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

MUHAMMAD AIMAN FAKRI1, MOHD NIZAM LANI1 AND CHUAH TSE SENG2

1Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
2Faculty of Plantation and Agrotechnology, Universiti Teknologi Mara (UiTM), 02600 Arau, Perlis, Malaysia

Corresponding author’s email: chuahs@ium.edu.my

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. kulaï, 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 106 spores/ml one day after treatment. After 7 days of treatment, the lesion area of treated and non-treated (negative control) C. annuum var. kulaï fruits did not differ significantly (p>0.05), whereas the lesion area of C. annuum var. longum fruits treated with Lc. lactis was twice as small as that of non-treated fruits. C. annuum 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. annuum var. longum without affecting physico-chemical properties of the fruits.

Key words: Capsicum frutescens L., Capsicum annuum var. longum, Capsicum annuum var. kulaï and Lactococcus lactis subspesies lactis.

Introduction

Chilli, (Capsicum annuum 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 (Kraikrua 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 chilli 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. annuum 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 (Meb) (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, bitox, babistin 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, reutil 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°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 OD₅₆₅ of 1.0 (7 x 10⁸ 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 10⁸ 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, darkening 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 mm² using equation 1 (Chanchaichaovivat et al., 2007).

\[ \text{Lesion area} = \pi a b, \text{where } a \text{ and } b \text{ are the length of major and minor axes} \]

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]½), 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 (Sukitanarak 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 brix⁰ value of Bird’s eye chilli, and brix⁰ 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°, indicating that the fruits had closely perfect diffused reflection. Similar trends were found in hue angle (H°) and chromas (C*) where the H° values and C* values ranged from 36 to 44° and 4.5 to 5.0°, 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 mm² as compared to the wounded area of 13mm² (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.

<table>
<thead>
<tr>
<th>Physico-chemical parameters</th>
<th>Treatments^</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sterilised distilled water</td>
<td>Fungicide-Mancozeb</td>
<td>Lactococcus lactis subsp. lactis without C. capsici inoculation</td>
</tr>
<tr>
<td>Colour (°)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>90.38 ± 0.71a</td>
<td>90.85 ± 1.34a</td>
<td>93.34 ± 3.49a</td>
</tr>
<tr>
<td>H°</td>
<td>36.81 ± 2.07ab</td>
<td>39.95 ± 5.57ab</td>
<td>43.18 ± 5.07ab</td>
</tr>
<tr>
<td>C*</td>
<td>4.94 ± 0.37a</td>
<td>4.88 ± 0.56a</td>
<td>4.53 ± 0.56a</td>
</tr>
<tr>
<td>Total Soluble Solid (%)</td>
<td>16.20 ± 0.29ab</td>
<td>13.96 ± 0.63ab</td>
<td>16.40 ± 0.68ab</td>
</tr>
<tr>
<td>Firmness (N)</td>
<td>0.49 ± 0.05a</td>
<td>0.42 ± 0.11a</td>
<td>0.53 ± 0.08a</td>
</tr>
<tr>
<td>Lesion Area (mm²)</td>
<td>19.81 ± 3.30a</td>
<td>18.05 ± 2.86a</td>
<td>17.92 ± 2.91a</td>
</tr>
</tbody>
</table>

^ 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.

<table>
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<td>Lactococcus lactis subsp. lactis without C. capsici inoculation</td>
</tr>
<tr>
<td>Colour (°)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>94.83 ± 2.44a</td>
<td>96.85 ± 0.47a</td>
<td>94.39 ± 2.59a</td>
</tr>
<tr>
<td>H°</td>
<td>45.59 ± 5.52ab</td>
<td>53.50 ± 4.45ab</td>
<td>44.36 ± 1.83ab</td>
</tr>
<tr>
<td>C*</td>
<td>3.83 ± 0.33a</td>
<td>3.39 ± 0.50a</td>
<td>4.02 ± 0.39a</td>
</tr>
<tr>
<td>Total Soluble Solid (%)</td>
<td>18.00 ± 1.32a</td>
<td>15.33 ± 3.27a</td>
<td>15.20 ± 1.94a</td>
</tr>
<tr>
<td>Firmness (N)</td>
<td>0.41 ± 0.06a</td>
<td>0.35 ± 0.04a</td>
<td>0.39 ± 0.09a</td>
</tr>
<tr>
<td>Lesion Area (mm²)</td>
<td>40.60 ± 2.18a</td>
<td>36.65 ± 3.87b</td>
<td>17.52 ± 2.32a</td>
</tr>
</tbody>
</table>

^ 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.

<table>
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<td></td>
<td>Sterilised distilled water</td>
<td>Fungicide-Mancozeb</td>
<td>Lactococcus lactis subsp. lactis without C. capsici inoculation</td>
</tr>
<tr>
<td>Colour (°)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>38.31 ± 0.94b</td>
<td>37.42 ± 1.13ab</td>
<td>39.18 ± 1.30ab</td>
</tr>
<tr>
<td>H°</td>
<td>23.19 ± 0.47ab</td>
<td>24.82 ± 1.14ab</td>
<td>26.53 ± 1.09ab</td>
</tr>
<tr>
<td>C*</td>
<td>37.49 ± 0.23ab</td>
<td>39.08 ± 0.85ab</td>
<td>39.23 ± 1.40ab</td>
</tr>
<tr>
<td>Total Soluble Solid (%)</td>
<td>8.22 ± 0.44b</td>
<td>8.65 ± 0.17b</td>
<td>6.68 ± 0.04ab</td>
</tr>
<tr>
<td>Firmness (N)</td>
<td>1.32 ± 0.18a</td>
<td>1.13 ± 0.07ab</td>
<td>0.90 ± 0.09ab</td>
</tr>
<tr>
<td>Lesion Area (mm²)</td>
<td>30.30 ± 2.21a</td>
<td>27.79 ± 2.64a</td>
<td>31.50 ± 0.33a</td>
</tr>
</tbody>
</table>

^ 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 inoculation (C) and Lactococcus lactis subspecies 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 inoculation (C) and Lactococcus lactis subspecies 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 inoculation (C) and Lactococcus lactis subspecies 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 ($L^*$) value, with light reflection values being in the range of 93 to 97$^\circ$. However, there was significant difference ($p<0.05$) among the treatments in $H^*$. The fruits treated with mancozeb had greater $H^*$ value ($p<0.05$) 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 ($p<0.05$) 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$^\circ$. 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 ($p<0.05$) 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 ($p>0.05$). 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 anti fungal 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<0.05), with light reflection values ranging from 36 to 40° 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* and C* values recorded among the treatments were significantly different (p<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<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<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 mm² as compared to the wounded area of 13 mm² (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. annuum var. kulai and C. annuum 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°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°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 snake root 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. annuum 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. annuum var. kulai were more affected 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 Capsicum 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 correlate exhibited antifungal activity against C. capsici (KraiKrualn et al., 2008a). Capsaicin (trans- 8- methyl- N-vanillyl- 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 capsaiacin. Cichewicz et al., (1996) found that there were no antifungal properties in pure capsaiacin and dihydrocapsaiacin, so the antifungal properties of *Capsicum* species were likely due to other compounds. Antifungal and sugar-binding characteristics are identified on lectins from *C. annuum* and *C. frutescens* (Sanatombi et al., 2007). The alternative mechanism of *C. frutescens* against *C. capsici* may also be due to phytoalexins and cytoene 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. annuum* 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 chili 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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