Ceylon Journal of Science 48(3) 2019: 261-269
DOI: http://doi.org/10.4038/cjs.v48i3.7650

RESEARCH ARTICLE

Characterization of fungal pathogens causing anthracnose in capsicum pepper (*Capsicum annuum* L.) and their seed borne nature

W.A. Welideniya[1], K.D.R.C. Rienzie[2], W.A.R.T. Wickramaarachchi[3,*] and A.G.B. Aruggoda[1]

[1]Department of Agricultural and Plantation Engineering, Faculty of Engineering Technology, The Open University of Sri Lanka, Nawala, Nugegoda 11222, Sri Lanka
[2]Agribusiness Centre, Faculty of Agriculture, University of Peradeniya, Peradeniya 20400, Sri Lanka
[3]National Plant Quarantine Service, Canada Friendship Road, Katunayake 11450, Sri Lanka

Received: 17/01/2019 ; Accepted: 08/07/2019

Abstract: Anthracnose caused by *Colletotrichum* spp. is a troublesome disease in all *Capsicum* spp. including capsicum pepper (*Capsicum annuum* L.), causing severe losses at both the pre-harvest and post-harvest stages. It is common throughout the world. In Sri Lanka, capsicum pepper is likely to be infected by both *Colletotrichum capsici* (*Colletotrichum truncatum*) and *C. gloeosporioides*, resulting in a reduction in the quantity and quality of the harvest. A detailed investigation was carried out to identify the different species of *Colletotrichum* that infect capsicum pepper, and the nature of this infection by employing standard tests. *Colletotrichum capsici* and *C. gloeosporioides* were isolated from the capsicum pepper seed samples collected from three different agro ecological regions in Sri Lanka. When capsicum pepper seeds collected from fruits categorized according to a standard rating scale were tested for germination, a gradual decrease of germination percentages and increased seed infection percentages were observed. After culturing different components of the seeds collected from infected fruits, it was found that both *C. capsici* and *C. gloeosporioides can* survive on the seed coat, pericarp and embryo. In addition, *Fusarium* spp. and *Aspergillus* spp. were also present in cultures of seed coat but not in cultures of the pericarp and embryo. The study revealed that both *C. capsici* and *C. gloeosporioides* can invade the important parts of the seeds internally and externally, causing higher germination losses, during both the pre and post emergence stages of capsicum pepper; the *Aspergillus* spp. and *Fusarium* spp. were only seed borne externally.

Keywords: Anthracnose, Capsicum pepper, *Capsicum annuum* L., *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, Seed borne pathogens.

INTRODUCTION

Capsicum pepper (*Capsicum annuum* L.) is a globally important vegetable crop that belongs to the family Solanaceae. It is recorded that the genus *Capsicum* originated in South America. There is a high market demand for capsicum pepper fruit due to its shape and colour. Kurunegala, Puttalam, Badulla, and Nuwara-Eliya are the major capsicum pepper growing districts in Sri Lanka since a favorable climate prevails in those areas for the growth of capsicum crop. Biotic factors such as pathogens (fungal, bacterial, viral, and nematodal) and insects can cause adverse effects on capsicum pepper cultivation (Senanayake *et al*., 2013; Kelaniyagoda *et al*. 2011). In Sri Lanka, bacterial wilt, anthracnose, various viruses, and several insect pests are the major biotic factors that constrain capsicum pepper cultivation. Out of those, anthracnose caused by *Colletotrichum* spp. accounts for the most significant crop losses globally to capsicum pepper cultivators (Mongkolporn & Taylor, 2018; Diao *et al*., 2015). Anthracnose sets in either during the pre-harvest or post-harvest period causing fruit rot, resulting in extensive losses when *Capsicum* is grown in tropical and subtropical climates, irrespective of season (AVRDC, 1998). Five species of *Colletotrichum* have been identified as being responsible for anthracnose disease in *Capsicum* in various countries. Among these are *Colletotrichum capsici*, also referred to as *Colletotrichum truncatum* according to the latest classification (Ranathunge *et al*., 2012), and *C. gloeosporioides*, which are the causal agents of anthracnose disease in green chilies (hot pepper) in Sri Lanka (Jayawardana *et al*., 2015; Rajapakse & Ranasighe, 2002).

In Sri Lanka, hot chili, which is one of the *Capsicum* spp. is mostly infected by *C. capsici,* while capsicum pepper is infected by both *C. capsici* and *C. gloeosporioides* (Rajapakse & Ranasighe, 2002). Seed borne nature of *C. capsici* and *C. gloeosporioides* has been proven by many studies throughout the world (Saxena *et al*., 2016). However, to date, no systematic studies have been conducted on seed borne nature of capsicum pepper anthracnose caused by *Colletotricum* spp. Furthermore, information on internationally recognized seed health testing procedures, which is a necessary part of the seed certification process for capsicum pepper seeds, is also scarce. Therefore, the present study was commenced with two objectives, namely to identify major fungal pathogens causing anthracnose in capsicum pepper and to evaluate the seed borne nature of *Colletotrichum* spp. using different seed health testing procedures.

**Corresponding Author's Email:* wartwa@gmail.com
https://orcid.org/0000-0002-5125-1091

MATERIALS AND METHODS

This research was conducted at the Division of Plant Pathology, Horticultural Crop Research and Development Institute (HORDI), Gannoruwa, Peradeniya, Sri Lanka.

Symptoms observed on infected fruits and isolation of fungal pathogens

Infected and healthy capsicum pepper fruits were randomly collected from three different agro ecological regions (AER), namely Low Country Dry Zone (LCDZ: 20 samples), Mid Country Wet Zone (MCWZ: 20 samples), and Up Country Wet Zone (UCWZ: 20 samples) in Sri Lanka. Fruits were placed in sealed polythene bags after wrapping with clean tissue paper and transported to the Laboratory. The visible symptoms of anthracnose in infected fruits were recorded while the manifestation of symptoms during development of the disease were also recorded with time. Table 1 and Table 2 present the information on healthy and diseased capsicum pepper fruits, respectively. Anthracnose-infected seeds and fruit tissues were cultured in potato dextrose agar (PDA) with 0.01 % Streptomycin, and incubated for 5 days at 25±1 °C. The mycelia growth and acervuli development around seeds and tissue were observed with the naked eye. Conidial masses suspected to be *Colletotrichum* spp. were picked from colonies and subcultured in PDA for further purification. Accordingly, seven isolates were designated with a reference number for each isolate. The collected *Colletotrichum* isolates were named in such a way that the place where the fruits were collected, cultivars, and maturity of the infected pods (whether ripe or unripe) could be identified.

For single spore isolation, spore suspensions from each sub-cultured fungal species were prepared. The concentration of all spore suspensions was adjusted to approximately 10 spores/µl with a Pipetman microliter pipette. A marker pen was used to draw circles (3 mm diameter) on the bottom of each 2% water agar plate. Ten microliters (10 µl) of the spore suspension was placed on the surface of the water agar above each circle. After incubation at 25±2 °C for 12 hours, each circle was inspected under a microscope at 100X magnification through the bottom of the plate. These marked agar areas were cut and transferred to PDA slants using a cork borer under aseptic conditions and incubated at 25±2 °C. The mycelia growth and acervuli development around seeds and tissue were observed through naked eye. Conidial masses picked from colonies that were suspected to be *Colletotrichum* spp., were sub cultured in PDA for further purifications. Accordingly, seven isolates were designated with reference numbers for each isolate. The collected *Colletotrichum* isolates were named using the combination of the place where the fruits were collected, cultivars, and maturity of the infected pods (unripe or ripe).

For single spore isolation, spore suspensions from each sub-cultured fungal species were prepared. The concentrations of the spore suspensions were adjusted to approximately 10 spores/µl with a Pipetman microliter pipet. A marker pen was used to draw circles (3 mm diam.) on the bottom of each 2% water agar plate. Ten micro liters (10 µl) of the spore suspension was placed on the surface of the water agar above each circle. After incubation at 25±2 °C for 12 hours, each circle was inspected under a microscope at 100× magnification from the bottom of the plate. These marked agar areas were cut and transferred to PDA slants using a cork borer under aseptic conditions and incubated at 25±2 °C.

Colony and spore morphology

Two *Colletotrichum* isolates were grown on PDA plates kept at 25±2 °C while subject to dark and light periods alternating every 12 hours. Plugs (5 mm) were aseptically

Table 1: Capsicum fruits collected from different cultivars from different locations.

AER	No. samples	District	Location	Cultivars
LCDZ	20	Kurunegala	Pure seeds farm (Nikaweratiya)	CA-8 and Bulnose
MCWZ	10	Kandy	Gannoruwa	CA-8 and Calister
MCWZ	10	Kandy	Kandy market	Hungarian Yellow Wax (HYW)
UCWZ	20	Nuwara Eliya	Rahangala	Hungarian Yellow Wax (HYW)

Table 2: Sources of seven isolates of *Colletotrichum* spp. obtained from infected fruits.

AER	District	Location	Cultivar	*Colletotrichum* spp.	Pod stage	Isolate name
LCDZ	Kurunegala	Nikaweratiya	CA-8	*C. capsici*	Ripe	NW-CA-R
			Bulnose	*C. capsici*	Ripe	NW-B-R
MCWZ	Kandy	Gannoruwa	CA-8	*C. capsici*	Ripe	G-CA-R
			Calister	*C. capsici*	Ripe	G-C-R
			Unknown	*C. capsici*	Ripe	G-UK-R
		Kandy market	HYW	*C. gloeosporioides*	Unripe	KM-HYW-UR
UCWZ	Nuwara Eliya	Rahangala	HYW	*C. gloeosporioides*	Ripe	HR-HYW-R

punched from the edges of actively growing areas of a 5-day-old culture of each isolate. Each plug was placed on PDA plates and incubated under the same conditions as starter cultures. After 7 days, the mean colony growth rate was determined. Colony characteristics of the mycelium such as color were recorded. Colony growth rate and texture were considered for species identification. The morphological characteristics of conidia were determined using 7-day-old fungal cultures. Fungal masses were harvested with sterile distilled water and the spores were observed under a compound light microscope. One hundred spores were measured under a high power objective using calibrated ocular and stage micrometers. Morphological characteristics such of acervuli, setae, and conidia were studied and the average spore sizes were calculated.

Koch's postulates

Koch's postulates were confirmed with fruits (pin-prick method/10 µL of conidia suspension per wound) by using a 7 day old inoculum grown on PDA at a concentration of $5X10^5$ conidia/ml standardized with a hemocytometer (Sharma *et al*., 2005). Re-isolation was performed from the lesions developed on artificially infected fruits. The isolated pathogen was compared with the original culture to prove Koch's postulates.

Determination of the seed borne nature of *Colletotrichum spp.* using seed health testing procedures

To discover the effectiveness of different seed health testing methods for detecting seed borne fungi of capsicum pepper, two methods were checked out according to the protocols published by the International Seed Testing Association (ISTA) (Anon, 1996), specifically standard blotter method and water agar method**.**

Standard blotter method

Standard Blotter Method developed by Doyer (1938) is a simple, convenient method, which was later approved by the International Seed Testing Association and included in the ISTA Rules of 1966. Accordingly, the seeds are rated using a scale developed for estimating disease severity of Anthracnose, and it includes the following levels: 0- No spots, 1- One or two small spots less than 1 cm^2 in size, 3- Two or three sunken spots of about 1 cm^2 in size, 6- Several bigger spots above 1 cm^2 in size, 9- More than half of entire surface of pod covered with discoloured or black spots and in a rotting state. Four hundred and fifty seeds infected by *C. capsici* (CA-8) and *C. gloeosporioides* (HYW) for each rating scale were tested by employing standard blotter method in three replicates of 150 seeds each (six plates with 25 seeds). Three circular pieces of blotting paper 90 mm in diameter were moistened with distilled water, and placed in 90 mm diameter sterilized culture plates after draining the excess water. 25 untreated seeds were placed and distributed uniformly on each culture plate so they were spaced equally. The plates were then incubated at room temperature (25±2°C) under alternate periods of 12 hr light and 12 hr darkness. After eight days of incubation, the seeds were examined under a light microscope to identify the associated fungi and to check their growth. Further confirmation of seed borne fungi was obtained by using a compound light microscope with temporary slides of the fungi. Disease incidence of fungi was calculated on a percentage basis by using the formula, (No. of infected seeds/ Total no. of seeds examined) X 100.

Water agar method

Four hundred and fifty seeds infected with *C. capsici* (CA-8) and *C. gloeosporioides* (HYW) were tested for each rating scale by placing 10 seeds per culture plate containing 20 ml of 2% agar; there were 3 replicates of 150 seeds each (15 plates with 10 seeds). The culture plates were incubated for 7 days and their fungal growth was examined under a dissecting microscope. The disease incidence was calculated using the same formula as described in the Standard Blotter Method.

Seed borne nature of C. capsici and C. gloeosporioides on seed quality parameters (pot culture studies)

Pot culture test was employed to test the transmissibility of pathogens from seeds to progeny plants potted with sterilized sand, topsoil, and compost in a 1:1:1 ratio (Dahanayake *et al*., 2012). A hundred seeds from each of the samples infected with *C. capsici* (CA-8) and *C. gloeosporioides* (HYW) were sown separately at the rate of 30 seeds per pot with 10 replicates. Seeds were misted with water and the pots were covered using transparent polyethylene sheets. Healthy seeds from varieties CA-8 and HYW were sown in pots to serve as controls. After 30 days, the symptoms were recorded and the germination rate, pre-emergence losses, and post emergence losses were calculated percentage-wise as described in Khare (1996).

Seed borne nature of C. capsici and C. gloeosporioides on seed quality parameters (Seedling symptom test)

Capsicum pepper seed samples infected with *C. capsici* (CA-8) and *C. gloeosporioides* (HYW), as well as healthy seeds were examined separately for seedling symptom test. Culture tubes were filled with 30 ml aliquots of 2% water agar. Three hundred seeds from each sample were placed individually in each tube (ten seeds per replicate) and incubated at 25±2 °C under alternating 12 hr light and dark periods for 21 days. Once the seedlings reached the tube brim, the cotton plug was removed and observations were recorded based on symptoms evident in the seedlings: percentage germination, pre-emergence losses and post emergence losses (Khare, 1996).

Determination of location of fungi in seeds

The location of fungi in seeds was studied by employing the component plating technique as described by Maden *et al*., (1975). Seed samples naturally infected from *C. capsici* (CA-8) and *C. gloeosporioides* (HYW), as well as healthy seeds were used separately in this study. Twenty-five seeds were washed four times with tap water, and surface sterilized in 1 % sodium hypochlorite solution for 2 minutes. The seeds were again washed with sterile water and soaked in water for 2 hr. Then the seeds were dissected aseptically using sterile needles and forceps. The

separated seed coat, pericarp, and embryo were placed immediately on water agar plates to prevent tissue drying. Healthy seed parts were used as the control. The plates were then incubated under room temperature (25±2 °C) under alternating 12 hr light and 12 hr dark periods. After seven days, the seed components were examined under a dissecting microscope to check for the presence of fungi in different seed parts. A compound light microscope was used for further confirmation of the pathogen.

Statistical analysis of data

All experiments were designed in accordance with Completely Randomized Design (CRD) approach. Data collected for experiments performed on seed borne nature of *Colletotricum* spp. using standard blotter method and water agar method were statistically analyzed with ANOVA, while the data obtained for the same experiment using pot culture studies and seedling symptom test methods were analyzed with Student's *t*-test using the SAS statistical software package (version 8) of SAS Institute Inc., Cary, NC, USA.

RESULTS AND DISCUSSION

Capsicum pepper (*Capsicum annuum* L.) is a globally important vegetable crop that is reported to be infected frequently by a complex of *Colletotrichum* species including *C. fructicola*, *C. siamense, C. gloeosporioides*, *C. truncatum*, *C. acutatum, C. coccodes, C. queenslandicum* and *C. simmondsii*. It has also been documented that both *C. gloeosporioides* and *C. capsici* are species complexes of which *C. capsici* has several pathotypes that are very destructive. However, up to now, with the exception of *C. gloeosporioides* and *C. capsici*, there have been no reports on multiple species associated with chili and capsicum anthracnose in Sri Lanka.

Symptoms of infected fruits and isolation of fungal pathogens

Development of symptoms on fruits

The symptoms on capsicum pepper fruits usually appear as small round dark brown areas on unripe fruits. These spots enlarge and rapidly develop into a brown superficial discoloration of the skin, which later turn into circular, slightly sunken, and water soaked lesions with darker margins and brown or black central portions as the fruit ripens. Gradually, the lesions coalesce and sparse mycelial growth often appears on the margins of these spots. In the *C. gloesporioides* infected fruits that were tested, an encrustation of salmon orange was observed on the lesion and *C. capsici* generated blackish acervulli, which were arranged in a concentric pattern (Fig. 1).

Colony growth, colony and spore morphology

Colony growth rate in the cultures of *C. capsici* isolate (G-CA-R) ranged between 5.5-7.5 mm/day and in *C. gloeosporioides* (NW-HYW-R), 6-9 mm/day. *C. gloeosporioides* (HR-HYW-R) produced colonies with orange cottony mycelia with alternating concentric rings and the bottom of the PDA culture was brown.

C. capsici (G-CA-R) produced colonies of whitish gray with more whitish aerial mycelial growth and concentric rings. The bottom of the PDA culture was dark brown in colour.

The colonies obtained through single spore isolation technique were scrutinized through the naked eye, while the spores were observed though a light microscope. The *C. gloeosporioides* isolate produced oblong conidia, which were hyaline, single celled and smooth walled, or tapered at both ends and rounded. Lengths and widths of conidia that were measured were in the range 9-20 µm X 3-7.5 µm (Fig. 2). Acervuli were circular to elliptical, measuring from 129 to 281.4 µm on average. Setae were erect in habit, measuring 37-89 µm X 1.4-4.0 µm on average. Setae were septate, dark brown, thick walled, circular, and up to 200 µm in length. Padmana and Janadhana (2011 and 2012) reported that a colony on PDA was grey in colour, with abundant production of acervuli and conidia. The conidiogenous cells were hyaline, oblong or tapered and were 20 X 3-4 µm in size on average. Setae were produced by most isolates (Fig. 2a). *C. capsici* isolates produced truncate conidia that were one celled, hyaline, smooth-walled, and with a central oil globule; they were curved in shape, tapering gradually at the ends and with an acute apex. Average size of setae ranged around 18 X 2.5-4 µm. Acervuli were circular to elliptical in shape and in the range of 70 to 165 µm in size, while the setae were erect in habit and of average dimensions 37-120 µm X 1.4-4.0 µm (Fig. 2b). Various parameters are used to characterize anthracnose at species level. Sangdee *et al*. (2011) used morphological characteristics and pathogenic variability of

Figure 1: Anthracnose symptoms on capsicum pepper pods. 1a: *C. gloeosporioides* symptoms (sunken lesions on ripe fruits) on HYW; 1b: *C. capsici* symptoms (distorted fruit: CA-8).

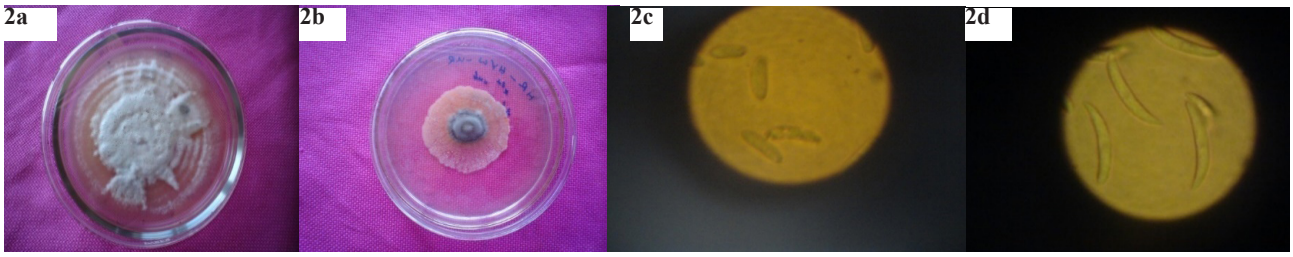

Figure 2: Pure cultures; *C. gloeosporioides* 2a: *C. gloeosporioides* and 2b: *C. capsici* and conidia; 2c: *C. gloeosporioides* and 2d: *C. capsici*.

fruits to characterize *C. capsici* infecting chili in Thailand, while Rajapakse and Ranasinghe (2002) characterized five isolates of *Colletotrichum* spp. using the colour, texture and appearance of colonies, along with the shape, size, and colour of acervuli.

Photita *et al*. (2005) isolated and identified 34 *Colletotrichum* spp. from various horticultural crops using colony morphology, and based on size and shape of appressoria and conidia. The genetic basis of the majority of *Colletotrichum* species has not yet been revealed in most of the Asian countries. Thus, having molecular evidences would be highly supportive when identifying pathogens. Primers that are designed based on ITS, β-tubulin, ACT, and GAPDH genes can be used to identify these species and are widely applied with other methods of identification; these methods have a greater level of sensitivity and specificity over conventional methods. However, such DNA based methods are complicated, and the associated cost factor is also high (Walcott, 2003), while it is also not possible to get an understanding on other parameters such as germinability, pre-emergence and post emergence infection. Therefore, employing a polyphasic methodology would increase the effectiveness of the process (Hunupolagama *et al*., 2017).

Confirmation of Koch's postulates using fruit inoculation test

Numerous oval or circular water soaked spots were observed on capsicum pepper fruits after seven days of inoculation. The fungus was re-isolated from such lesions and the culture obtained was found to be the same as that of the original culture of *C. capsici* and *C. gloeosporioides*.

Seed borne nature of the fungal pathogens

Researchers have employed various seed health testing methods. Standard blotter method (Rathod *et al.,* 2012; Nagaraja & Krishnappa, 2006), agar plate and seed wash methods (Rathod *et al*., 2012), and deep-freezing method (Nahar *et al*., 2004) are popular seed screening methods when testing for pathogens. Each of these methods possesses advantages as well as disadvantages, depending on the nature of seeds of the crop species tested and resource availability. Standard blotter method, agar plate method and paper towel methods are widely used seed testing methods in Sri Lanka (Priyantha *et al*., 2016).

Standard blotter and water agar methods

Seed samples from healthy and infected capsicum pepper fruits were categorized based on the rating scale used. Accordingly, infected seeds with *C. capsici* (capsicum pepper variety: CA-8) and *C. gloeosporioides* (varieties: CA-8 and HYW) were used for the evaluation of seed borne nature of the pathogens. Results obtained through two seed health testing methods revealed that seed germination was significantly reduced in line with the severity of infection of pods. Germination of seeds obtained from apparently healthy pods displayed a 77.77% germination rate with standard blotter method and 68.82% with the water agar method. However, germination of seeds obtained from highly infected pods with anthracnose was reduced in both methods to 37.03 % (corresponding to standard scale level 9) with standard blotter method and 12.82% (corresponding to standard scale level 9) with water agar method, respectively. These values were statistically significant to germination percentages corresponding to other levels from 0 to 3, except level 6. Infection percentage of seeds in *C. capsici* using standard blotter method gradually increased with the level of infection of pods from which the seeds were obtained. Accordingly, highest seed infection percentage of 46.89% was recorded with respect to *C. capsici*, while it was 61.25% for *C. gloeosporioides.* These percentages were significantly different from other corresponding percentages (0-6) of the scale. These seeds were collected from highly infected pods that were rated 9 on the rating scale (Table 3).

According to Vinaya *et al*. (2009), among different seed health testing methods experimented with, standard blotter method proved the most efficient for quick and accurate diagnosis of *C. capsici*. Standard blotter method indicated a greater incidence of fungi on seed parts followed by agar plate and deep-freezing methods (Alves & Pozza, 2009), but the agar plate method was found most suitable for the isolation of saprophytic fungi from maize (Niaz & Dawar, 2009). Bhale *et al*. (2000) reported that the test tube water agar seedling symptom test was a better method for detecting pathogens in seedlings. In Thailand, Sangchote and Juangbhanich (1984) confirmed the preference of *C. capsici* for the seed coat using blotter and agar methods. According to Sharfun-Nahar *et al*. (2004), occurrence of fungal species is detected more frequently using blotter test, compared to deep-freezing method in the case of both seeds and pericarps. Kumar *et al.* (2004) reported that *C. capsici* is transmitted to young seedlings through inoculum developed on infected seeds by local contact. The seed testing methods employed in the present study also can be further recommended for detecting *C. capsici* and *C. gloeosporioides* infections in capsicum pepper. Asalmol *et al*. (2001) reported that the seed borne fungi of chili were

capable of causing pre-emergence and post emergence mortality. In keeping with that, the results of this study indicated that the highest mortality (88%) occurred with *C. capsici* while the lowest was with *Aspergillus flavus* (59%). Similar findings indicating loss in seed germination and reduced vigour of *C. capsici* were reported by Perane and Joi (1988), and Mesta (1996). Yesuf and Sangchote (2004) reported that typical bean anthracnose symptoms became visible on the cotyledon and the first true leaves within a few days after emergence, indicating the important role of seed borne fungus as primary source of inoculum. Many seedlings died within 4-5 days of seed emergence. However, pre-emergence and post-emergence seedling mortality rates were higher in both seed samples of the naturally infected seeds.

Seed borne nature of C. capsici on seed quality parameters (pot culture studies)

Results of the experiment presented in Table 4 indicate that naturally infected seed sample manifests lower germination values of 30.00% (*C. capsici*) and 33.33% (*C. gloeosporioides)* and with higher pre-emergence losses of 70.00% (*C. capsici*) and 66.66% (*C. gloeosporioides)*. In contrast, healthy seeds showed higher germination percentages of 93.33% and 96.66%, with lower pre-emergence and post-emergence losses of 6.66% and 3.33% respectively for *C. capsici* and *C. gloeosporioides.* Student's *t*-test revealed that values obtained for germination, pre-emergence and post-emergence infection percentages were significantly different for both healthy and infected seeds at $P < 0.05$. Results of pot culture test conducted to analyze

Table 3: Seed borne nature of *C. capsici* and *C. gloeosporioides* at different levels of infection.

	Seed borne nature of *C. capsici* (standard blotter method)		**Seed borne nature of *C. gloeosporioides* (Water agar method)**	
Rating scale value	Seed germination %	Seed infection%	Seed germination %	Seed infection%
0	77.77[a]	0.00[c]	68.82[a]	0.00[d]
1	67.45[b]	3.84[c]	58.99[a]	28.78[c]
3	46.89[c]	33.56[b]	43.07[b]	39.1 5[b]
6	36.04[d]	35.78[b]	28.78[c]	47.04[b]
9	37.03[d]	46.89[a]	12.82[d]	61.25[a]
Mean	58.66	24	62.39	26.66
LSD at 0.05	7.979	8.597	12.07	8.024
CV%	8.16	19.01	16.41	13.15

Table 4: Seed borne nature of *C. capsici* and *C. gloeosporioides* on seed quality parameters (pot culture studies).

	C. capsici			***C. gloeosporioides***		
Seed category	**Germination %**	**Pre emergence infection %**	**Post emergence infection%**	**Germination %**	**Pre emergence infection %**	**Post emergence infection %**
Healthy seeds (Mean)	93.33[a]	6.66[a]	11.1[a]	96.66[a]	3.33[a]	0[a]
Infected seeds (Mean)	30[b]	70[b]	68.57[b]	33.33[b]	66.66[b]	27.76[b]

Values within a column and not followed by the same letter are significantly different at (P<0.05) according to the Student's *t*-test.

Table 5: Seed borne nature of *C. capsici* and *C. gloeosporioides* on seed quality parameters (seedling symptom test).

	C. capsici			***C. gloeosporioides***		
Seed category	**Germination %**	**Pre emergence infection %**	**Post emergence infection %**	**Germination %**	**Pre emergence infection %**	**Post emergence infection %**
Healthy seeds (Mean)	100[a]	0[a]	0[a]	100[a]	0[a]	0[a]
Infected seeds (Mean)	20[b]	80[b]	100[b]	20[b]	80[b]	100[b]

Values within a column and not followed by the same letter are significantly different at (P<0.05) according to the Student's *t*-test.

the seed-to-seedling transmission of *C. capsici* and *C. gloeosporioides* revealed that seedlings that emerged from naturally infected seeds could exhibit severe reduction in the percentage that germinated and higher seedling mortality about 21 days after sowing. Germination was adversely affected by the presence of seed borne fungi. Typical *Capsicum* anthracnose symptoms on the first true leaves became visible within 3-4 days after emergence.

Seed borne nature of C. capsici on seed quality parameters (Seedling symptom test)

Selected seed samples (both healthy and infected) of the varieties HYW and CA-8 were subjected to water agar seedling symptom test to diagnose seed borne infection and to observe the seed-to-seedling disease transmission. Experimental results as shown in Table 5 indicated that naturally infected seeds could suffer a lower germination rate of 20.00% and higher pre-emergence loss of up to 80.00%. Healthy seeds displayed a higher germination rate of 100% with no pre- and post-emergence losses. Accordingly, the results were similar in respect of both *C. capsici* and *C. gloeosporioides*. Student's *t*-test revealed significant differences ($P < 0.05$) with respect to germination, pre-emergence and post-emergence infection percentages in the case of both healthy and infected seeds. The infected seed samples exhibited the anthracnose symptoms with the presence of acervuli on the seed coat. These seed samples aborted and failed to germinate while exhibiting seed rot after 21 days of incubation. In this test, after 21 days of incubation, germinated seedlings from the batches of infected seeds developed acervuli on the seed coats that were attached to the cotyledonary leaves. Most of the infected seeds failed to germinate and manifested seed rotting symptoms with increased post-emergence losses.

Location of fungi in seeds

Naturally infected capsicum pepper seed sample of cultivar CA-8 and HYW were used for the study. The presence of seed borne pathogens in the separated seed parts of both infected and healthy seeds, viz. seed coat, pericarp, and embryo were recorded (Tables 6). Results revealed that both *C. capsici* and *C. gloeosporioides* can reside in seed coat, pericarp and embryo, indicating their internal and external seed borne nature, but *C. capsici* and *C. gloeosporioides* were not detected in the seed coat of healthy seeds. Research evidences indicate that both *C. capsici* and *C. gloeosporioides* could be seed borne, both internally and externally (Gawade, 2013; Hemannavar, 2008). Similarly, Kumar *et al.* (2004) reported that *C. capsici* could be both externally and internally seed borne and that *C. capsici* and *C. gloeosporioides* were not visible on the seed coat, pericarp and in embryo of healthy seeds. In contrast, two other fungal species, namely *Fusarium* and *Aspergillus* species were associated only with the seed coat, indicating their externally seed borne nature. Both of these genera have been reported to be acting as pathogens and non-pathogens or saprophytes on pepper seeds and fruits (Chigoziri & Ekefan, 2013; Asalmol *et al*., 2001). *Aspergillus* sp. is a commonly found fungal species associated with chili seeds. It is noted for causing reduction in germination by inducing crown rot/ collar rot (Chigoziri & Ekefan, 2013), while *Fusarium* acts as a member of the damping off complex in *Capsicum* spp. Moreover, these pathogens can produce toxins that render agricultural produce unsuitable for consumption.

CONCLUSIONS

C. capsici and *C. gloeosporioides* were observed in the seed coat, pericarp, and embryo, whereas *Fusarium* and *Aspergillus* were observed only on the seed coat of infected seeds. *C. capsici* and *C. gloeosporioides* were not present in the seed coat, pericarp, and embryo of apparently healthy seed samples tested while *Aspergillus* and *Fusarium* spp. were visible only in the seed coats of the same seeds. It would be worthwhile to experiment on how these two species would intensify the impact of the infection by acting in combination with *C. capsici* and *C. gloeosporioides* . The study revealed that both *C. capsici* and *C. gloeosporioides* can invade the important components of the seeds internally and externally, causing higher losses at germination, and at both the pre- and post-emergence stages of capsicum pepper, whereas *Aspergillus* and *Fusarium* spp. are externally seed borne.

REFERENCES

Alves, M.D.C. and Pozza, E.A. (2009). Scanning electron microscopy applied to seed-borne fungi examination. *Microscopy Research and Technique* **72**(7): 482-488.

Anonymous (1996). International rules for seed testing. *Seed Science and Technology* **24**: 1-335.

Asalmol, M.N., Kale, V.P. and Ingle, S.T. (2001). Seed borne fungi of chilli, incidence and effect on seed germination. *Seed Research* **29**(1): 76-79.

AVRDC (1998). Annual Report, Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Bhale, U., Bhale, M.S., Pandey, B.R. and Pandey, R.P. (2000). Seed borne fungi of chili in Madhya Pradesh and their significance. *Journal of Mycopathological Research* **38**(2): 117-119.

Chigoziri, E. and Ekefan, E.J. (2013). Seed-borne fungi of Chilli pepper (*Capsicum frutescens*) from pepper producing areas of Benue state, Nigeria. *Agriculture and Biology Journal of America* **4**: 370-374.

Dahanayake, N., Madurangi, S.A.P., Ranawake, A.L. (2012). Effect of potting mixture on growth and yield of chilli varieties (*capsicum* spp) and microbial activity. *Tropical Agricultural Research and Extension* **15** (3): 33-35.

Diao, Y., Zhang, C., Xu, J., Lin, D., Liu, L., Mtung'e, O.G. and Liu, X. (2015). Genetic differentiation and recombination among geographic populations of the fungal pathogen *Colletotrichum truncatum* from chili peppers in China. *Evolutionary Applications* **8**(1): 108-118.

Doyer, L.C. (1938). Manual for the determination of seed-borne diseases.

Gawade, S.B. (2013). Studies on seed borne pathogens of soybean (*Glycine max* (L.) Merrill), PhD Dissertation. Mahatma Phule Krishi Vidyapeeth, Maharashtra, India.

Hemannavar, V. (2008). Studies on seed borne aspects of anthracnose of chilli and its management, MSc dissertation, University of Agricultural Sciences, Dharwad, India.

Hunupolagama, D.M., Chandrasekharan, N.V., Wijesundera, W.S.S., Kathriarachchi, H.S., Fernando, T.H.P.S. and Wijesundera, R.L.C. (2017). Unveiling members of *Colletotrichum acutatum* species complex causing Colletotrichum leaf disease of *Hevea brasiliensis* in Sri Lanka. *Current Microbiology* **74**(6): 747-756.

Jayawardana, H.A.R.K., Weerahewa, H.L.D. and Saparamadu, M.D.J.S. (2015). Enhanced resistance to anthracnose disease in chili pepper (*Capsicum annuum* L.) by amendment of the nutrient solution with silicon. *The Journal of Horticultural Science and Biotechnology* **90**(5): 557-562.

Kelaniyangoda, D.B., Salgadoe, A.S.A., Jayasekera, S.J.B.A. and Banda, R.G., 2011. Wilting of bell pepper (*Capsicum annuum* L.) causal organism isolation and a successful control approach. *Asian Journal of Plant Pathology* **5**:155-162.

Khare, M.N. (1996). Methods to test seeds for associated fungi. *Indian Phytopathology* **49**: 319-328.

Kumar, K., Singh, J. and Khare, A. (2004). Detection, location, transmission and management of\seed borne *Colletotrichum dematium* causing dieback and anthracnose in chili. *Farm Science Journal* **13**(2): 152-153.

Maden, S., Singh, D., Mathur, S.B. and Neergard, P. (1975). Detection and location of seed borne inoculum of *Ascochytarabei* and its transmission in chickpea. *Seed Science and Technology* **3**: 667-671.

Mesta, R.K. (1996). Studies on fruit rot of chili caused by *Colletotrichum capsici* (Sydow.) Butler and Bisby.M.Sc.(Agri.) Thesis, University of Agricultural Sciences, Dharwad, India.

Mongkolporn, O. and Taylor, P.W.J. (2018). Chili anthracnose: *Colletotrichum* taxonomy and pathogenicity. *Plant Pathology* **67**(6): 1255-1263.

Nahar, M.M.S., Mushtaq, M. and Pathan, I.H. (2004). Seed- borne mycoflora of *Capsicum annuum* imported from India. *Pakistan Journal of Botany* **36**(1): 191-197.

Nagaraja,O. and Krishnappa,M. (2006). Detection of seed borne nature *Cercospora guizoticola***,**
location and its transmission in niger **[***Guizotia abyssinica* (L F). Cass]**.** *Journal of Plant Disease Sciences* **5**(1): 16-22.

Niaz, I. and Dawar, S. (2009). Detection of seed borne mycoflora in maize (*Zea mays* L.). *Pakistan Journal of Botany* **41**(1): 443-451.

Padman, M. and Janardhana, G.R. (2012). Screening for inhibitory activities of essential oils on the growth of *Colletotrichum gloeosporioides* (Penz.) Penz. And Sacc., the causal agent of leaf spot disease of *Murrayakoenigii* L. *Archives of Phytopathology and Plant Protection* **45**(13): 1575-1581.

Padman, M. and Janardhana, G.R. (2011). Occurrence and characterization of *Colletotrichum gloeosporioides* isolated from *Murrayakoenigii. New York Science Journal* **4**(8): 70-76.

Perane, R.R. and Joi, M.B. (1988). Studies on seed borne infection of fruit rot and dieback of chilies. *Journal of Maharashtra Agricultural University* **13**: 231-232.

Photita, W., Taylor, P.W.J., Ford, R., Hyde, K.D. and Lumyong, S. (2005). Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand. *Fungal Diversity* **18**: 117-133.

Priyantha, M.G.D.L., Athukorala, A.R.J., Jayasinghe, J.A.V.J., Sato, M., and Takahashi, H. (2016). Seed borne pathogens associated with seed lots of major food crops of Sri Lanka. *Annals of Sri Lanka Department of Agriculture* **18**: 26-27.

Rajapakse, R.G.A.S. and Ranasinghe, J.A.D.A.R. (2002). Development of variety screening method for anthracnose disease of chili (*Capsicum annuum* L.) under field conditions. *Tropical Agricultural Research and Extension* **5**(1-2): 7-11.

Ranathunge, N.P., Mongkolporn, O., Ford, R. and Taylor, P.W.J. (2012). *Colletotrichum truncatum* Pathosystem on *Capsicum* spp: infection, colonization and defence mechanisms. *Australasian Plant Pathology* **41**(5): 463-473.

Rathod, L.R.M.D., Jadhav, S.K., Mane, S.M., Muley, and Deshmukh, P.S. (2012). Seed borne mycoflora of legumes seeds. *International Journal of Advanced Biotechnology and Research* **3**(1):530-532 Available at: http://www.bipublication.com.

Sangchote, S. and Juangbhanich, P. (1984). Seed Transmission of *Colletotrichumcapsici* on Pepper (*Capsicum* spp.). *Kasetsart Journal: Natural Science* **18**(1): 7-13.

Sangdee, A., Sachan, S. and Khankhum, S. (2011). Morphological, pathological and molecular variability of *Colletotrichum capsici* causing anthracnose of chili in the North-east of Thailand. *African Journal of Microbiology Research* **5**(25): 4368-4372 Available online at: http://www.academicjournals.org/AJMR

Saxena, A., Raghuwanshi, R., Gupta, V.K. and Singh, H.B. (2016). Chilli anthracnose: The epidemiology and management. *Frontiers in Microbiology* **7**, No. 1527.

Senanayake, D.M.J.B., Jayasinghe, J.E.A.R.M., Shilpi, S., Wasala, S.K. and Mandal, B. (2013). A new begomovirus–betasatellite complex is associated with chilli leaf curl disease in Sri Lanka. *Virus Genes* **46**(1): 128-139.

Sharfun-Nahar, S.N., Mushtaq, M. and Pathan, I.H. (2004). Seed-borne mycoflora of *Capsicum annuum* imported from India. *Pakistan Journal of Botany* **36**(1): 191-198.

Sharma, P.N., Kaur, M., Sharma, O.P., Sharma, P. and Pathania, A. (2005). Morphological, pathological and molecular variability in *Colletotrichum capsici*, the cause of fruit rot of chillies in the subtropical region of north-western India. *Journal of Phytopathology* **153**(4): 232-237.

Vinaya, H., Rao, M.S.L., Yashoda, H. and

Mohankumar, H.D. (2009). Status of seed borne incidence of anthracnose of chilli in northern Karnataka and evaluation of seed health testing methods for the detection of *Colletotrichum capsici*. *Karnataka Journal of Agricultural Sciences* **22**(4): 807-809.

Walcott, R.R. (2003). Detection of seed borne pathogens. *Horticultural Technology* **13**(1): 40-47.

Yesuf, M. and Sangchote, S. (2004). Seed transmission and epidemics of *Colletotrichum lindemuthianum* in the major common bean growing areas of Ethiopia (Jan-Mar 2005). *Kasetsart Journal: Natural Science* **39**: 34-45.