PREHARVEST APPLICATION OF POTASSIUM ON ENHANCING RESISTANCE TO ANTHRACNOSE IN TOMATO (*LYCOPERSICON ESCULENTUM* L.)

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ABSTRACT

Anthracnose is one of the major fungal diseases of tomato caused by many Colletotrichum spp. Colletotrichum gloesporioides and Colletotrichum dematium are two major Colletotrichum spp causing anthracnose diseases in tomato in Sri Lanka. Resistance against anthracnose caused by C. dematium and C. gloesporioides was investigated in tomatoes cv. 'Padma 108 F1', by soil application of KCl as a source of potassium at three different levels: 6.9 g per plant or 4.6 g per plant or 2.3 g per plant(control). Disease resistance in the fruit of plants treated with different levels of K was assessed by artificial inoculation with Colletotrichum gloeosporioides or C. dematium. Significant reductions in lesion areas were observed in fruit from plants treated with K compared to the fruit from control plants. There were 73% and 95% reductions in disease development against C. gloeosporioides in fruit of plants treated with 4.6 g/ plant and 6.9 g per plant and the reductions were 59% and 81% against C. dematium. Similarly a delay in anthracnose symptom development was observed in fruits obtained from plants treated at higher levels of K (6.9 g/ plant). The mechanisms underlying effect of potassium were investigated by measuring the thickness of the cell wall of the fruit exocarp and by counting the number of appressoria in the inoculated area. The cell wall thickness and appressoria count were significantly higher in fruits of plants treated with highest K (6.9g per plant) than fruit of control plants. The thicker cell wall may have acted as a physical barrier for the Colletrotrichum spp to invade the tissue and the appressoria may have formed as a latent structure for the infection by Colletrotrichum spp. Thickness of cell wall and the presence of significantly higher number of aspersoria may have contributed the delay in symptom development of K treated tomato fruits.

Key words: Potassium application, tomato anthracnose, *Colletotrichum* spp.

Introduction

Anthracnose is one of the major diseases caused by *Colletotrichum* species affecting severe post- harvest losses in number of vegetables, fruits, legumes and cereals (Bailey et al., 1992). Many cultivated fruit crops are infected by *Colletotrichum* species. However, the significant economic loss occurs when the fruiting stage is attacked (Bailey et al., 1992) which is encounted up to 50% crop loss (Smith and Black, 1990). A single host plant can be infected by numerous *Colletotrichum* species and likewise one *Colletotrichum* species can be pathogenic to many species (Freeman et al., 1998).

In tomato, anthracnose is considered as a major post harvest disease causing heavy post harvest loss and the quality. *C. gloesporioides*, *C. dematium* (Barksdale, 1972), *C. coccodes* (Byrne, 1997), *C. acutatum* (Martínez, 2009, Živković, 2010) are some of the major *Colletotrichum* species causing anthracnose in tomato. The disease is controlled by seed treatments and/or contact and systemic fungicides which are environmentally unsound. Thus, environmentally friendly measures of controlling anthracnose should be introduced and implemented.

Anticipated pre-harvest practices are one aspectwhich reduces postharvest loss by improving the durability and quality. Pre-harvest application of high doses of potassium has been confirmed to be effective in decreasing the incidence of many diseases in crops (Fuchs and Grossmann 1972). Application of thrice the recommended dose of potassium had provento reduce stem-end rot significantly in mango cv. 'Karuthacolomban' by over 45% compared to the recommended dosage (Karunanayake, 2008).

Anthracnose, stalk-end rot and freckle disease in banana cv. 'Embul' could effectively be supressed by twice the recommended dosage of K (Weerakoon et al., 2005). This paper describes the potential of enhancing resistance against anthracnose in tomato caused by two *Colletotrichum* species, *C. gloesporioides and C. dematium* by pre-harvest application of extra doses of potassium.

Research design

Plant material

Seeds of tomato genotype *Lycopersicon esculentum* L. cv. 'Padma 108 F1' (East-West Seed International Ltd., Nonthaburi, Thailand.) were sown on a 1:1 mixture of compost and coir dust. The plant nursery was maintained at 28 – 30 ºC, 80 – 85% relative humidity (RH) and 12h photoperiod for six weeks.

Treatments and experimental design

The basal fertilizer application comprising 2.3g of urea, 13g of triple super phosphate (TSP) and 2.3g of murate of potash (MOP or KCl) was provided to each pot four days prior to transplanting. Mature, healthy plants were transplanted to pots (of 0.004 m^3) containing top soil under the same environmental conditions as mentioned above. At the first and second top dressing 2.3g of urea was provided for each plantafter 3 and 6 weeks of transplanting. Three K treatments at the second top dressing i.e. recommended dosage (2.3g/ plant) as the control, double (4.6g/ plant) and triple the recommendation (6.9g/ plant) were used in the experiment (Table 1).Treatments were arranged in a complete randomized design (CRD) with four replicates.

Table 1: Mass and time duration of fertilizer application in the experiment.

Treatments	Urea g/per plant	TSP g/per plant	KCl g/per plant
Basal	2.3	13	2.3
1st top dressing (3 weeks after transplanting)	2.3	-	-
2nd top dressing (6 weeks after transplanting)	2.3	-	2.3
	2.3	-	4.6
	2.3	-	4.6

Pathogen identification and isolation

Samples of *Colletotrichum* species were obtained from young anthracnose colonies of naturally infected tomato fruits. The diseases tissues were washed thoroughly with sterilized distilled water (SDW). Infected tissues were cut along with unaffected adjacent tissues into 2-5 mm pieces and were washed with 1% (v/v) sodium hypochlorite solution followed by a series of SDW. The tissues were then transferred to potato dextrose agar (PDA) and incubated at 27-30 ºC (Narayanasamy, 2010). Ten culture plates per species were maintained.Pure cultures of *C. gloeosporioides* and *C. dematium* were obtained after series of sub culturing. Pure *C. gloeosporioides* and *C. dematium* were identified using the Commonwealth Mycological Institute (CMI) descriptions via their characteristic colony and spore features observing under a compound microscope (Daffodil MCX100, Vienna, Australia). Koch's postulates were performed by inoculating *C. gloeosporioides* or *C. dematium* on mature tomato fruits. The pathogenswere re-isolated from the infected fruits. Seven to ten day old cultures were used for all experiments.

Inoculum preparation and disease assessment followed challenged inoculation of *Colletotrichum* species

Seven day old pure cultures were used for preparing conidial suspension of *C. gloeosporioides* and*C. dematium.* Mycelium was scraped and suspended in SDW and filtered through glass wool to remove the mycelium. The conidial suspension was adjusted to 10^5 conidia/ ml using a haemocytometer. Fifteen tomato fruits from each replicate were washed in 70% ethanol followed by dipping in 0.1% sodium hypochlorite solution and rinsed with SDW (Etebarian et al. 2005). Each fruit was inoculated by placing drops of (20µl) conidial suspension at three places on fruit surface. Inoculated fruits were incubated in moist chambers with 95-100% RH at 28±2 °C. The number of days taken for disease initiation in each treatment was recorded. The development of lesion area was recorded daily for 10 days after inoculation (DAI) and the mean lesion area per fruit was calculated.

Assessing the degree of appressoria formation at inoculated zones

One cell layer thick fruit peels were taken from the *C. gloeosporioides* or *C. dematium* inoculated areas and were observed under the light microscope after 2, 3, 4, 5 and 6 DAI. The total number of spores and the number of appressoria formed spores were counted in five fields of vision under 400x using the light microscope. The percentage of appressoria formed spores per vision was calculated and averaged per treatment. The procedure was repeated four times per treatment for either organism.

Exocarpic cell wall thickness

Twenty fruits from each KCl-treated plant were used for measuring the cell wall thickness of the exocarp. Three cross-sections (0.1 mm thick) of each fresh fruit were mounted on a glass slide and the exocarpic cell wall thickness was measured using a calibrated ocular micrometer at a magnification 400x using a compound microscope (Daffodil MCX100, Vienna, Australia).

Statistical analysis

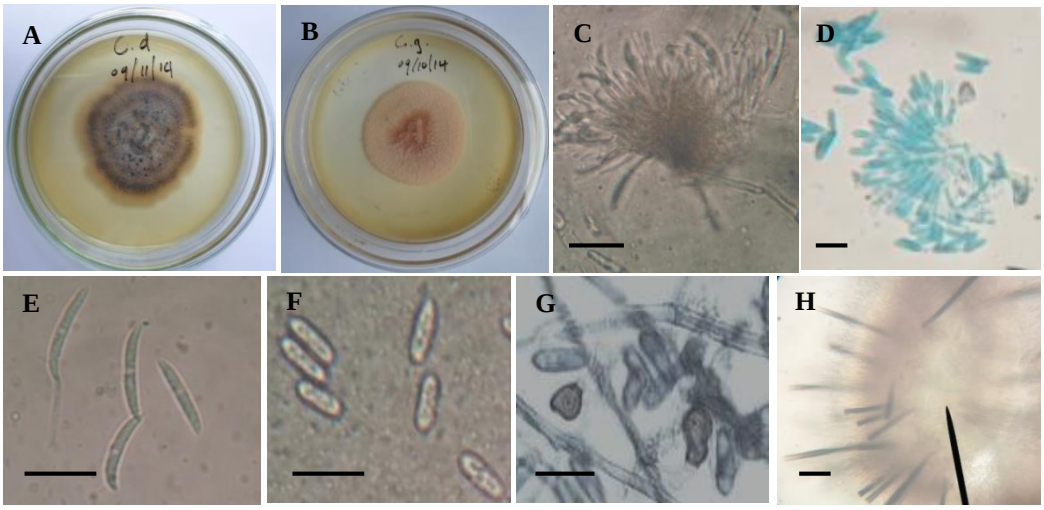
All the collected data were analysed using one way ANOVA in SPSS 16.0 statistical package and treatment means were compared by using the Duncan's multiple range test (DMRT) at $P \leq 0.05$ confidence interval.

Results and discussion

Pathogen identification and isolation

C. gloeosporioides was identified by its orange cotton-like mycelium (Sutton, 1980) and ovoid-shape conidia (Du *et al*., 2005). *C. dematium* was identified by its falcate conidia (Cano et al., 2004) the presence of prominent setae and its brown colour colony (Sutton, 1992). The conidiogenous cells were hyaline in both species and cylindrical (*C. gloeosporioides*) or tapered (*C. dematium*) in shape. Setae wereobserved only in cultures of*C. dematium.* They were dark brown, septate and acicular in shape. The conidia produced by *C. gloeosporioides* were cylindrical and straight. *C. dematium* produced relatively slenderer, olive green coloured and falcate conidia (Figure 1).

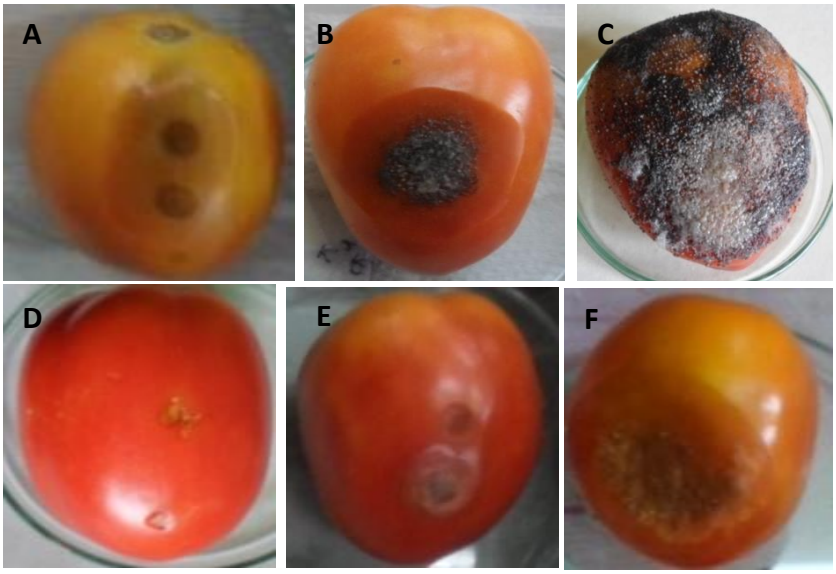
Figure1. Colonies and microscopic features of *Colletotrichum gloeosporioides* and *Colletotrichum dematium*. A, 7 day old colony of *C. dematium* grown on PDA. B, 6 day old colony of *C. gloeosporioides* grown on PDA; C, fruiting structure of *C. dematium* along with conidiospores; D, fruiting structure of C. *gloeosporioides* with conidiopores; E, Conidia of *C. dematium*; F, Conidia of *C. gloeosporioides*; G, Appressoria of *C. gloeosporioides*; H, Sporodicial fruiting structure of *C. dematium* along with conidiopores and setae protruding from the structure. (Scale bars = 15 µm)



Assessment of anthracnose disease development followed challenged inoculation with *Colletotrichum* species

Resistance to anthracnose disease in tomato cv. 'Padma 108 F1' was investigated by challenged-inoculation of fruit with either of two *Colletotrichum* spp. A significant reduction of anthracnose lesion areas (at $P \leq 0.05$) caused by either fungi was observed in fruits of plants treated with KCl at 4.6 and 6.9g per plant compared to the control. The fruits challenged-inoculated with *C. gloeosporioides* showed a greater reduction in lesion areas compared to fruits inoculated with *C. dematium* (Figure 2). The highest reduction in lesion area against *C. gloeosporioides* (95%) was observed in fruits of plants treated at 6.9g K where as it was (81%) in fruits obtained from plants treated by the same treatment of K against *C. dematium* (Table 2). Similarly, the severity of anthracnose disease was reduced by K in crops such as mango (Karunanayake, 2008) and banana (Weerakoon et al., 2005) by application of higher the recommended dose of potassium.

Figure 2: Anthracnose lesion development on tomato fruits challenge- inoculated with *C. gloeosporiodes* and *C. dematium* 10 DAI. A, B, and C: fruits inoculated with *C. dematium*. D,E and F:fruits inoculated with *C. gloeosporiodes*. A, D: fruits from plants treated with 6,9 g KCl/ plant, B, E: fruits from plants treated with 4.6 g KCl/ plant, C, F: fruits from plants treated with 2.3 g KCl/ plant.



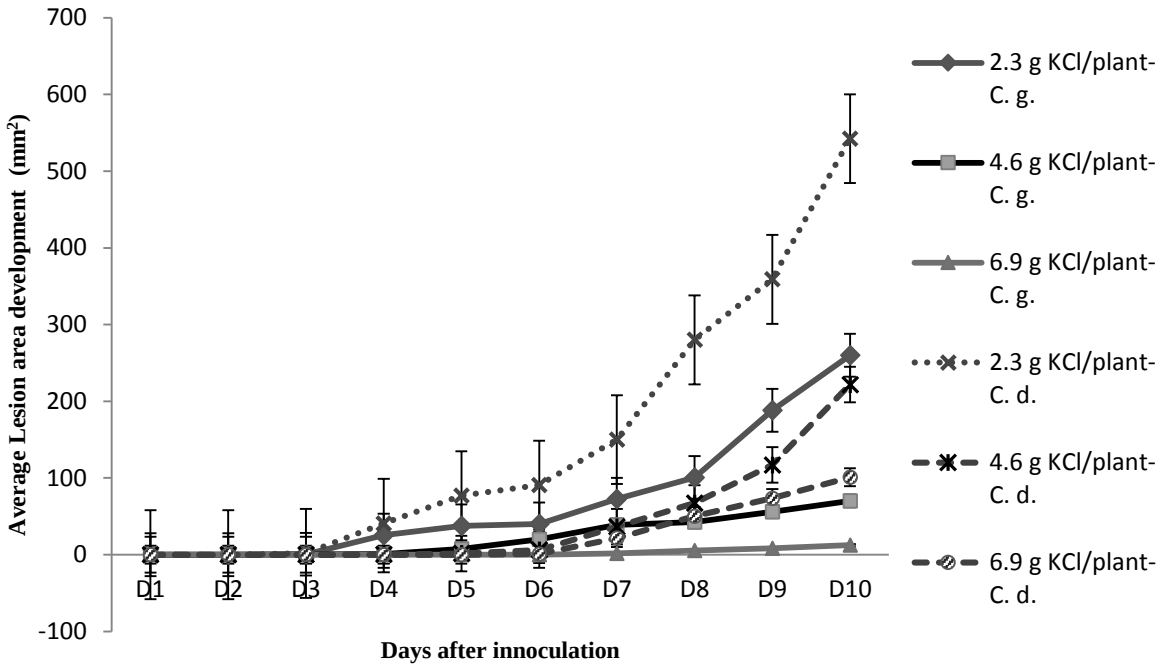
Appearance of anthracnose lesions on tomato fruits after challenged-inoculation with either *C. gloeosporioides*or *C. dematium* was delayed in fruit from higher doses of potassium-treated plants compared to the fruit from the control plants. The anthracnose lesions were appeared early (3rd – 4th DAI) on fruits from plants treated at 2.3 g KCl/ plant (control). However, it was delayed by two days in fruit from plants treated at 6.9 g KCl/ plant (Table 2).

Table 2: Cell wall thickness of the exocarp on potassium application and anthracnose disease development on inoculated fruits with *C. gloeosporioides* or *C. dematium*

Treatment g KCl/ plant	Cell wall thickness of the exocarp (µm)	Anthracnose disease development			
		C. gloeosporioides		*C. dematium*	
		Total lesion area (mm^2)	Day of disease initiation	Total lesion area (mm^2)	Day of disease initiation
2.3 (control)	0.2 ^{c}	260 ^{a}	3 ^{b}	542.3 ^{a}	3 ^{c}
4.6	0.24 ^{b} (20%)	70.2^{b} (73%)	3 ^{b}	221.8 ^{b} (59.1%)	4 ^{b}
6.9	0.29 ^{a}(45%)	12.4^{c} (95.2%)	7 ^{a}	101 ^{c} (81.4%)	6 ^{a}

The rate of lesion development by *C. dematium* on fruits of either treatment was comparatively greater than those of *C. gloeosporioides* suggesting a higher virulence of *C. dematium,* for the tomato cultivar irrespective of the dose application of potassium. However, it was highest in fruit obtained from 2.3g KCl/ plant or control treatments inoculated with *C. dematium* (Figure 03). The lesion development was slower in fruit harvested from plants treated at 6.9 g KCl/ plant regardless of the causal organism.

Figure 3: Anthracnose lesion development on tomato fruits (cv. 'Padma 108 F1') followed challenged- inoculated with *Colletotrichum gloeosporioides* and *C. dematium* for10 DAI. Fruits (*n* = 24) harvested from plants treated with different concentrations of potassium (2.3, 4.6 and 6.9 g/ plant) in pot experiments were used for the disease assessment. C. g.= *Colletotrichum gloeosporioides* and C. d. = *Colletotrichum dematium.* Bars represent ± Standard Error.



Number of appressoria development at inoculated zones

There was a significance difference in the percentage of appressoria formation among the treatments. The percentage of appressoria formation of both the *Colletotrichum* spp. seemed to be more or less related to the date of disease initiation. Although the disease initiation caused by *C. gloeosporioides* on tomato fruits from 6.9 g KCl/ plant treatments was observed in 7 DAI of the pathogen, there was a more than 23% of appressoria formation from 4 DAI. In contrast, such increase in % appressoria formation of *C. dematium* was not observed in the fruits obtained from the same treatment until the date of disease initiation (6 DAI) and there was an impulsive increase in the % of appressoria formation on that day (Table 3.).In a similar study, Weerahewa and David (2015) reported an increase of formation of appressoria of *C. gloeosporioides* on tomato fruits (cv. 'Thilina' and 'Maheshi') of plants treated at higher doses of K.

Table 3: Formation of appressoria by *Colletotrichum* spp. on inoculated tomato fruits of different treatments

Colletotrichum spp.	Treatment (g KCl/ plant)	% Appressoria formation				
		2DAI	3DAI	4DAI	5DAI	6DAI
C. gloeosporioides	2.3	0 ^{c}	4.12 ^{a*}	17.89 ^{d}	**	**
	4.6	1^{bc}	5.42 ^{a}	18.24 ^{d*}	20.35 ^{b}	**
	6.9	1^{bc}	6.14 ^{a}	23.07 ^{a}	25.45 ^{a}	30.15
C. dematium	2.3	0 ^{c}	5.32 ^{a*}	18.23 ^{d}	**	**
	4.6	2.1^{ab}	6.21 ^{a}	16.98 ^{c*}	**	**
	6.9	1.6 ^{a}	5.45 ^{a}	11.23 ^{b}	18.15 ^{c}	26.42*

Means in each column followed by different letters are significantly different at $P \leq 0.05$ according to the DMRT. ($n = 20$).
*The day of disease initiation
**Samples were difficult to obtain due to lesion sporulation on fruits

The occurrence of a higher percentage of appressoria of both *Colletotrichum* spp. without progressing infection and/ or formation of germ tube which was observed in fruits harvested from 6.9 g KCl/ plant treated tomato plants might be due to an obstacle present in the fruits. In the present study, it was observed a significant increase (45%) in cell wall thickness of the exocarp in 6.9 g KCl/ plant treated fruits (Table 2) and that might have acted as a physical barrier against the penetration. It was said that adequate supply of potassium results thicker cell walls making it harder for disease organisms to penetrate plant cells and establish an infection (International Plant Nutrition Institute, 2010).

Conclusion

Since the application of 6.9 g KCl/ plant significantly reduced the anthracnose lesion areas caused by both *C. gloeosporioides* and *C. dematium*, KCl at 6.9 g KCl/ plant can be considered as the optimum concentration for suppressing the disease caused by either species in the tomato cultivar 'Padma 108 F1'. The anthracnose disease development was more obvious on fruits challenged inoculated with *C. dematium* than that of *C. gloeosporioides.* Thus it can be assumed that the cultivar is more

vulnerable to anthracnose caused by *C. dematium* than *C. gloeosporioides.* The increased cell wall thickness of the exocarp in fruits of extra dose of K application possibly could have contributed the enhanced disease resistance against anthracnose.

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