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# ANTIFUNGAL ACTIVITY OF *LACTOCOCCUS LACTIS* AGAINST ANTHRACNOSE PATHOGEN, *COLLETOTRICHUM CAPSICI* OF CHILLI

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**Abstract**

Anthracnose, caused by *Colletotrichum capsici*, has impeded output in major chilli-growing regions, resulting in yield losses of 10 to 25%. Our previous *In vitro* study demonstrated that Lactic acid bacteria of *Lactococcus lactis* subsp. *lactis* had potential to inhibit *C. capsici*. Thus, this study aimed to investigate efficacy of *L. lactis* subsp. *lactis*, against *C. capsici* in three chilli varieties: Kulai chilli (*Capsicum annuum* var. *kulai*, Sakata 469), Hot chilli pepper (*Capsicum annuum* var. *longum*), and Bird's eye chilli (*Capsicum frutescens* L.) under glasshouse conditions. At the maturity index of 7, the wounded fruits were sprayed with sterilized distilled water (negative control), mancozeb (positive control), and *Lc. lactis* subsp. *lactis* solution. The fruits were then infected with spore suspension of *C. capsici* at 1 x $10^8$ spores/ml one day after treatment. After 7 days of treatment, the lesion area of treated and non-treated (negative control) *C. annum* var. *kulai* fruits did not differ significantly ($p$>0.05), whereas the lesion area of *C. annum* var. *longum* fruits treated with *Lc. lactis* was twice as small as that of non-treated fruits. *C annum* var. *longum* showed no significant difference ($p$>0.05) in chroma, hue angle, lightness, fruit firmness, and total soluble solid between treated and non-treated fruits. There was no need to apply *Lc. lactis* subsp. *lactis* treatment to inhibit *C. capsici* in *C. frutescens* because it was tolerant to *C. capsici* infection. This finding implies that *Lc. lactis* subsp. *lactis* has the ability to serve as an antifungal agent against *C. capsici* in *C annum* var. *longum* without affecting physico-chemical properties of the fruits.

**Key words:** *Capsicum frutescens* L., *Capsicum annum* var. *longum*, *Capsicum annum* var. *kulai* and *Lactococcus lactis* subspecies *lactis*.

## Introduction

Chilli, (*Capsicum annum* L.) commonly known as chilli pepper, hot pepper, and chile belongs to the family Solanaceae (Farhan *et al*., 2014). Chilli is grown worldwide as a vegetable and spice. In Thailand, pungent chilli is an economically important crop grown for local consumption and for domestic and international food industry market (Kraikruan *et al*., 2008b). Bird's eye chilli (*Capsicum frutescens* L.) is one of the two chilli types widely available in Thailand (Wangcharoen *et al.*, 2009). There are approximately more than 30 species and 200 varieties of *Capsicum* (Hernandez *et al.*, 1999) in which five are domesticated as *C. annuum, C. frutescens, C. chinense, C. baccatum* and *C. pubescens* (Than *et al.*, 2008). The most cultivated varieties are *C. annuum* (Tong & Bosland, 1999) and *C. frutescens* (Bosland & Votava, 2003). Considering its high nutritive and economic value, it was planted on a land of approximately 3,380 ha and had produced about 37, 856 Mt every year in Malaysia (Anon., 2008).

Chilli plants are easily infected with a large number of microorganisms because of their contact with soil during growth and harvesting. *Colletotrichum capsici*, the fungal infection that causes chili anthracnose disease, is the most common fungal pathogen involved in chilli deterioration (Jinantana *et al*., 1998). Anthracnose disease hampering the production in major chilli growing regions has led to 15% yield loss in Malaysia between 2009 and 2010 (Anon., 2012) and in India, accounting 12 to 25% yield loss (Sharma *et al.*, 2005). In the field, disease incidence has been recorded from 20 to 80% on fruits of *C. annum* and 5 to 20% on fruits of *C. frutescens* (Taylor, 2007). It has been reported that a part of postharvest losses of fruit quality deterioration of chilli is due to anthracnose ranged from 21 to 47% (Rajapakse *et al.*, 2007). Fresh chillies are living tissues and have higher moisture content even after harvest. When fresh chilli is removed from the plant, it is highly vulnerable to desiccation (drying), mechanical injury, decay and also infection (Anon., 2015). These physiological changes generally lead to quality loss and shorten the shelf life of chilli fruits (Anon., 1989).

As the demand for agricultural products has grown in recent decades, farmers have become increasingly reliant on agrochemicals as a crop protection tool (Saxena *et al.*, 2016). Fungicides such as manganese ethylenebis dithiocarbamate (Maneb) (Smith, 2000) and carbendazim have been recommended for controlling the disease, although it is found that the usages of both fungicides are ineffective under severe disease outbreak. Anon., (2007) mentioned that chemical fungicides namely mancozeb, ziram, blitox, bavistin and bordeaux mixtures are normally recommended for controlling anthracnose disease in seed dressings. However, evolution of fungicide resistance (Staub, 1999), health issues of farmers, economic status, and toxic environmental pollution (Voorrips *et al.*, 2004) reported particularly in developing countries, cannot be ignored (Garg *et al.*, 2014). Hence, there is an interest that focuses on potential of environmental friendly microorganisms with their metabolites to improve and extend the shelf life of agricultural produces (De Martinas *et al*., 2001).

Lactic acid bacteria (LAB), commonly known as beneficial and harmless microbes, have been widely investigated in agriculture (Nordin *et al.*, 2017; Fakri *et al*., 2018; Zakaria *et al.*, 2018). Sjogren (2005) has described a variety of LAB against different mould and yeast species with antifungal compounds (Bulgasem *et al.*, 2016). Studies on antifungal compounds have shown that several different species of LAB could produce fungal inhibitory substances. LAB produces antifungal compounds such as hydrogen peroxide, carbon dioxide, diacetyl, acetaldehyde, amino acids, reutin and also bacteriocins (Cintas *et al.*, 2001; Lani *et al.*, 2015). In

addition, some studies on antifungal LAB-strains are also available but the active compounds have not yet been reported (Schwenninger *et al*., 2005). In most studies, LAB species belonging to the *Lactobacillus* genus are reported to have inhibitory substances against fungal pathogens (Karami *et al*., 2017). For example, our previous *In vitro* study demonstrated that *Lactococcus lactis* subspecies *lactis* had potential to inhibit the growth of *Colletotrichum capsici* (Fakri *et al.*, 2018), but *In vivo* antifungal activity of *L. lactis* subsp. lactis on chilli is still scarce. Thus, this work presents evaluation on biological control of anthracnose disease by applying *L. lactis* subsp. *lactis* towards *C. capsici* on different cultivars of chilli under glasshouse conditions.

## Materials and Methods

**Growing of chilli plants:** Three chilli varieties, Kulai chilli (*Capsicum annuum* var. *kulai*, Sakata 469) (GM Peladang Sdn. Bhd.), Hot chilli pepper (*Capsicum annuum* var. *longum*) (Baba Smart Grow, Kean Beng Lee Industries (M) Sdn. Bhd) and Bird's eye chilli (*Capsicum frutescens* L.) (Baba Smart Grow, Kean Beng Lee Industries (M) Sdn. Bhd.) were examined in this study. The chilli plants were cultivated using the Department of Agriculture's (DOA) method (2010). In the glasshouse of the Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu (UMT), the seeds of each chilli variety were placed in seedling trays containing peat moss at a temperature of 27–35$^{o}$C, 12 hours of photoperiods, and 70–80% relative humidity. For optimal growth, the seedlings were transplanted four weeks after sowing to polyethylene bags containing 5 kg of mixed coco peat, peat moss, and mineral soil at a 2:1:1 ratio for Kulai chilli, and at a 1:1:1 ratio for Hot chilli pepper and Bird's eye chilli. Commercial granular chemical fertilizers were dissolved in water tanks and irrigated the chilli plants for 12 weeks using the fertigation system. An electrical conductivity (EC) meter was used to monitor fertiliser concentrations on a regular basis. Pest infestations were controlled using commercial pesticides such as cypermethrin, amitraz and carbofuran at the recommended rates, respectively.

**Application of treatment and inoculation of *Colletotrichum capsici* on chilli fruit:** The chilli fruit grown in the glasshouse was firstly wounded using sterilized needle within a range of 2 mm in diameter. Then, sterilized distilled water with 1% Tween 80 was applied as negative control treatment, whereas mancozeb was applied at the recommended rate of 2000 g a.i ha$^{-1}$ as positive control treatments. Pure culture of *Lc. lactis* subsp. *lactis* was obtained from Fakri *et al.*, (2018) and suspended with PBS and 1% Tween 80 before being adjusted to $OD_{560}$ of 1.0 (7 x $10^8$ CFU/ml). A total of 3 mL *Lc. lactis* subsp. *lactis* solution was sprayed onto the surface of each chilli fruit covering the wounded area at the maturity index of 7 (FAMA, 1984). One day after treatment, *C. capsici* spores suspended in 1% Tween 80 (adjusted to 0.5 McFarland standards turbidity) (1 x $10^8$ spores/ml) were inoculated using the same technique. After 7 days, the chilli fruits were harvested and physico-chemical analyses were carried out.

**Lesion area:** The infection level was estimated based on lesion development of the fruit caused by *C. capsici* with symptoms of sunken necrotic lesion, darkenings and spore production by measuring the diameter (mm) of lesion on the wounded area of the fruit using a digital Vernier calliper and expressed as mm$^2$ using equation 1 (Chanchaichaovivat *et al.*, 2007).

Lesion area = πab, where a and b are the length of major and minor axes -------- (1)

**Colour:** The skin colours of chilli fruits were taken at one point (middle part) using a Chroma meter (CR-400 Chroma Meter, Konica Minolta Sensing Americas Inc, USA) based on CIE L*, a* and b* system. The L* coordinate was a measure of lightness (white-black; ranged from no reflection L = 0 to perfect diffused reflection L=100), the 'a' scales ranged from negative values for green to positive values for red, while the 'b' scale ranged from negative for blue to positive values for yellow. The instrument was calibrated against standard white colour plate (Y= 93.9, x= 0.313, and y= 0.321) (Anon, 1993). The samples were analysed in triplicate. These L*, a* and b* values then were used to calculate hue angle degree (h°= arctan [b* a* $^{-1}$]), where 0°= red-purple; 90°= yellow, 180°= bluish-green and 270°= blue and chroma (C*= [a*$^2$ + b*$^2$]$^{1/2}$), indicative of the intensity or colour saturation (McGuire, 1992).

**Firmness:** Using a texture analyzer (TA. XTPlus, Stable Micro System Ltd, UK), the firmness of chilli fruits was determined by calculating the maximum penetration force needed using the same chilli samples from colour measurement analyses. At the depth of 3 mm with a rate of 5mms$^{-1}$, the P2N probe was used to penetrate into the tissue. The downward distance was set at 10 mm and return was automatic (Rojas-Grau *et al*., 2007). At the middle part of chilli fruit, the firmness of each sample was stated as peak force and expressed in Newton.

**Total soluble solid (TSS):** Using the same chilli from texture analyses, the chilli was cut into small pieces and put in muslin cloth and then squeezed to get the juice (aqueous solution). Two drops of juices were put on the digital refractometer prism and total soluble solid contents were recorded using a digital refractometer (MA871 Digital Brix Refractometer, Milwaukee Instruments Inc, USA) with a scale of 0-85% Brix (Suktanarak *et al*., 2013).

**Experimental design and statistical analysis:** The glasshouse experiments were laid out in a complete randomized design (CRD) with five replications of fruits. The data of colour, firmness, total soluble solid and lesion areas were expressed as mean and checked for normality and homogeneity of variance before being subjected to one-way analysis of variance (ANOVA). A post-hoc Tukey test was done in order to compare the mean treatments at 5% of significance level. In certain case, colour L* and firmness vale for Hot chilli, colour L* and brix$^{o}$ value of Bird's eye chilli, and brix$^{o}$ and firmness value of Kulai chilli were subjected to Kruskal-Wallis. Mean comparisons were performed by Tukey Test at 5%

of significance level. All statistic procedures were conducted using SPSS software version 20.

## Results

### Physico-chemical analysis

**Bird's eye chilli:** Table 1 shows the effects of different protective treatments on physico-chemical properties of Bird's eye chilli fruits inoculated with spores of *C. capsici* seven days after treatment under glasshouse conditions. For lightness value, treated and non-treated chilli fruits (distilled water) were not significantly different ($p$>0.05), with light reflection values ranging from 90 to 94$^o$, indicating that the fruits had closely perfect diffused reflection. Similar trends were found in hue angle (H$^o$) and chromas (C*) where the H$^o$ values and C* values ranged from 36 to 44$^o$ and 4.5 to 5.0$^o$, respectively, indicating that the chilli fruits were red in colour with low colour saturation. Likewise, the treated and non-treated fruits did not differ significantly ($p$>0.05) in fruit firmness and lesion area. The fruit firmness values were recorded in the range of 0.4 to 0.5 N while the lesion area was in the range of 17 to 20 mm$^2$ as compared to the wounded area of 13mm$^2$ (Fig. 1). Interestingly, there was significant difference ($p$≤0.05) among the treatments in total soluble solid content. Fruits inoculated with *C. capsici* and treated with fungicide-mancozeb had lower brix values compared to fruits treated with *Lc. lactis* subsp. *lactis* with or without *C. capsici* inoculation. This result implied that Bird's Eye chilli was likely to be tolerant to fungal pathogen of *C. capsici*.

**Table 1. Effects of different protective treatments on physico-chemical properties of Bird's eye chilli (*Capsicum frutescens*) fruit inoculated with spores of *Colletotrichum capsici* under glasshouse conditions.**

| Physico-chemical parameters | | Treatments^ | | | |
|---|---|---|---|---|---|
| | | **Sterilised distilled water** | **Fungicide-Mancozeb** | ***Lactococcus lactis* subsp. *lactis* without *C. capsici* inoculation** | ***Lactococcus lactis* subsp. *lactis*** |
| Colour ($^o$) | L* | 90.38 ± 0.71$_a$ | 90.85 ± 1.34$_a$ | 93.34 ± 3.49$_a$ | 91.36 ± 2.81$_a$ |
| | H$^o$ | 36.81 ± 2.07$_a$ | 39.95 ± 5.57$_a$ | 43.18 ± 5.07$_a$ | 38.86 ± 3.63$_a$ |
| | C* | 4.94 ± 0.37$_a$ | 4.88 ± 0.56$_a$ | 4.53 ± 0.56$_a$ | 4.89 ± 0.73$_a$ |
| Total Soluable Solid (%) | | 16.20 ± 0.29$_{ab}$ | 13.96 ± 0.63$_a$ | 16.40 ± 0.68$_b$ | 16.60 ± 1.11$_b$ |
| Firmness (N) | | 0.49 ± 0.05$_a$ | 0.42 ± 0.11$_a$ | 0.53 ± 0.08$_a$ | 0.51 ± 0.06$_a$ |
| Lesion Area (mm$^2$) | | 19.81 ± 3.30$_a$ | 18.05 ± 2.86$_a$ | 17.92 ± 2.91$_a$ | 19.49 ± 2.59$_a$ |

^ Sterilised distilled water and fungicide – mancozeb denote negative and positive control treatments, respectively. Data were tabulated with mean ± standard deviation of mean. Mean followed by the similar letter with the same row has no significant difference at $p$>0.05 after determined by Tukey test

**Table 2. Effects of different protective treatments on physico-chemical properties of Hot chilli pepper (*Capsicum annuum* var. *longum*) fruit inoculated with spores of *Colletotrichum capsici* under glasshouse conditions.**

| Physico-chemical parameters | | Treatments ^ | | | |
|---|---|---|---|---|---|
| | | **Sterilised distilled water** | **Fungicide-Mancozeb** | ***Lactococcus lactis* subsp. lactis without *C. capsici* inoculation** | ***Lactococcus lactis* subsp. *lactis*** |
| Colour ($^o$) | L* | 94.83 ± 2.44$_a$ | 96.85 ± 0.47$_a$ | 94.39 ± 2.59$_a$ | 93.96 ± 2.77$_a$ |
| | H$^o$ | 45.59 ± 5.52$_{ab}$ | 53.50 ± 4.45$_b$ | 44.36 ± 1.83$_a$ | 45.46 ± 2.69$_{ab}$ |
| | C* | 3.83 ± 0.33$_a$ | 3.39 ± 0.50$_a$ | 4.02 ± 0.39$_a$ | 4.51 ± 0.73$_a$ |
| Total Soluable Solid (%) | | 18.00 ± 1.32$_a$ | 15.33 ± 3.27$_a$ | 15.20 ± 1.94$_a$ | 17.46 ± 2.26$_a$ |
| Firmness (N) | | 0.41 ± 0.06$_a$ | 0.35 ± 0.04$_a$ | 0.39 ± 0.09$_a$ | 0.43 ± 0.13$_a$ |
| Lesion Area (mm$^2$) | | 40.60 ± 2.18$_b$ | 36.65 ± 3.87$_b$ | 17.52 ± 2.32$_a$ | 21.10 ± 0.78$_a$ |

^ Sterilised distilled water and fungicide – mancozeb denote negative and positive control treatments, respectively. Data were tabulated with mean ± standard deviation of mean. Mean followed by the similar letter with the same row has no significant difference at p>0.05 after determined by Tukey test

**Table 3. Effects of different protective treatments on physico-chemical properties of Kulai chilli (*Capsicum annuum* var. *kulai*, Sakata 469) fruit inoculated with spores of *Colletotrichum capsici* under glasshouse conditions.**

| Physico-chemical parameters | | Treatments ^ | | | |
|---|---|---|---|---|---|
| | | **Sterilised distilled water** | **Fungicide-Mancozeb** | ***Lactococcus lactis* subsp. *lactis* without *C. capsici* inoculation** | ***Lactococcus lactis* subsp. *lactis*** |
| Colour ($^o$) | L* | 38.31 ± 0.94$_b$ | 37.42 ± 1.15$_{ab}$ | 39.18 ± 1.30$_b$ | 35.47 ± 1.33$_a$ |
| | H$^o$ | 23.19 ± 0.47$_a$ | 24.82 ± 1.14$_{ab}$ | 26.53 ± 1.09$_b$ | 24.27 ± 1.02$_a$ |
| | C* | 37.49 ± 0.23$_{ab}$ | 39.08 ± 0.85$_b$ | 39.23 ± 1.40$_b$ | 36.93 ± 1.17$_a$ |
| Total Soluable Solid (%) | | 8.22 ± 0.44$_b$ | 8.65 ± 0.17$_b$ | 6.68 ± 0.04$_a$ | 7.86 ± 0.18$_{ab}$ |
| Firmness (N) | | 1.32 ± 0.18$_b$ | 1.13 ± 0.07$_{ab}$ | 0.90 ± 0.09$_{ab}$ | 0.71 ± 0.01$_a$ |
| Lesion Area (mm$^2$) | | 30.30 ± 2.21$_a$ | 27.79 ± 2.64$_a$ | 31.50 ± 0.33$_a$ | 30.50 ± 1.60$_a$ |

^ Sterilised distilled water and fungicide – mancozeb denote negative and positive control treatments, respectively. Data were tabulated with mean ± standard deviation of mean. Mean followed by the similar letter with the same row has no significant difference at $p$>0.05 after determined by Tukey test

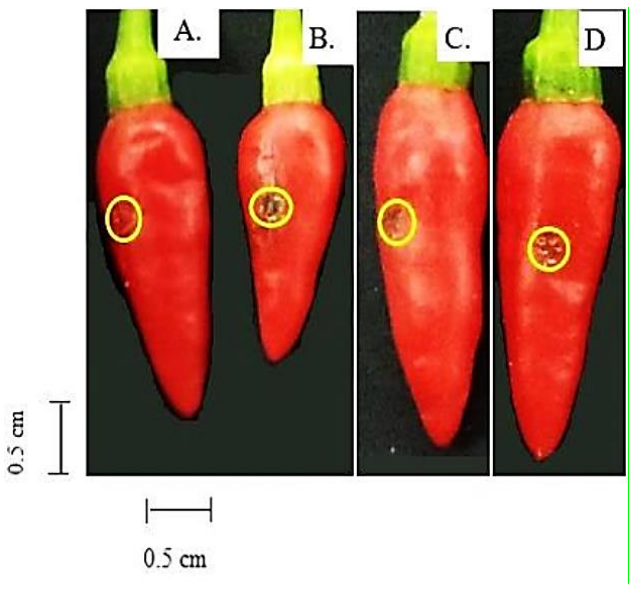


Fig. 1. Lesion area of *Capsicum frutescens* 7 days after treatments of sterilised distilled water fb. fungal inoculation (A), fungicide mancozeb fb. fungal inoculation (B), *Lactococcus lactis* subspecies *lactis* without fungal incolulation (C) and *Lactoccus lactis* subspecis *lactis* fb. fungal inoculation (D). fb denotes followed by.

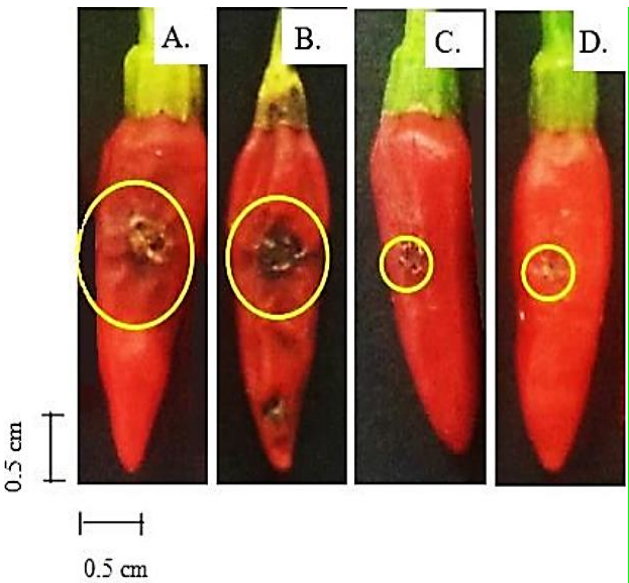


Fig. 2. Lesion area of *Capsicum annuum* var. *longum* 7 days after treatments of sterilised distilled water fb. fungal inoculation (A), fungicide mancozeb fb. fungal inoculation (B), *Lactococcus lactis* subspecies *lactis* without fungal incolulation (C) and *Lactoccus lactis* subspecis *lactis* fb. fungal inoculation (D). fb denotes followed by.

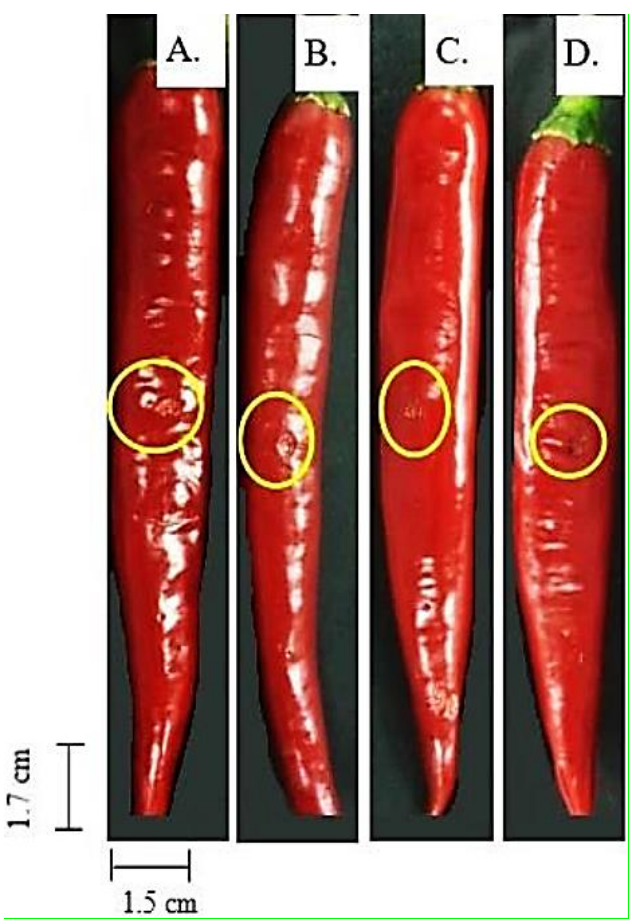


Fig. 3. Lesion area of *Capsicum annuum* var. *kulai* 7 days after treatments of sterilised distilled water fb. fungal inoculation (A), fungicide mancozeb fb. fungal inoculation (B), *Lactococcus lactis* subspecies *lactis* without fungal incolulation (C) and *Lactoccus lactis* subspecis *lactis* fb. fungal inoculation (D). fb denotes followed by.

**Hot chilli:** Table 2 presents the effects of different protective treatments on physico-chemical properties of hot chilli pepper fruits inoculated with spores of *C. capsici* seven days after treatment under glasshouse conditions. Treated and non-treated (distilled water) chilli fruits were not significantly different ($p>0.05$) in lightness value, with light reflection values being in the range of 93 to 97°. However, there was significant difference ($p\leq0.05$) among the treatments in H°. The fruits treated with mancozeb had greater H° value ($p\leq0.05$) than those treated with *Lc. lactis* subsp. *lactis* without *C. capsici* inoculation. On the other hand, there was no significant difference ($p>0.05$) among the treatments in C*. The fruits subjected to *Lc. lactis* subsp. *lactis* with *C. capsici* inoculation gave greater C* value ($p\leq0.05$) compared to those subjected to mancozeb treatment. By contrast, the treated and non-treated fruits were not significantly different ($p>0.05$) in total soluble solid content, with the brix value ranging from 15 to 18°. A similar trend of insignificant difference ($p>0.05$) was observed in fruit firmness in both treated and non-treated fruits, where the firmness values were in the range of 0.35 to 0.43 N. It is interesting to note that the treated and non-treated fruits exhibited significant difference ($p\leq0.05$) in lesion areas. The fruits subjected to sterilise distilled water had lesion areas of 41 $mm^2$. The lesion areas could be reduced twice after treated with *Lc. lactis* subsp. *lactis*. The fruits treated with *Lc. lactis* subsp. *lactis* with or without any fungal inoculation had similar lesion area ($p\geq0.05$). Surprisingly, the mancozeb-treated fruits which served as positive control treatment had similar insignificant difference ($p>0.05$) of lesion areas with those of distilled water-treated fruits which served as negative control treatment (Fig. 2). These findings suggested that *Lc. lactis* subsp. *lactis* had potential to be applied as antifungal agent against *C. capsici*-infected hot chilli pepper.

**Kulai chilli:** Table 3 shows the effect of different protective treatments on physico-chemical properties of Kulai chilli fruits inoculated with spores of *C. capsici* seven days after treatments under glasshouse conditions. For lightness value, treated and non-treated chilli fruits (distilled water) were significantly different ($p \leq 0.05$), with light reflection values ranging from 36 to 40$^{o}$ except that fruits treated with *Lc. lactis* subsp. *lactis* without fungal inoculation had a greater L* value than those treated with *Lc. lactis* subsp. *lactis* with fungal inoculation, suggesting that the dark colour of *C. capsici* spores might be able to reduce L* value. Although H$^{o}$ and C* values recorded among the treatments were significantly different ($p \leq 0.05$) but these differences were not apparent. Interestingly, the fruits treated with *Lc. lactis* subsp. *lactis* without fungal inoculation had lower brix value (6.7) in comparison to the mancozeb-treated fruits (8.7) ($p \leq 0.05$). On the other hand, the non-treated fruits (1.3 N) had greater fruit firmness than those of fruits treated with *Lc. lactis* subsp. *lactis* (0.7 N) ($p \leq 0.05$). However, the treated and non-treated fruits did not differ significantly ($p > 0.05$) in lesion area. The lesion area was in the range of 28 to 32 mm$^2$ as compared to the wounded area of 13 mm$^2$ (Fig. 3). These results showed that Kulai chilli was susceptible to fungal pathogen of *C. capsici* and all tested treatments failed to reduce the lesion area caused by of the fungal pathogen. Furthermore, application of *Lc. lactis* subsp. *lactis* could cause lesion to this variety.

## Discussion

Surprisingly, in the present study, it showed that mancozeb failed to control *C. capsici* when being applied to *C. annum* var. *kulai* and *C. annum* var. *longum*. It is likely that the timing of application influenced fungicide efficacy under glasshouse conditions. The efficacy was mostly reduced by rising the time between application and infection, especially in protectant fungicides such as mancozeb. New tissue was vulnerable from following application, and through growing time between application and infection, there was more vulnerable tissue. It is especially crucial to give vulnerability to the infection of newly formed tissue (Diggle *et al*., 2002). The authors also stated that the development of sporulating lesion in lupin because anthracnose emerged on the 6th days after 24 hours' inoculation at 24$^{o}$C. Horoszkiewicz-Janka *et al*., (2002) also noted that contact fungicide was extremely effective when applied near to the time of infection, but its effectiveness was decreased as the duration between application and infection increased. In this study, the spore suspension of *C. capsici* was inoculated onto chilli fruits after 24 hours upon application of treatments under glasshouse conditions at a higher temperature of approximately 28 to 30$^{o}$C. Therefore, low efficacy of mancozeb against *C. capsici* under glasshouse conditions in the present study is most likely not due to inappropriate period between application and inoculation.

Abundant previous studies have shown that regulation of chilli anthracnose and fruit rot disease induced by *C. capsici* could be accomplished by mancozeb spraying (Das & Mohanty, 1988; Biswas, 1992; Ebenezar & Alice, 1996). Shukla *et al*., (2010) recorded that mancozeb was not only highly effective at lower concentrations against conidial germination of *C. capsici*, but it also gave promising protection to Indian snakeroot from field infections of *C. capsici*. By contrast, high efficacy of mancozeb against *C. capsici* was evident in the *In vitro* experiment where it showed excellent antifungal activity at a concentration as low as 1% (w/v) (Fakri *et al*., 2018), but it exhibited poor efficacy on chilli plants infected with *C. capsici* at the glasshouse (*In vivo*) in the present study. Shukla *et al*., (2010) also reported a similar finding where propiconazole was highly effective against *C. capsici* spore germination but when sprayed over the flowers and foliage of Indian snake root infected with *C. capsici*, it was ineffective in the field. Margina & Zheljazkov (1994) who examined the fungicide effect on mint rust of mint, also obtained similar result where most of the fungicides were successfully applied during vegetative period of mint and not during matured stages. The distinct response observed in the *In vitro* and *In vivo* studies in this study was due to the non-absorption or decomposition of mancozeb by the variety-dependent chilli plant and environmental factors such as temperature, relative humidity and others.

During fruit ripening, plant responses to *Colletotrichum* spp. morphogenesis are essential signs in assessing the resistance or susceptible interactions that have occurred (Oh *et al*., 1999). The inoculation of the fungal spore onto the chilli fruit was carried out at maturity index 7 and the fruit was fully ripened when the assessment was conducted 7 days after treatment in the *In vivo* study. In the present study, physico-chemical analysis showed that fruit colour and total soluble solid were not much affected either of any chilli cultivars but fruit firmness was affected due to the occurrence of lesion. Interestingly, lesions of *C. annuum* var. *longum* and *C. annum* var. *kulai* were apparent one day after inoculation with *C. capsici* whereas *Capsicum frutescens* was not affected, suggesting that *C. frutescens* was more tolerant to *C. capsici* whereas *C. annuum* var. *longum* and *C. annum* var. *kulai* were more afflicted by *C. capsici*. Prior to adaption towards the necrotrophic mode of nutrition in the host plant, Liao *et al*., (2012) observed a middle phase displaying partial endophytic life style of the anthracnose pathogen. Different species of *Colletotrichum* have been shown to exhibit different infection mechanisms, depending on the infected host. Necrosis is due to intramural necrotrophy by *C. capsici* which caused the breakdown of cell wall structures that damaged cowpea (Pring *et al*., 1995).

The harmony of plant-pathogen interactions is usually driven by the gene-for-gene model in a lot of pathosystems (Flor, 1971). A partial resistance genotype will result in lower infection levels, thus decreasing the amount of inoculum in the field and restricting the ability for epidemics to occur. *Colletotrichum acutatum*, a relatively virulent species (Than *et al*., 2008) against chilli genotypes, was examined by Kim *et al*., (2004) and they discovered that C*apsicum baccatum* genotype 'PBC 80' was an anthracnose-resistant genetic resource pool. Another genotype of *C. baccatum*, 'PBC81', however, was highly susceptible to certain isolates of *C. acutatum*. Many studies have demonstrated that capsaicin and its correlative exhibited antifungal activity against *C. capsici* (Kraikruan *et al*., 2008a). Capsaicin (trans- 8- methyl- N - vanillyn- 6- noneamide) is the major alkaloid responsible

for the mucosal irritant properties of plant species from the genus *Capsicum* (Buck & Burks 1985). It is suggested that the resistance of *C. frutescens* towards *C. capsici* in this study may be due to the existence of capsaicin and its analogues which have antifungal properties.

Concurring with the results of the present study, Soetarno *et al*., (1997) documented that antibacterial and antifungal properties were known to exist in *C. frutescens*. CAY-1, a novel *C. frutescens* saponin, has been shown to have antifungal activities against many fungi. CAY-1 was found to be active against 16 different fungi including *Aspergillus fumigatus*, and interfered with fungal cell membrane integrity (De Lucca *et al*., 2006). There is, however, a different view of the antifungal properties generated by capsaicin. Cichewicz *et al*., (1996) found that there were no antifungal properties in pure capsaicin and dihydrocapsaicin, so the antifungal properties of *Capsicum* species were likely due to other compounds. Antifungal and sugar-binding characteristics are identified on lectins from *C. annum* and *C. frutescens* (Sanatombi *et al*., 2007). The alternative mechanism of *C. frutescens* against *C. capsici* may also be due to phytoalexins and cysteine proteins associated with pathogenesis, such as lipid transfer protein and thionins (Oh *et al*., 1999) which induce rapid cell death to halt *C. capsici* tissue colonisation.

Roy *et al*., (1996) examined the impact of incubation time and temperature on the development of *Lc. lactis* subsp. *lactis* antifungal compounds, and found that the optimum production was at 30°C after 48 hours of incubation. In this study, the temperatures that ranged from 27 to 35°C under glasshouse conditions were conducive for *Lc. lactis* subsp. *lactis* to produce antifungal compounds, but all the three chilli varieties responded differently with *C. annuum* var. *longum* having a smaller lesion size compared to *C. annuum* var. *kulai* after treating with *Lc. lactis* subsp. *lactis*. It was surprising to note that the antifungal activity exhibited by *Lc. lactis* subsp. *lactis* against *C. capsici* was quite promising for *C. annuum* var. *longum* although *Lc. lactis* subsp. *lactis* only provided a small inhibition zone in the present *In vitro* study (Fakri *et al*., 2018).

There are a few possibilities which explain the effectiveness of *Lc. lactis* subsp. *lactis* against *C. capsici* for *C. annuum* var. *longum*. The rapid growth of *Lc. lactis* subsp. *lactis* which occur within 48 hours may create a nutrient competition condition with *C. capsici,* thereby decreasing infiltration of *C. capsici* into fruits cells (Roy *et al*., 1996). Plants contain several phytochemicals compounds, which are known to play an important role in defence against bacteria, fungi, herbivores, insects and viruses (Duke *et al*., 1999). In another study it was demonstrated the introduction of plant activator such as strobilurins that could initiate protective responses in the plant, a process called systemic acquired resistance against pathogen infection and disease (Ypema *et al*., 1999). *Lc. lactis* subsp. *lactis* from this current study may act as plant activator which induces the defence mechanism of *C. annuum* var. *longum* against *C. capsici*. When initiating this process, energy can be aimed at raising the thickness of plant cell walls, increasing the concentration of phytoalexin, and also initiating cell death, thereby decreasing the amount of plant energy being utilized into growth and fruit production (Romero *et al*., 2001). Strobilurin compounds have been shown to suppress several different fungi. Strobilurins inhibit mitochondrial respiration by preventing the oxidation of quinol in the complex of cytochrome bc1, thus supressing the production of ATP (Ypema *et al.*, 1999). This behaviour is not lethal, but it is inhibitory and can make parasitism more vulnerable to the fungus.

On the other hand, the lesion on *C. annuum* var. *kulai* caused by *Lc. lactis* subsp. *lactis* is not a new phenomenon as some LAB such as *Leuconostoc* spp. and *Lactobacillus* spp. have been documented on many vegetables as the common weak pathogens (Lund, 1992) and causing for the fermentation of brined vegetables (Samish *et al*., 1963). Similarly, *Leuconostoc mesenteroides* that caused a sour rot type decay of tomatoes was identified by Conn *et al*., (1995). Bartz *et al.* (1995) also isolated both *Leuconostoc mesenteroides* and *Lactobacillus* sp. from lesions on tomatoes induced by the fungus *Geotrichum candidum* that seemed to have sour rot.

## Conclusion

Preventive treatment of *Lc. lactis* subsp. *lactis* was evaluated against *C. capsici* in three chilli varieties. *Lc. lactis* subsp. *lactis* exhibited excellent antifungal potential against *C. capsici* when treated on C. *annuum* var. *longum.* By constrast*, Lc. lactis* subsp. *lactis* treatment not only failed to inhibit *C. capsici* infection on *C. annum* var. *kulai* fruit but it also caused lesion on the fruits. There is no need to apply *Lc. lactis* subsp. *lactis* treatment to inhibit *C. capsici* in *C. frutescens* because it was tolerant to *C. capsici* infection. Physico-chemical assessment revealed that *Capsicum annuum* var. *longum* was not significantly different ($p$>0.05) in chroma, hue angle, lightness, fruit firmness and total soluble solid between treated and non-treated (distilled water) fruits. This finding suggests that *Lc. lactis* subsp. *lactis* has potential to antagonize *C. capsici* infection in *Capsicum annuum* var. *longum* without affecting physico-chemical properties of the fruits. The results of present study could lead to the development of an environmentally benign method for managing anthracnose disease utilizing Lactic acid bacteria (LAB), with the goal of reducing the heavy reliance on fungicides for anthracnose disease control in chili plants.

Further research is required to determine the effectiveness of cell-free supernatant of *Lc. lactis* subsp. *lactis* and compounds which act as the antifungal agents since the present study only examined antifungal activity from whole cell of *Lc. lactis* subsp. *lactis*. It is necessary to further examine effects of other LAB species such as *Lactobacillus* and *Leuconostoc* isolated from different soil types for suppression of *C. capsici* in chilli plants. In addition, the experimental period should be prolonged from pre-harvest to postharvest stages to reveal effectiveness of *Lc. lactis* subsp. *lactis* for fungal control at different maturity stage of chilli fruits.

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