The effect of fruit characteristics of cayenne pepper (*Capsicum frutescens*) and biocontrol agents (*Trichoderma* sp and azoxystrobin) on severity of anthracnose (*Colletotrichum acutatum*)

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Abstract. Anthracnose disease is the most important constraint to cayenne pepper (*Capsicum frutescens*) production in most major chili pepper - growing regions of the world, and often results in high yield losses. Anthracnose disease is due to several species of *Colletotrichum* (*C. gloeosporiodes*, *C. capsici* and *C. acutatum* are considered the major species). The incidence of anthracnose depends on cultivar susceptibility and weather. In warm and humid conditions cayenne pepper varieties are susceptible to anthracnose. Anthracnose is difficult to control after the symptoms appear, particularly when environmental conditions are favorable for infection. In order to identify an effective control measure, 4 varieties of cayenne pepper with 2 different color of immature fruits (green and yellow) and 2 type of fruit (straight up and decline) and used of 4 bio-control agents (*Trichoderma* species and azoxystrobin) were measuring the effects on disease incidence and yield. The experiment was conducted at Indonesian vegetables Research Institute from July 2014 to February 2015. The experiment was arranged as a split plot block design with four replications. Result of this experiment indicated that combination between tolerant varieties and application of bio control agent were the best option to control anthracnose of cayenne pepper rather than chemical control as it is cost-effective and environment friendly. Application of all bio control agents on immature green cayenne pepper + straight up type of fruit significantly reduced the incidence of anthracnose diseases which ranged between 3.51–6.81%. Application of *T. harzianum* and azoxystrobin were significantly more effective than *T. koningii* and *T. viride* in reducing the incidence of anthracnose on different fruit characteristic of cayenne pepper. This study revealed that in green immature straight up type fruit cayenne pepper application of *T. harzianum* and azoxystrobin have good potential in controlling the anthracnose disease of *C. acutatum* and resulted higher yield than in other treatments.

Key Words: *Colletotrichum* spp., tolerant variety, diseases management, bio control agent, fruit color, type of fruit.

Introduction. Cayenne pepper (*Capsicum frutescens*) is of the most important crops in Indonesia. It is cultivated about 125,122 ha with a total production of 713,502 t (BPS 2014). The average yield of cayenne pepper is 5.70 t ha^{-1} which is low compared to the yield of other cayenne peppers growing worldwide. Colletrotrichum diseases is one of the major economic constrain to chili production worldwide especially in tropic and subtropical regions (Than et al 2008a). Typical anthracnose symptoms on chilli fruit include sunken necrotic tissues, with concentric rings of acervuli (Mistry et al 2008). In some cases, the lesions are brown and then turn black, due to the formation of setae and sclerotia. Fruit rot reduces dry weight, capsaicin and oleoresin content of affected fruits (Mistry et al 2008), leading to reduction in the medicinal properties of chili. Kim et al (2004) reported that different species of *Colletotrichum* cause diseases in different organs of the chilli plant. Harp et al (2008) reported the occurrence of anthracnose on immature pepper fruit caused by *C. acutatum*. *C. capsici* generally causes disease on ripe red fruit, while *C. gloeosporioides* produces disease on both green and ripe fruits. Than et al (2008b) and Mongkolporn et al (2010) reported that among the three species of *Colletotrichum*, *C. acutatum* appears to be the most aggressive pathogen, which can

infect all tested four *Capsicum* species i.e. *C. annuum*, *C. frutescens*, *C. chinense* and *C. baccatum*.

It has been reported that apart from pre-harvest losses, fruit quality deterioration due to anthracnose range from 21-47% in Sri Lanka (Anonim 1993). Yield losses of 13% have been recorded in Korea (Yoon et al 2004), about 50% in Malaysia (Sariah 1994), 66-84% in India (Thind & Jhooty 1985) and 75% in Indonesia (Kusandriani & Permadi 1996). In Thailand, anthracnose can cause fruit yield losses ranging between 10–80% (Poonpolgul & Kumphai 2007).

To control anthracnose diseases, frequent spraying of fungicides are required, so some times every 3 days throughout the plant cycle or up to the fruit ripening stage often combining two or more products. Cayenne pepper farmers may spray up to 25 times in a season with a mixture of fungicides. Despite its high fungicide use, the average yield loss due to anthracnose incidence was still high (Setiawati et al 2011). Extensive and repeated use of fungicides have destroy useful microorganism with positive effect in fertility of soil and growth of plant (Chandra et al 2010), *C. gloeosporioides* developed resistant to systemic fungicide (Ziogas et al 2005; Sundravadana et al 2006) and caused environmental problems (Voorrips et al 2004).

The best way to control this disease is by planting cayenne pepper tolerant to anthracnose pathogens and the use of bio control agents. Some genetic resources resistant to anthracnose in chili have been independently reported from different countries and regions (Cheema et al 1984; Kim et al 1986; Kim et al 1987; Park et al 1987; Hong & Hwang 1998; Yoon & Park 2001; Rajapakse & Ranasinghe 2002; AVRDC 2004; Voorrips et al 2004; Prasath & Ponnuswami 2008; Syukur et al 2009; Hasyim et al 2014). Commercial cultivars of chili pepper resistant to the pathogens that cause anthracnose have not yet been developed (Park 2007). Chili pepper cultivars can be classified into three categories of susceptibility to anthracnose: highly susceptible, moderately susceptible and resistant.

Some field observations indicated that low incidence of anthracnose diseases is commonly found on cayenne pepper of some commercial varieties whose have green color in immature and fruits are straight up type (Setiawati at al 2013). These varieties may have physical and chemical adaptations that allow them to repel, tolerate, or even kill disease. Manandhar et al (1995) reported that diseases incidence was correlated to cuticle and exocarp thickness and varied by fruit maturity. Prasath & Ponnuswami (2008) reported biochemical factors and enzyme activities. The disease is frequently observed on the fruit during high humidity. Conditions such as environmental stress or borer damage may contribute to the severity of the disease. These cultivars could be tested in the field condition to control anthracnose disease. There is a need to incorporate alternative control components that are effective in field.

Biological control is an important method in the management of plant pathogens. Advantages include reduction of dependence of high-risk chemicals for diseases, control or other ecological and economical benefits (Bale et al 2008). The use of *Trichoderma* as biological control agent is being considered because of its antagonistic properties against pathogenic microorganisms and its beneficial effect to the environment. This alternative method may help reduce pesticide use. In spite of several studies on the antifungal effect of the biocontrol agents, *T. harzianum* and *T. viride* were reported to be effective in controlling the anthracnose (Rajathilagam & Kannabiran 2001). It is also believed that *Trichoderma* species are able to effectively compete for surface area, thereby reducing pathogen infection success (Jeffries & Koomen 1992; Maymon et al 2004). Boonratkwang et al (2007) reported that *Trichoderma* species effective to control *Colletotrichum* species in chili, citrus (Moretto et al 2001), strawberries (Freeman et al 2001), grape (Senthil et al 2011), bean (Amin et al 2014) with concomitant disease reduction. *Trichoderma* help in the maintenance of soil health and protect crops from pathogen.

Azoxystrobin was produced by the Basidiomycetes fungus, *Strobilurus tenacellus* having novel mode of action (Hewitt 1998). Azoxystrobin shows a unique spectrum of disease control. It is active against the fungi belong to oomycetes, ascomycetes, basidiomycetes and deuteromycetes (Sauter et al 1995). Azoxystrobin 25 SC was highly compatible commonly used with insecticides *viz.,* Profenphos, Dichlorvos, Monocrotophos,

Carbaryl, Dimethoate, Triazophos and Quinalphos and bioagents such as *Pseudomonas fluorescens* and *Bacillus subtilis* (Devi & Prakasam 2013).

Therefore, the present study has been carried out to study the compatibility of tolerant varieties of cayenne pepper and bio control agents (*Trichoderma* species and azoxystrobin) against incidence of anthracnose diseases on cayenne pepper in field conditions.

Material and Method. The study was conducted at the Experimental Station of the Indonesian Vegetables Research Institute (IVEGRI) in Lembang-West Java (1250 m above sea level) from July 2014 to February 2015. The soil of the experimental field is classified as Andisols. During the experiment average temperature was 20.91^{0}C and Rainfall was 4.51 mm. The variety of chili pepper used was hybrid variety, which was commonly grown by the farmers in Lembang, the main chili pepper production area in West Java. Local variety bordered the experimental plants. The experiment was arranged as a split plot block design with four replications as followed:

Main plot : Immature fruit color + Type of fruit

- A1. Green cayenne pepper + Straight up
- A2. Yellow cayenne pepper + Straight up
- A2. Green cayenne pepper + Decline
- A4. Yellow cayenne pepper + Decline

Main plot : Biocontrol agents

- B1. *Trichoderma koningii*
- B2. *Trichoderma viride*
- B3. *Trichoderma harzianum*
- B4. Azoxystrobin

Green Cayenne pepper + Straight up

Yellow Cayenne pepper + Straight up

Green Cayenne pepper + Decline

Yellow Cayenne pepper + Decline

Figure 1. Immature fruit colors and fruit type of Cayenne pepper (original).

Cayenne pepper seedling was prepared previously at nursery protected using bio-agent and nylon net to minimize attack of pests and diseases at very early stages. Transplanting was conducted when the seedling was 4 weeks old. Plots consisted of raised beds that were 40 cm high, 2 m wide, and 10 m long. Cayenne pepper was transplanted using 40 plants per plot in double rows (80 plants/plot) with spacing of 70 cm between rows and 50 cm between plants in the row. Land preparation started with ploughing and making beds. Organic materials consisting of mature compost were applied as basal fertilizer along with NPK composite fertilizers. The dose of organic materials and NPK was 30 t/ha and 1 t/ha respectively. The beds were covered with silvery plastic mulch.

The first spray of bio control agents (*Trichoderma* sp. and azoxystrobin fungicide) were done after the flowering stage appears (50% flowering). The second, third and four sprays were done at seven days interval with same dose. *Trichoderma* sp. was sprayed into the soil (soil drench application) at the rate of 1 kg/20 L of water or 10 g/plant for each treatment and azoxystrobin applied as foliar spray at a rate of 20 mL/20 L of water.

Weeding was conducted when necessary. Weeding was only applicable to the space of inter beds. Data on fruit yield were recorded at plot basis. The intensity of anthracnose attack on chili fruit was measured using formula as follow:

$$\text{Intensity} = \frac{\text{\# of infected fruits}}{\text{\# observed fruits}} \times 100\%$$

Statistical analysis. The data were subjected to two-way ANOVA followed by a Least Significant Difference Test comparison of means test was used to determine if means were significantly different (α=0.05) from each other. Count data was transformed using $\sqrt{x}$ +0.5 and percentage data arcsine-square root transformed. Statistical analysis was conducted with the software package SPSS 17.0 for Windows (SPSS Inc., Chicago, IL). In graphs and figure, the original data and their standard errors are presented.

Results and Discussion. Anthracnose incidence was observed every harvest period (8 times) and from total harvest (Table 1, Figure 1). Generally, anthracnose infection at all treatments was higher at sixth harvest period comparable to another harvest periods. Significant ($P<0.05$) differences among treatments of cayenne pepper tested has been observed. The incidence of anthracnose was varied from 4.46 % to 41.26 % depending on the treatments. Different cayenne pepper varieties had varying responses to fruit infection due to anthracnose. The incidence of anthracnose was most severe on yellow fruits than on green fruits. In the immature yellow fruit, the diseases spread rapidly. Incidence of anthracnose was found more severe in line with the harvest time. The incidence of anthracnose was varied between 8.73–37.92%. Meanwhile, in the immature green fruits, the incidence of anthracnose was varied between 4.46–28.89%. In Figure 2 is showed that all the treatments were superior over control against anthracnose disease. However, minimum disease incidence (13.53 %) was recorded in green cayenne pepper + straight up, which was found the most effective treatment against anthracnose disease of cayenne pepper. Green cayenne pepper + decline (17.16 %) was next in order of efficacy, followed by yellow cayenne pepper + straight up (21.26 %) and yellow cayenne pepper + decline (21.34 %). Agrios (2005) reported that the use of resistant varieties not only eliminated losses from diseases, but also eliminated chemical and mechanical expenses of disease control.

There is an interaction effect between fruit characteristic of cayenne pepper that were used with bio-control agents treatments on anthracnose incidence. Combination between green cayenne pepper and *T. harzianum* had significantly reduced the incidence of anthracnose disease followed by green cayenne pepper and azoxystrobin. The highest incidence of anthracnose was found in combination treatment of yellow cayenne pepper + decline and *T. koningii* (Table 2). The use of each combinations in the present study is easily producible, biodegradable, less expensive and cause no environment hazards to human health. These are ecologically safe and culturally more acceptable among the cayenne pepper farmers.

Table 1

Anthracnose incidence in various treatments of cayenne pepper

Treatments	*Anthracnose (%) at …… harvest period*							
Type of chili	*1st*	*2nd*	*3rd*	*4th*	*5th*	*6th*	*7th*	*8th*
Green cayenne pepper + Straight up	5.16 b	4.46 b	5.78 c	12.93 b	16.64 a	27.18 b	13.18 b	24.04 b
Yellow cayenne pepper + Straight up	9.68 a	9.27 a	12.06 ab	21.45 a	18.88 a	32.29 ab	37.34 a	35.91 a
Green cayenne pepper + Decline	11.12 a	8.33 a	9.40 bc	14.72 b	18.42 a	28.14 b	17.2 b	28.89 ab
Yellow cayenne pepper + Decline	10.35a	8.73 a	16.30a	17.46 ab	19.06 a	34.72 a	37.92 a	26.90 b
LSD 5 %	3.28	2.90	4.45	5.19	3.03	9.94	6.53	7.84
Bio-control agents								
Trichoderma koningii	10.27 ab	9.48 a	10.70 a	17.62 a	17.78 ab	36.92 a	32.98 a	41.26 a
Trichoderma viride	13.43 a	7.38 ab	11.02 a	13.78 a	20.06 a	31.74 a	25.23 b	31.60 b
Trichoderma harzianum	8.43 b	8.19 ab	12.29 a	18.21 a	18.18 ab	28.06 a	25.97 b	20.95 c
Azoxystrobin	4.57 c	5.73 b	9.53 a	16.94 a	16.98 b	31.78 a	24.18 b	27.31 bc
LSD 5 %	3.28	2.90	4.45	5.19	3.03	9.94	6.53	7.84

It was observed that all bio-control agent treatments had significantly reduced the incidence of anthracnose disease. However, the reduction of anthracnose varied between the treatments. Azoxystrobin (2.0 mL/L) significantly suppressed the fruit infection, followed by *T. harzianum, T. viride* and *T. koningii* both in every harvest period and total harvested fruits (Table 1, Figure 1). This result is in agreement with earlier results (Rahman et al 2004). *T. pseudokoningii* had better inhibition of the mycelia growth of *C. destructivum* than *T. harzianum. T. pseudokoningii* and *T. harzianum* have good antagonistic potentials against *C. destructivum (*Akinbode & Ikotun 2011). *T. viride* and *T. harzianum* are effective to control anthracnose *C. capsisi* (Rajathilagam & Kannabiran 2001). Based on the study, the degree of control on anthracnose by *T. harzianum* is comparable with the use of commercial bio fungicide (Azoxystrobin). Soytong et al (2005) reported that the rhizosphere component of bio control agents such as *Trichoderma* sp. have potential efficacy as far as introduced fungi are concerned; a root colonizer is a fungi that becomes distributed along the root in soil, propagates, and survives for long time, increasing colonization and reduction of pathogens. The inhibition of pathogen may be due to attributed to the production of secondary metabolites by antagonists such as glioviridin, viridin and gliotoxin (Shabir & Rubina 2010).

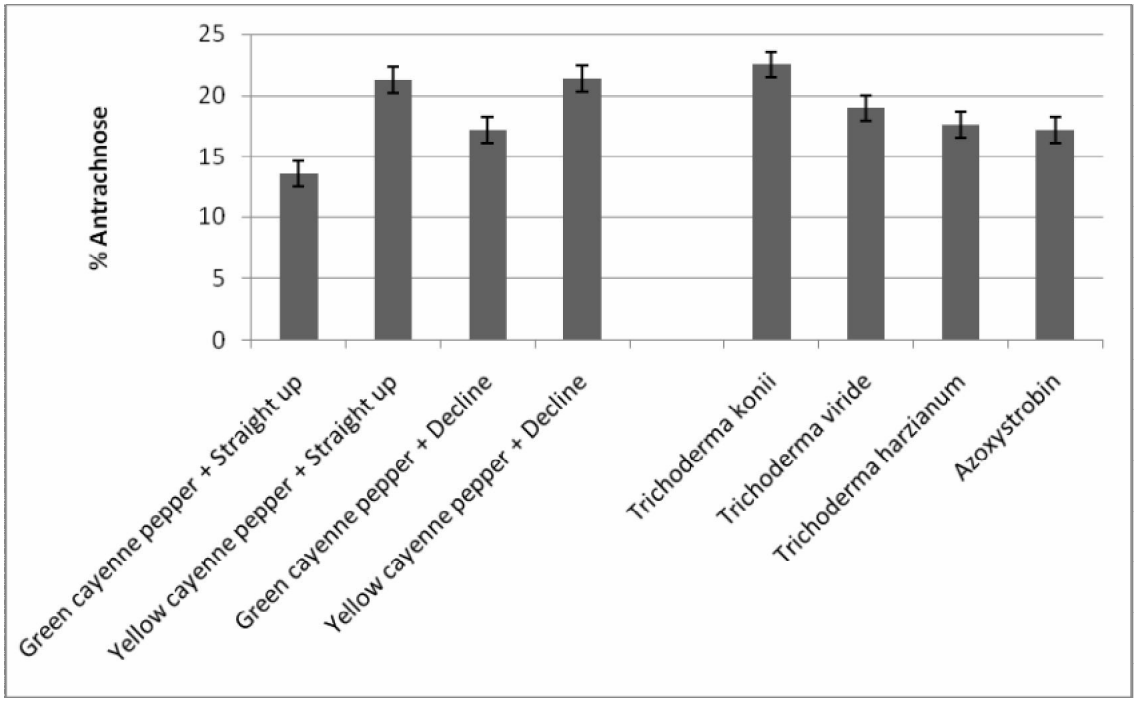

Figure 2. The average incidence of anthracnose in different treatments.

Azoxystrobin is one of the strobilurin class fungicides developed from naturally occurring antifungal compounds found in wood-decaying mushroom fungus like *S. tenacellus*. It has broad spectrum activity against the four major groups of plant pathogenic fungi including ascomycota, basidiomycota, deuteromycetes, and oomycetes. There are many reports on efficacy of azoxystrobin against plant diseases such as gray mold (*Botrytis cinerea*) of fruits and vegetables, leaf spot (*Cercospora beticola*) and powdery mildew (*Erysiphe betae*) of sugar beet, black spot (*Guignardia citricarpa*) of citrus, post-harvest rot (*Colletotrichum gloeosporioides*) of avocado (Reuveni & Sheglov 2002; Anesiadis et al 2003; Miles et al 2004; Lange 2004).

Table 2

Interaction between fruit characteristic of cayenne pepper and bio control agents to incidence of anthracnose

Bio – control agents / *Type of fruit*	*Trichoderma koningii*	*Trichoderma viride*	*Trichoderma harzianum*	Azoxystrobin
Green cayenne pepper + Straight up	6.05 ab C	6.81 a C	3.51 b C	4.28 ab A
Yellow cayenne pepper + Straight up	13.88 a B	10.90 ab B	9.79 b AB	4.15 c A
Green cayenne pepper + Decline	13.84 b B	17.41 a A	9.97 c A	4.84 d A
Yellow cayenne pepper + Decline	19.98 a A	10.44 b B	5.98 c BC	4.99 c A

Fruit color is related to antioxidants (AO) content such as capsanthin, zeaxanthin, β cryptoxanthin, β-carotene, ascorbic acid, α-tocopherol, γ-tocopherol, total phenolics (Hanson et al 2004; AVRDC 2004). Prasath & Ponnuswani (2008) reported that total phenol and enzyme plays a role in resistance against fungal infection on chili pepper. For example purple chili is immune to anthracnose (Komariah & Amalia 2011). Purple pod of bean is resistance to anthracnose (Gantet 1991). Fruit color is attributable to anthocyanins, which has often been correlated with diseases resistance in plants (Chiang & Norris 1984; Close & Beadle 2003). Chili pepper fruits at ripe red stage are more susceptible to *C. acutatum* than green fruits (Hasyim et al 2014) due to a number of preformed antifungal compounds detected in unripe fruits, which declined during ripening (Rajapakse & Ranasinghe 2002). Orange fruit color contains a very low amount of capsanthin, zeaxanthin (AVRDC 2004). Straight up fruit received full sunlight may directly affect disease development by helping to reduce the period of free moisture on fruit surfaces, thereby creating an environment that is unfavorable for spore germination and leaf infection. Sunlight also heats the fruit surface, which reduces the number of spots lesion and the production of fungal fruiting bodies. Both the direct affects of sunlight and the reduction in humidity typical of exposed plantings may have contributed to the reduced disease severity in sunny sites. Hammerschmidt & Nicholson (1977) demonstrated that the size of anthracnose lesions on lines of corn resistant and hypersensitive-resistant to *C. graminicola* were smaller when inoculated plants were grown under high light intensity.

The green cayenne pepper have thicker fruit wall compare to yellow cayenne pepper. The tinniest was established in yellow cayenne pepper + decline fruit. Fisher (1992) categorizes pepper varieties according to the exocarp thickness in the following groups: under 40 µ - determinate white varieties; from 40–80 µ interdeterminate varieties, above 80 µ red chili varieties. Manandhar et al (1995) reported that diseases incidence was correlated to cuticle and exocarp thicknesses. Cuticle thickness was significantly correlated to conidial production and lesion expansion. In another host-pathogen interaction, infection was shown to be controlled by chemical stimuli.

Observations recorded on cayenne pepper yield are presented in Figure 3. As indicated in the figure the highest fruit yield was obtained from green cayenne pepper (straight up or decline) and use of bio-control agents (*T. harzianum* and azoxystrobin) applications whereas the yellow cayenne pepper (decline) and *T. koningii* application have the lowest yield. The highest yield was generally attributed to the combination of fruit number per plant and incidence of anthracnose. This clearly revealed from the present investigation that green cayenne pepper and application with *T. harzianum* or bio fungicide azoxybtrobin contributed significantly in reducing the incidence of anthracnose on cayenne pepper.

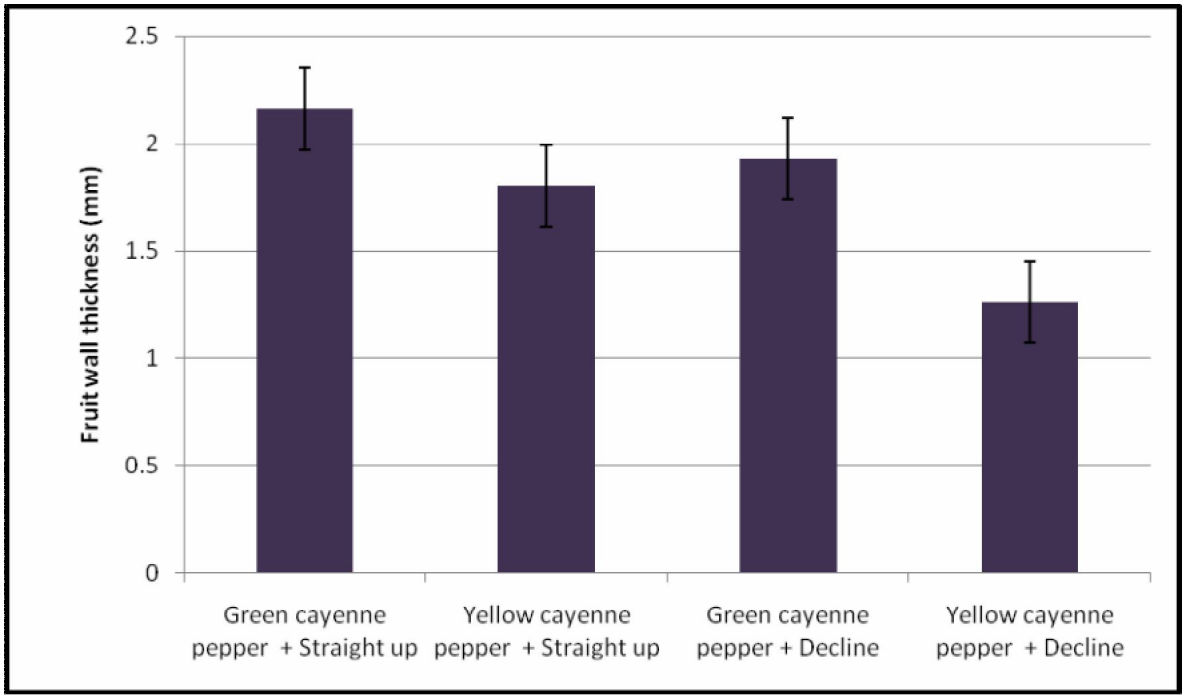

Figure 3. The fruit wall thickness of cayenne pepper.

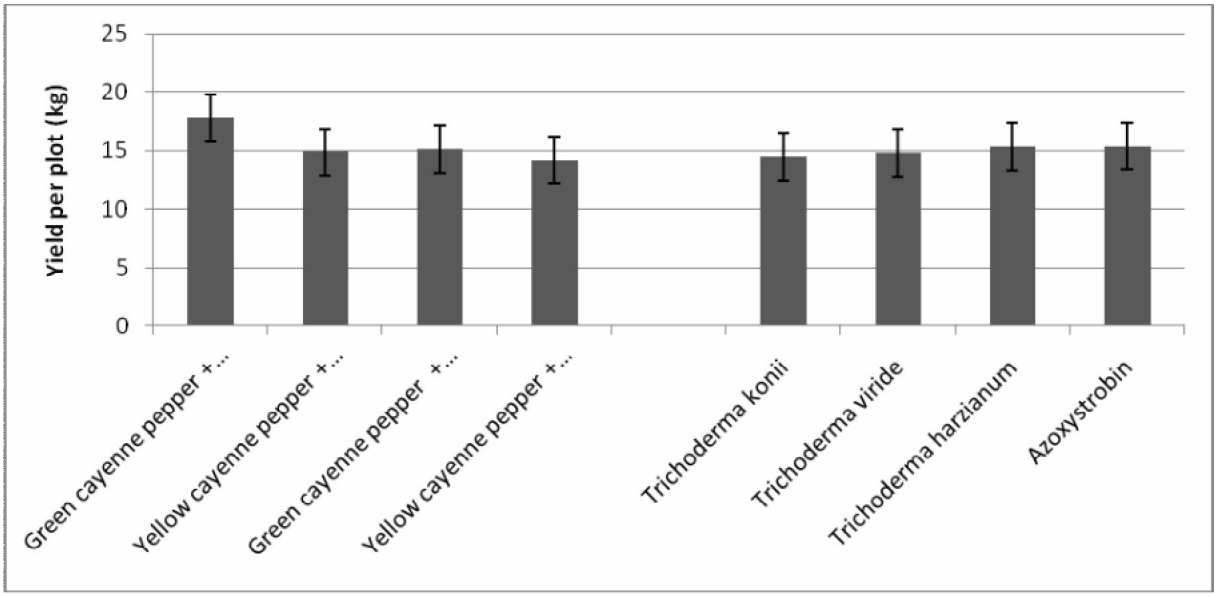

Figure 4. Cayenne pepper yield.

Conclusions. Combination between tolerant varieties and application of bio control agent were the best option to control anthracnose of cayenne pepper rather than chemical control as it is cost-effective and environment friendly. Application of all bio control agents on immature green cayenne pepper + straight up type of fruit significantly reduced the incidence of anthracnose diseases ranged between 3.51–6.81%. Application of *T. harzianum* and azoxystrobin were significantly more effective than *T. koningii* and *T. viride* in reducing the incidence of anthracnose of cayenne pepper with different fruit type. This study revealed that immature green cayenne pepper and straight up type of fruit with application of *T. harzianum* and azoxystrobin have good potential in controlling the anthracnose disease of *C. acutatum* on cayenne pepper and resulted higher yield than in other treatments.

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