Method)

Twenty-five non-surface sterilized *A. cepa* L. seeds were placed in petri plates containing Potato Dextrose Agar medium (PDA) supplemented with tetracycline. The plates were then incubated at room temperature for seven days. The isolated fungal genera were identified based on their macro and micro-morphological characteristics, using the sticky tape method.

Blotter method

The blotter method was utilized to detect fungi associated with the surface of *A. cepa* L. seeds. In this method, two layers of sterile Watman No. 1 filter paper were moistened with sterilized water and placed at the bottom of a 15 cm diameter petri dish. Batches of 40 non-sterilized seeds were then placed on the moist filter paper. After plating, the seeds were incubated at room temperature for seven days. At the conclusion of the incubation period, the fungi were identified based on observed characteristics of the colony and microscopic structures.

RESULTS AND DISCUSSION

Effect of pre-harvest treatments on control of diseases during the storage

Two post-harvest fungal storage diseases were identified as black mold and basal rot based on previously available data (Table 1). Furthermore, a certain level of storage loss was attributed to the sprouting of onion bulbs. The study revealed that bulb diseases associated with fungi accounted for approximately 33.33% to 65.33% of the losses during storage.

The results of percent diseased bulbs indicated that there was no significant effect of pre-harvest treatments, whether pre-treated with *Trichoderma* spp. or pre-treated with fungicides, on the control of diseases during storage (Table 2).

Determination of weight loss during the storage

The average percent weight loss during storage ranged from 6.26% to 34.53%.

Isolation and identification of fungi associated with diseased big onion bulbs during storage

a) Percentage occurrence of isolated fungi associated with basal rot disease of *A. cepa* L. during the storage

Mucor sp. had the highest frequency of occurrence (50%) among the surface-sterilized infected scales of the onion bulbs, followed by *Fusarium* sp. (44%). *Penicillium* sp. recorded the lowest frequency of occurrence (6%).

b) Percentage occurrence of isolated fungi associated with black mould disease of *A. cepa* L. during storage

Aspergillus niger had the highest frequency of occurrence (44.4%), followed by *Aspergillus flavus* (40.0%), among the black mould disease-bearing non-surface sterilized dry scales. *Penicillium* sp. had a frequency of occurrence of 6.7%. *Fusarium* sp. and *Mucor* sp. had the lowest frequency of isolation, both at 4.4%.

Isolation of associated fungi from roots/leaves of sprouted onion bulbs during the storage

The following fungal genera were isolated by tissue plating method (Table 3).

Determination of seed quality

a) Seed infestation test (Agar Plate Method)

The fungal pathogens isolated from the *A. cepa* L. seeds were *Aspergillus* spp., *Curvularia* spp., *Alternaria* sp. , *Fusarium* sp.

b) Blotter method

Aspergillus niger was isolated from non-surface sterilized seeds using Blotter method.

Table 1 : Post-harvest fungal storage diseases, symptoms and causal agents

Storage disease	Symptoms	Causal Agent
Black mould disease	Infected bulbs may develop a black discoloration, clusters of black spores generally form along veins and on or between the outer papery scales of bulbs	*Aspergillus niger*
Basal rot disease	The basal part of the onion bulb exhibits a brown discolouration, softening of the bulbs, when affected bulbs are cut vertically, they show a watery brown discoloration	*Fusarium oxysporum* f. sp. *cepae*

Table 2: Percent diseased bulbs after storage period with different treatments

Pre-treatment during field condition	Percent diseased bulbs (%)*
Neither pre-treated with *Trichoderma* spp. nor fungicides (Control)	56.44 (48.70)[a]
Pre-treated with *Trichoderma asperellum*	51.55 (45.89)[a]
Pre-treated with *Trichoderma virens* and *Trichoderma asperellum*	48.89 (44.36)[a]
Pre-treated with fungicides	40.00 (39.23)[a]

*Means of three replicates in each pre-treatment. Figures in parentheses are arc sin transformed values.

Table 3 : Fungal genera isolated from root and leaf samples after a storage period of 14 weeks

Pre-treatment during field condition	Tissue sample	Fungal genera isolated
Neither pre-treated with *Trichoderma* spp. nor fungicides (Control)	Roots	*Fusarium* sp., *Mucor* sp.
	Leaves	*Fusarium* sp. , *Penicillium* sp., *Mucor* sp.
Pre-treated with *Trichoderma* spp.	Roots	*Fusarium* sp., *Aspergillus* sp., *Mucor* sp.
	Leaves	*Aspergillus* sp.
Pre-treated with fungicides	Roots	*Mucor* sp.

Big onions are an essential condiment in the daily diet of Sri Lankans, with a consistent year-round demand totaling approximately 200,000 tonnes annually (Customs report, 2009). The production of big onions is limited to a seasonal cultivation period from April to September, known as the yala season, with harvesting taking place in September and October. However, the off-season shortage and restricted cultivation result in the importation of big onions to meet the demand.

Proper preharvest and postharvest conditions are essential for the storability of onion bulbs, as they play a significant role in ensuring a fresh market supply and availability for processing purposes (Adamicki, 2005). Additionally, *Allium cepa* L. seeds play a crucial role in disseminating and developing diseases during different growth stages. Seed-borne diseases can also be transmitted through seeds, causing damage at various stages of *Allium cepa* L. growth, from germination to crop maturity, and even during storage, resulting in substantial losses (Dumbre et al., 2011). Therefore, determining seed quality is equally important.

The current study revealed that the surfaces of *Allium cepa* L. seeds were contaminated with *Aspergillus niger* and other internal contaminants, including *Aspergillus* spp., *Curvularia* spp., *Alternaria* sp., and *Fusarium* sp., using the Agar plate method, similar to the work conducted by Dumbre et al., in 2011. In their study, Dumbre et al., (2011) identified 12 different fungi, including five species of *Aspergillus*, two species of *Fusarium*, and one each from *Penicillium*, *Drechslera*, *Curvularia*, *Rhizopus*, and *Alternaria*. Among the various fungi, *Aspergillus niger* was found to be dominant in *Allium cepa* L. seeds collected from different locations in India, with a frequency of 58.88% in non-surface treated seeds and 33.55% in seeds treated with $HgCl_2$.

Further, Koycii and Ozer (1997) reports, high frequency of *Aspergillus* and also Nagerabi and Abdalla (2004) established that *Aspergillus* was the most prevalent genus among all the seed borne fungi of onion. The findings of Adongo et al., (2015) reported that *A. niger* was the most frequently encountered fungus from the seeds of Bawku Red onion variety. *A. flavus, A. niger* were also isolated by Jidda and Benjamin (2016) from seed samples of Monguno Red onion variety. Additionally, *Alternaria alternate*, *Alternaria porri*, *Rhizopus stolonifer*, *Botrytis allii* and *Penicillium roqueforti*, *Fusarium* spp. were also isolated from onion seeds other than *Aspergillus* spp. (Adongo et al., 2015; Jidda and Benjamin 2016).

During the current study, two major diseases were encountered during the storage period: black mould disease and basal rot disease. In addition to diseases, storage losses occurred due to sprouting and rooting of *Allium cepa* L. bulbs. Similar findings were reported by Currah and Proctor (1990), who identified sprouting and bulb rotting as major causes of losses. The present study revealed that five fungal genera were associated with post-harvest storage diseases: *A. niger, A. flavus, Fusarium* sp*., Mucor* sp*.,* and *Penicillium* sp. These findings are consistent with those reported by Jidda and Benjamin (2016), who also isolated *A. niger* and *Fusarium* sp. from rotten red onion bulbs.

Adongo et al., (2015) conducted a survey of fungi associated with postharvest deterioration of onion bulbs and found that rotten onion bulbs were infected by *Aspergillus niger*, *A. flavus*, *Penicillium* sp., *Rhizopus stolonifer*, and *Fusarium oxysporum*. Major storage rots identified included black mould, blue mould, soft rot, neck rot, and basal plate rot. Similar to the current findings, black mould was the most predominant storage disease. Mahmud and Monjil (2015) also reported that losses were mainly due to black mould rot and *Fusarium* bulb rot. Similarly, Ko et al., (2002) stated that black mould caused by *A. niger* is the major disease during storage under ambient conditions in the tropics.

Seeds are considered to be sources responsible for the transmission of pathogens. The presence of *Aspergillus* spp. and *Fusarium* sp. in seed samples, as well as in black mould and basal rot diseased bulbs during storage, suggests the possibility of fungal transmission through seeds, which later act as agents of storage diseases. The Food and Agriculture Organization of the United Nations has indicated that *A. niger* infection occurs during onion production, but the development of the fungus on the bulbs takes place during storage when the environmental conditions are conducive to their growth.

Furthermore, the results revealed that pre-harvest treatments, such as pre-treatment with *Trichoderma* spp. or fungicides, did not have a significant effect on disease control during storage. However, standard fungicide treatments during field conditions reduced the percentage of diseased bulbs after harvest (40%), compared to control treatment (56.44%) or pre-treatments with *Trichoderma* spp., such as pre-treated with *Trichoderma asperellum* (51.55%) and pre-treated with *Trichoderma virens* and *Trichoderma asperellum* (48.89%). Nevertheless, *Trichoderma virens* and *Trichoderma asperellum*, isolated from the soils of *A. cepa* L. growing areas and applied as seed coating, seedling root dip, or soil inoculation, could be effectively used in the management of *Allium cepa* L. damping off and basal rot diseases under greenhouse and field conditions (Gunaratna et al., 2020(a), Gunaratna et al., 2020(b)).

Additionally, some onions were spoiled due to sprouting. Suojala (2001) reported that storage losses were primarily caused by sprouting, rooting, and wilting. The prevalent temperature (~30-32°C) and relative humidity (~77%-79%) during the storage period might have enhanced sprouting. Appropriate storage temperature and relative humidity can help prevent sprouting. According to the Food and Agriculture Organization of the United Nations, storage at temperatures of 25-30°C has been shown to reduce sprouting and root growth compared to low-temperature storage (10-20°C). However, high-temperature storage can lead to weight loss, bulb desiccation, and rot. Successful low-temperature storage requires proper ventilation and a relative humidity range of 70-75%. However, for tropical climates, the organization recommends high-temperature storage with proper ventilation, temperatures above 25°C, and humidity levels of 75-85%.

Moreover, during post-harvest, fungal genera such as *Fusarium* sp. and *Mucor* sp. were found to be associated with the root tissues of sprouted onion bulbs during storage. Moreover, *Aspergillus* sp. was isolated from the root tissues of sprouted onion bulbs that were pre-treated with *Trichoderma* spp., while only *Mucor* sp. was isolated from the root tissues of sprouted onion bulbs pre-treated with fungicides. *Aspergillus* sp. was also isolated from the leaf tissues of sprouted onion bulbs that underwent pre-treatment with *Trichoderma* spp., whereas *Fusarium* sp., *Penicillium* sp., and *Mucor* sp. were isolated from the leaf tissues of sprouted onion bulbs that were neither pre-treated with *Trichoderma* spp. nor fungicides.

CONCLUSIONS

Five fungal genera, namely *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* sp., *Mucor* sp., and *Penicillium* sp., were found to be associated with post-harvest storage diseases in *Allium cepa* L. Additionally, the major contaminants of *Allium cepa* L. seeds were

identified as *Aspergillus* spp. The main post-harvest diseases observed in *Allium cepa* L. were black mould disease and basal rot disease. Furthermore, significant losses were attributed to sprouting, and some weight loss was also reported during storage. It was determined that neither pre-harvest treatments with *Trichoderma* spp. nor fungicides were effective in controlling diseases during the storage of *Allium cepa* L.

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