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Research Article

A Simple Seed Disinfection Technique to Eliminate Anthracnose Pathogen from Capsicum Seeds

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Abstract: Anthracnose disease significantly affects the yield and quality of capsicum (*Capsicum annum* L.). Pathogen/s disperses from infected plants to another through soil, water, air and seeds. It has been reported that seed lots of capsicum received to seed health testing laboratory of Seed Certification Service, Department of Agriculture from various sources were highly infected with anthracnose and failed to fulfill the requirements of seed health standard due to seed infection with anthracnose pathogen. Therefore, studies were conducted to identify effective hot water treatment for elimination of anthracnose pathogen from capsicum seeds. The predominant anthracnose pathogen found in the capsicum seeds of tested samples was *Colletotrichum gloeosporioide*. However, *Colletotrichum capsici* was rarely found. Among the hot water treatments tested, most effective seed disinfection technique to eliminate anthracnose pathogen and other fungi was hot water treatment of infected seeds at 53^0C for 30 min followed by seed treatment by fungicide, Thiram 80WP at the rate of 5g of fungicide in one kg of seeds.
Keywords: Anthracnose, Capsicum, *Colletotrichum*, Seed disinfection.

Introduction

Anthracnose disease is one of the major constraints to the profitable cultivation of capsicum of Sri Lanka. Anthracnose is seed-borne disease and recently, a serious incidence of anthracnose of capsicum was observed in many areas of Sri Lanka. Direct losses occurred in the field and during transit. In addition to direct losses, it has been reported that pod quality deterioration of capsicum due to anthracnose are very high and varied with grown varieties, weather conditions of the growing season in Sri Lanka (Rajapakse *et al.,* 2008).

Anthracnose of capsicum is caused by species of the genus *Colletotrichum*. Characteristically, it is predominantly associated with lesions on matured fruit, but also causes the dieback of stems and branches in capsicum. Several species of *Colletotrichum*, including *C. capsici*, *C. gloeosporioides*, *C. acutatum*, *C. coccodes*, *C. dematium*, *C. truncatum* have been implicated in the anthracnose disease of capsicum from various part of the world (Amrita *et al.,* 2016; Hadden & Black, 1989; Kiran *et al.,* 2020). Among them *C. gloeosporioides* and *C. capsici* are the major causal agents of anthracnose disease of capsicum in Sri Lanka (Rajapakse *et al.,* 2008). It has reported that anthracnose is seed, soil, air and water-borne and may causes significant losses of fruit quality as well as yield of capsicum (Amrita *et al.,* 2016). Anthracnose pathogens are transmitted from infected seed to seedling and reduced germination in severe infection (Khilendra Singh *et al.,* 2009).

It has been reported by seed health testing laboratory of Seed Certification Service (SCS), Department of Agriculture (DOA), Sri Lanka that some seed lots of Capsicum received from many sources were failed to fulfill the requirements of seed health standard due to seed infection with

anthracnose pathogen resulting shortage of quality capsicum seeds in the market. Therefore, studies were conducted to identify simple seed disinfection technique to eliminate anthracnose pathogen from capsicum seeds.

Material and Methods

Identification of causal agent/s:

Anthracnose affected capsicum seed lots were collected from Seed Certification Service (SCS), Department of Agriculture (DOA). Pathogens were isolated from disease-affected seeds on Potato Dextrose Agar (PDA). Single spore isolates of pathogen were prepared from mycelia of single conidia cultures grown on PDA. Pathogens were identified on the basis of morphological observations of conidia, setae, acervuli by microscope and culture characteristics of isolates on PDA. Pathogenicity of all isolates was tested by wound inoculation with conidia suspension ($4x10^5$ conidia/ml) i.e. pin prick method and subsequent anthracnose development on fruit surfaces (Rajapakse, 1999).

Seed disinfection

Diseased seed samples (each 5g) were subjected to hot water treatments with different temperature regimes ranged from 47 ^{0}C to 60 ^{0}C and time period ranged 15 min to 60 min. After the hot water treatments, seeds of each treatment were kept on tissue papers to drain off excess water and then half of the samples of each treatment were treated with fungicide- Thiram 80WP (5g/kg of seeds). Both treated and untreated control seed samples were allowed to dry in room temperature (24-30 ^{0}C) for two days and incubated at 10-12 ^{0}C for two weeks before laboratory test conducted. Experiment was repeated two times. All seed samples were initially tested in laboratory at Horticultural Research and Development Institute (HoRDI), DOA to calculate percentage seed germination and anthracnose infection. Standard blotter method was used for detection of the pathogen from seeds (Limonard, 1996). Microscopic observations were performed to calculate percentage seed infection of each seed lot with anthracnose pathogen.

Based on the preliminary laboratory test results, hot water treated (53 ^{0}C for 30 min) seed lots and hot water treated (53^{0}C for 30 min) followed by fungicide, Thiram 80WP (5g/kg of seeds) treated seed lots and untreated control sample were handed over to seed health test laboratory, SCS/DOA to carry out further laboratory test for detection and quantifying the pathogenic microorganisms associated with submitted seed lots.

ESTA Standard laboratory techniques were applied to identify associated pathogens and their percent seed infection of seed samples at seed testing laboratory of SCS/DOA. Associated pathogens were identified by culturing test, microscopic observations and relevant molecular tools.

Result and Discussion

Isolates of anthracnose pathogen of capsicum seeds were identified by comparison of their morphology and culture characters on PDA with published data. According to the colony morphology on PDA medium and the shape and size of conidia, isolates were identified as *C. capsici* and *C. gloeosporioides* (Table 1). Pring *et al*., (1993) and Sutton (1992) showed that variation between isolates is typical for many species in the genus *Colletotrichum*. However, characteristics of tested isolates were within the published range of *C. capsici* and *C. gloeosporioides* (Sutton, 1992). Isolates were tested for their ability to induce lesions in mature fruits using the pin prick method indicated that tested isolate of *C. capsici* and *C. gloeosporioides* had the ability to develop anthracnose lesions on capsicum fruits. The disease was identified by large 1-1.5 cm diameter, brown, circular depressions and the fungus appeared as brown acervuli on the surface of inoculated capsicum fruits. Most of the seed lots collected in this study were found infected with *C. gloeosporioides* and a few were found with *C. capsici* infection. The predominant pathogen of anthracnose disease of the capsicum seeds was *C. gloeosporioides*.

Table 1. Characters of fungal isolates of *Colletotrichum gloeosporioides* and *Colletotrichum capsici* collected from anthracnose affected capsicum seeds.

Characters of fungi isolates	Morphology and culture characters of isolates	
	Colletotrichum capsici	***Colletotrichum gloeosporioides***
Colony colour on PDA	White initially then turned light brown or brown, colony margin smooth	Grey initially then turned light brown or brown, colony margin wavy
Reverse colony colour on PDA	Dark brown	Brown
Acervuli	Black colour masses, conidia present inside.	Brown colour masses, conidia present inside.
Setae	Black colour, straight, septate, 60-192 µm in length & 4-6 µm in width	Absent
Conidia	Sickle shaped, aseptate, vacule present in centre, 16-32 µm in length & 2-4 µm in width, Conidia germinate and produce appressoria at the end of germ-tube on plant surfaces, but rarely in distilled water	Cylindrical with round end shaped, aseptate, Fat globules present inside, 12-18 µm in length & 2-4 µm in width, Some conidia germinate in distilled water and produce appressoria at the end of germ-tube
Shape of the appressoria	Oval shape, abundant and black colour	Oval shape, abundant and black colour

Preliminary laboratory test results conducted at HORDI indicated that two treatments i.e. hot water treated seeds (53^0C for 30 min) and hot water treated (53^0C for 30 min) followed by fungicide Thiram 80WP (5g/kg of seeds) treated seeds were free from anthracnose pathogen and recorded higher seed germination (above 90%) compared to other treatments tested (Data not presented). Seed health tests conducted by SCS/DOA were confirmed that hot water treated seeds (53^0C for 30 min) as well as hot water treated (53^0C for 30 min) followed by fungicide Thiram 80WP (5g/kg of seeds) treated seeds were free from anthracnose pathogen (Test report No: SHTU/20/CR/0012, SHTU/20/CR/0011 and SHTU/20/CR/0013 respectively (Table 2). However, there were higher percentage of *Aspergillus* spp. in hot water treated seeds compared to hot water and Thiram treated seeds. Untreated disease seeds were showed higher percentage of *Colletotrichum* as well as several other fungi (Table 2).

Table 2. Fungal species detected in different treatments by standard bottler test

Hot water treated seeds (Report no: SHTU/20/CR/0012)		Hot water followed by Thiram treated seeds (Report no: SHTU/20/CR/0011)		Untreated diseased seeds (control) (Report no: SHTU/20/CR/0013)	
Aspergillus spp.	99.0	*Aspergillus* spp	0.25	*Colletotrichum* spp.	9.25
Curvularia spp.	0.25	*Rhizopus* spp.	0.25	*Fusarium* spp.	6.5
Unidentified saprophytes	0.5			*Curvularia* spp.	2.0
				Penicillium spp.	3.75
				Alternaria alternate	0.75
				Rhizopus spp.	88.0
				Unidentified saprophytes	86.75

Conclusion

The predominant pathogen of anthracnose disease of the capsicum seeds was *C. gloeosporioides*. However, *C. capsici* was rarely found. Most effective seed disinfection technique to eliminate anthracnose pathogen was hot water treatment of infected seeds at 53^0C for 30 min followed by seed treatment by fungicide, Thiram 80WP at the rate of 5g of fungicide in one kg of seeds.

Conflicts of interest

The authors declare no conflicts of interest.

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