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Short Communication

Antifungal property of chili, shallot and garlic extracts against pathogenic fungi, *Phomopsis* spp., isolated from infected leaves of para rubber (*Hevea brasiliensis* Muell. Arg.)

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ABSTRACT

The aim of this study was to evaluate the antifungal property of extracts of chili, shallot and garlic (local varieties in Sisaket, Thailand) against pathogenic fungi, *Phomopsis* spp., which were isolated from infected leaves of para rubber (*Hevea brasiliensis* Muell. Arg.). Seven isolates of *Phomopsis* spp. namely *Phomopsis* sp. SSK1.1, SSK1.2, SSK3.1, SSK4.1, SSK5.1, SSK5.2 and SSK7.1 were identified on the basis of morphological characteristics. Fresh plants were extracted with water to obtain crude extracts and their antifungal properties were tested on potato dextrose agar (PDA) media. The study demonstrated that increasing the concentrations (20%, 40%, 60% or 80%) of the chili extract exhibited a dependent increase in the inhibitory level on mycelial growth of *Phomopsis* spp. SSK3.1, SSK4.1 and SSK5.2. The inhibitory level on mycelial growth of shallot extract also increased in a dose-dependent manner in all isolates of *Phomopsis*. The garlic extract had significant inhibition on the growth of all isolates with complete inhibition at 80% concentration. The highest levels of percentage inhibition of mycelial growth were with garlic extract followed by shallot and chili extracts, respectively. The study also showed that these plant extracts contained some polyphenols (apigenin, gallic acid, catechin, quercetin, kaempferol and tannic acid) which are well-known compounds possessing antifungal activity. Therefore, it is possible that the antifungal properties of these plant extracts were partly due to these polyphenols or unknown active compounds which could not be analyzed in this study. Collectively, these results suggest that local varieties of both shallot and garlic possess strong antifungal properties.

Introduction

The genus *Phomopsis* (Sacc.) Bubak contains more than 800 species from a wide range of hosts known to be plant pathogenic fungi (Uecker, 1988). *Phomopsis* spp. cause cankers, diebacks, fruit rots, root rots, leaf spots, blights, decays and wilts on a wide range of plant hosts including economic plants such as *Amaranthus* sp., *Vaccinium vitis-idaea*, fruits of grapevines, strawberry, *Photinia serrulata*, *Oryza sativa* and para rubber trees (Udayanga et al., 2011). The para rubber tree (*Hevea brasiliensis* Muell. Arg., Euphorbiaceae) is a major economically important plant because its white sap-like extract, commonly known as latex, is the primary source of natural rubber (Venkatachalam et al., 2013). *Phomopsis* sp. is commonly found in newly fallen leaves and middle stage decaying leaves of the *H. brasiliensis* (Seephueak et al., 2010). Sophon et al. (2014) reported *Phomopsis* sp. in the leaves of infected young seedlings of *H. brasiliensis* and non-symptom exhibiting seedlings after cultivation by grafting para rubber tree in southern Thailand. In addition, they found that *Phomopsis* sp. could cause dieback in seedlings resulting in the retarded growth and death of trees both in the nursery and the field, as well as a reduction of latex in mature trees. Furthermore, these infections of *Phomopsis* sp. in the rubber seedlings could also spread and cause problems for rubber seedling producers. For this reason, chemical pesticides are used in agriculture for plant pest control with various undesirable and harmful effects to humans, leaving toxic residue in food and the environment (Aktar et al., 2009). The negative consequences especially include contaminating agricultural products such as fruits and vegetables leading to problems in agricultural exports (Dinham, 2003). Recently, many researchers have taken an interest in using

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medicinal plant extracts for controlling plant pests, as such extracts are biodegradable, barely leave residue in the soil, and are also less likely to cause harm to animals and humans (Zarins et al., 2009).

The inhibitory effect of plant extracts against plant pathogenic fungi has been studied (Aba Alkhail, 2005; Kongkaew and Phichai, 2010). Shallot (*Allium ascalonicum* Hort.), garlic (*Allium sativum* L.) and chili (*Capsicum frutescens* L.) are economically important crops in Thailand (Limnirankul and Gypmantasiri, 1998). These plants are reported to possess various biological activities including antifungal activity (Mahmoudabadi and Nasery, 2009; Garcia, 2011; Soumya and Nair, 2012). Ogbebor et al. (2007) found that garlic extract exhibited potential inhibition on the mycelial growth of *Colletotrichum gloeosporioides* which was isolated from leaves of the para rubber tree. Noengpa (2004) reported that water extracts of garlic and shallot showed inhibitory effects on *C. gloeosporioides* and *Fusarium* sp. spore growth. Kongkaew and Phichai (2010) also found that dried shallot powder, which was extracted using a maceration method in distilled (DI) water and 95% ethanol solvent, was effective at inhibiting the growth of *Trichoderma* spp. isolated from Yanagi mushroom. Soumya and Nair (2012) reported that the dried leaves and fruits of chillies extracted using a Soxhlet extractor in DI water solvent were capable of preventing fungal infection in groundnut seeds.

Native varieties of chili, shallot and garlic (Sisaket variety) are economic plants in Sisaket province, Thailand and are well-known national products (Office of Agricultural Economics, 2016). However, there are no known reports of these varieties and their efficacy against the pathogenic fungi, *Phomopsis* sp. isolated from the leaves of para rubber. Therefore, the aim of the study was to investigate the antifungal effects of chili, shallot (Sisaket variety) and garlic (Kaew variety) extracts against *Phomopsis* sp., which is a pathogenic fungus of the para rubber tree.

Materials and methods

Isolation of pathogenic fungi of para rubber tree

Infected leaves of *H. brasiliensis* (RRIM 600) were collected from rubber plantations (aged 2–7 yr) in Sisaket province, Thailand. Bits of 1 cm × 1 cm cut across lesions of the leaf were surfaced and sterilized by dipping in 0.1% sodium hypochloride for 1 min. The cut pieces were subsequently rinsed in five changes of sterilized DI water. Excess water was decanted by soaking with sterilized blotting paper. The cut pieces were then placed on potato dextrose agar (PDA) + 50 mg/L chloramphenicol in a glass Petri dish and incubated at 28 ± 2 °C for 3–5 d (Evueh and Ogbebor, 2008). Pure colonies of fungi were isolated using the hyphal tip isolation technique (Strobel et al., 1996), then further cultured in PDA media and incubated at 28 ± 2 °C for 7 d for mycelial formation in a Petri dish. Pure cultures were kept at 4 °C for further use.

Phomopsis sp. was identified on the basis of morphological characteristics by the Center for Genetic Engineering and Biotechnology (BIOTEC), Pathum Thani Thailand. First, evaluation was carried out of pure cultures colony in PDA media under a stereo microscope at 4.5× magnification. The mycelium formation which was picked from the pure colonies of fungi was cultured in PDA media and incubated for 1–2 wk. After incubation, fungal types were identified using the characteristics of conidia under a compound microscope at 40× magnification.

Plant materials and extracts preparation

Local varieties in Sisaket of chili, shallot and garlic were collected from a local home garden in Sisaket province, Thailand. These plants were grown under organic agriculture. Fresh cloves of shallot and garlic and fresh fruits of chili were washed with tap water and then placed on tissue paper to remove residual water. Each crude plant extract was prepared as described by Evueh and Ogbebor (2008). Briefly, parts of the plants used were blended in sterilized DI water (100 g:100 mL, weight:volume). This suspension was then filtered through several layers of gauze. The pooled crude extracts were centrifuged at 3500 × *g* and 4 °C for 5 min for further use. The supernatant was filtered using vacuum filtration with a pore size of 0.22 μm. All sterilized crude extracts were then diluted with PDA media to obtain final concentrations (20%, 40%, 60% or 80%). The final concentration of 2% captan (1% N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) was diluted in PDA media and then used as a standard group.

Identification and quantification of polyphenols in plant extracts

Polyphenols in the crude extracts of each plant were analyzed by the Central Laboratory Co., Ltd, Chiang Mai, Thailand. Polyphenols from these extracts were characterized by high performance liquid chromatography (HPLC) diode array detection mass spectrometry analysis. HPLC was performed using an Agilent 1100 series instrument (Agilent Technologies; Waldbroon, Germany), diode array detection, and a Purospher STAR RT-18 endcapped LiChroCART column (Merck KGaA; Darmstadt, Germany). The mass spectral characteristics of the polyphenols were compared to chemical standards. Quantification was performed by comparison of retention times; diode array spectra were matched against chemical standards.

Effect of plant extracts against mycelial growth of Phomopsis spp.

Six millimeters agar plugs of *Phomopsis* sp. from 7 d-old culture were taken using a cork borer and placed at the center of a Petri dish after solidification of diluted concentrations of plant extracts or 2% captan with PDA media (20 mL/plate). The experiment was conducted with three replications and repeated thrice. The observation of inhibition diameter (in millimeters) of mycelial growth was carried out for 7 d after incubation at 28 ± 2 °C. The percentage inhibition of mycelial growth was evaluated using the poisoned food technique, and calculated using the formula for percent inhibition = (C–T)/C × 100, where C and T are mycelial growth diameter of *Phomopsis* sp. on a negative control plate (NC; plate alone without the standard chemical and plant extracts) and treatment plate (plate with standard chemical or plate with plant extracts), respectively.

Statistical analysis

Statistical significance was determined using one-way analysis of variance with a *post hoc* Turkey's analysis (GraphPad Prism 5; San Diego, CA, USA) to determine differences between treatments and control groups. Values were considered statistically significant when $p < 0.05$. Data were presented as mean ± SD ($n = 3$) and were representative of at least three independent experiments.

Results and discussion

Isolation of Phomopsis spp.

Phomopsis sp. was isolated from five samples of infected leaves of para rubber trees from Sisaket province, Thailand. Seven fungal isolates were identified using morphological characteristics, namely *Phomopsis* sp. SSK1.1, *Phomopsis* sp. SSK1.2, *Phomopsis* sp. SSK3.1, *Phomopsis* sp. SSK4.1, *Phomopsis* sp. SSK5.1, *Phomopsis* sp. SSK5.2 and *Phomopsis* sp. SSK7.1. These isolates were named by the

sources and the orders of fungal collection. At present, the genus *Phomopsis* cannot be identified to the species level, either by using morphological characteristics alone or in conjunction with molecular techniques, because their nucleotide sequences are still recorded as *Phomopsis* sp. in the nucleotide database.

The characteristics of *Phomopsis* sp. colonies cultured on PDA media were white to brown hyphae (Fig. 1A–G). Fruiting bodies were pycnidia and black in color and were commonly distributed in the colony. Old pycnidia had ostiole-producing conidiophores which were long in shape, hyaline or bright, and were septate. Pycnidia with both alpha and beta types of conidia were identified (Fig. 1A–G). Beta conidia were single cells, hyaline or bright and filiform-shaped at the end (Fig. 1A–G). Alpha conidia were single cells, hyaline or bright, and ovoid-shaped (Fig. 1A–G). Likewise, Ko et al. (2011) suggested that the dominant characteristics of the genus *Phomopsis* were conidia of both alpha and beta types, whereas others produce only one type of conidia in culture media.

Phomopsis sp. is a pathogen which causes damage to economically important crops including the para rubber tree (Udayanga et al., 2011). The para rubber tree is an economically important plant species in southern Thailand and today it covers large areas in the eastern, northeastern, northern and central regions of Thailand (Saengruksawong et al., 2012). Infection of *Phomopsis* sp. on leaves of para rubber in southern Thailand has been reported (Seephueak et al., 2010; Sophon et al., 2014). The current study is the first regarding *Phomopsis* sp. infecting the leaves of para rubber trees in plantations in Sisaket province, northeastern Thailand. Therefore, the possibility of infection by *Phomopsis* sp. in para rubber trees is not only restricted to southern and northeastern Thailand, but may also be found in other areas of Thailand and throughout other countries that cultivate this plant.

Identification and quantification of polyphenols in plant extracts

The type and concentration of polyphenols in chili, shallot and garlic (Sisaket varieties) extracts are given in Table 1. Eriodictyol (0.37 mg/mL), kaempferol (0.66 mg/mL) and quercetin (35.91 mg/mL) were only found in shallot extract, whereas rutin (3.22 mg/mL) was only found in chili extract. However, catechin was found in both chili (8.50 mg/mL) and garlic (6.93 mg/mL) extracts. This study indicated that gallic acid (32.77 mg/mL), quercetin (35.91 mg/mL) and catechin (6.93 mg/mL) were the major components in chili, shallot and garlic extracts, respectively. This study also suggested that tannic acid is the dominant phenolic compound in chili (66.33 mg/mL), shallot (21.71 mg/mL) and garlic extracts (13.18 mg/mL).

Plant phenolics are secondary metabolites involved in the defense mechanisms of plants against fungal pathogens (Lattanzio

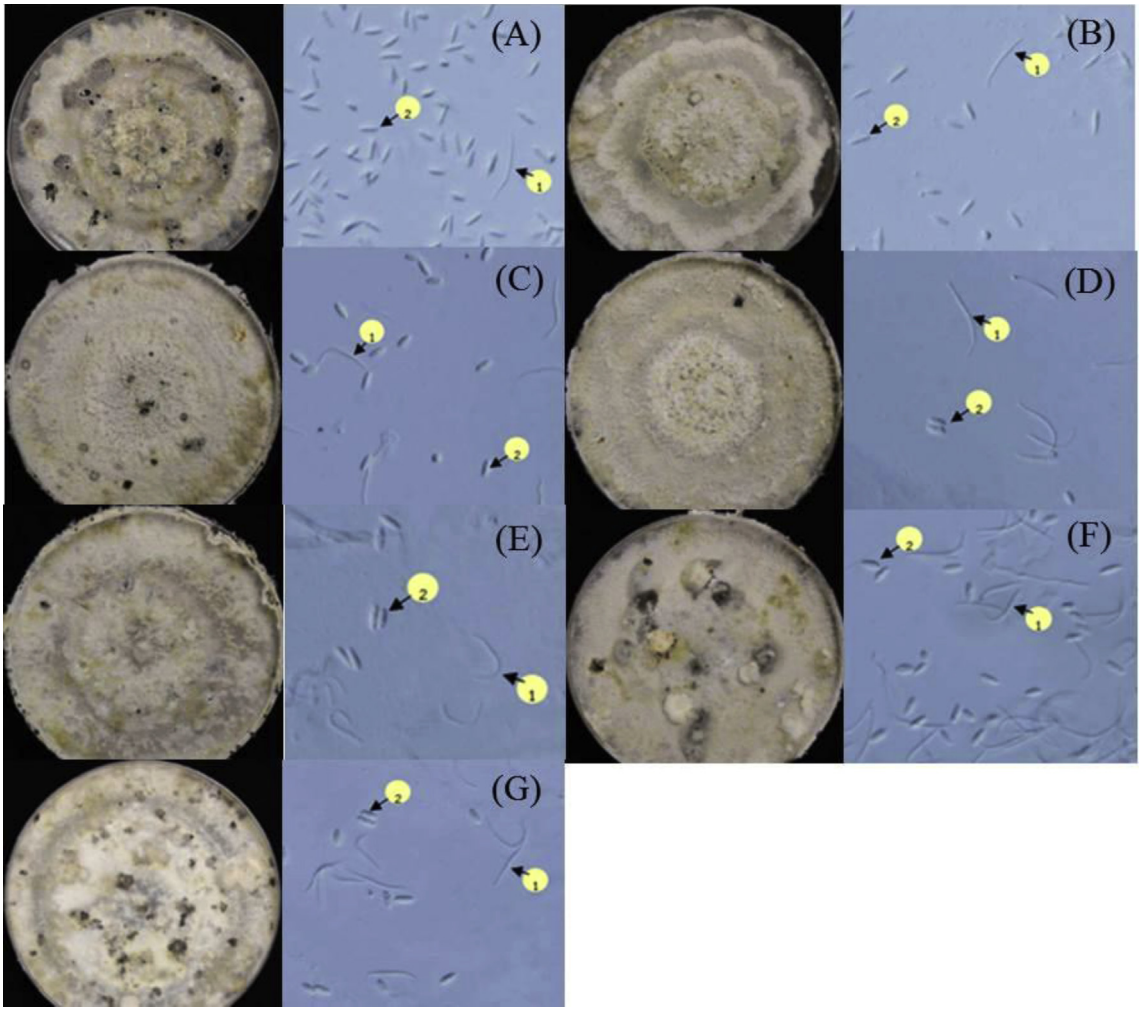

Fig. 1. Morphology of seven strains of *Phomopsis* spp.: (A) *Phomopsis* sp. SSK1.1; (B) *Phomopsis* sp. SSK1.2; (C) *Phomopsis* sp. SSK3.1; (D) *Phomopsis* sp. SSK4.1; (E) *Phomopsis* sp. SSK5.1; (F) *Phomopsis* sp. SSK5.2 (G) *Phomopsis* sp. SSK7.1, where left-hand side of figures show characteristics of the colonies which were observed under a microscope at 4.5× magnification and right-hand side of figures show types of conidia: 1) beta and 2) alpha, which were observed under a compound microscope at 40× magnification.

Table 1
Contents of polyphenols in extracts of chili, shallot and garlic (Sisaket varieties).

Phenolic compound	Plant extract (mg/mL)		
	Chili	Shallot	Garlic
Gallic acid	32.77	2.13	3.14
Eriodictyol	–	0.37	–
Apigenin	11.49	0.11	0.32
Isoquercetin	2.82	10.55	0.33
Kaempferol	–	0.66	–
Quercetin	–	35.91	–
Rutin	3.22	–	–
Catechin	8.50	–	6.93
Tannic acid	66.33	21.71	13.18

et al., 2006). From analyzing polyphenols in this study, chili, shallot or garlic extracts contained some polyphenols such as apigenin, gallic acid, quercetin, kaempferol, catechin or tannic acid. All aforementioned compounds have been shown to possess antifungal plant properties (Singh et al., 1988; Lattanzio et al., 2006; Alves et al., 2014). The analysis of polyphenols in chili, shallot and garlic extracts also indicated that other constituents could not be analyzed and identified in the current experimental system. Thus, the extracts may contain other unknown compounds.

Effect of plant extracts against mycelial growth of Phomopsis spp.

The poisoned food technique was conducted to determine whether plant extracts (from chili, shallot or garlic) inhibit the mycelial growth of *Phomopsis* spp. strains. The efficacy of these plant extracts was tested at various concentrations (20%, 40%, 60% or 80%) on the mycelial growth of *Phomopsis* spp. and the results were expressed as the percentage inhibition of mycelial growth (Table 2). Increasing concentrations of chili extract demonstrated a dependent increase in the level of percentage inhibition on growth of *Phomopsis* sp. SSK3.1 (7.65–41.97%), *Phomopsis* sp. SSK4.1 (3.83–35.24%) and *Phomopsis* sp. SSK5.2 (0.78–32.53%). Therefore, this study clearly revealed that chili extracts possessed antifungal activity against the mycelial growth of some isolated *Phomopsis* sp. such as *Phomopsis* sp. SSK3.1, *Phomopsis* sp. SSK4.1 and *Phomopsis* sp. SSK5.2. However, the levels of percentage inhibition of mycelial growth of all isolates of *Phomopsis* were significantly lower than the standard group (2% captan). The present study also showed that all concentrations of chili extract had no effect on inhibition (promotion) of the growth of *Phomopsis* sp. SSK1.1. Low concentrations of chili extracts had no effect on inhibition (promotion) of the growth of some isolates such as *Phomopsis* sp. SSK1.2, SSK5.1 and SSK7.1. Only the highest concentration (80%) had a clear effect on inhibition of the mycelial growth of these isolates. Lagrange et al. (2001) reported that rutin extracted from *Eucalyptus globulus* ssp. *bicostata* root exudates could stimulate *Pisolithus* hyphal growth at picomolar concentrations. This promotion by chili extract is notable in that it is caused by treatment with low concentrations. Therefore, this promotion by chili extracts may be due to the effect of low concentrations of rutin which is only contained in this plant when compared to shallot and garlic. In addition, the sensitivity of treated concentrations by chili extract may be dependent on the strain of *Phomopsis*.

Of the other plant extracts, shallot increased the levels of percentage inhibition of mycelial growth in a dose-dependent manner and these levels of percentage inhibition were also significantly less than captan (2%) in all isolates of *Phomopsis*. In addition, all concentrations of garlic extract significantly inhibited mycelial growth by more than 89% in all isolates of *Phomopsis*. Concentrations of garlic extract at 40%, 60% or 80% could totally inhibit (100%) myceial growth in *Phomopsis* sp. SSK1.1, *Phomopsis* sp. SSK3.1 and *Phomopsis* sp. SSK7.1, and showed higher inhibition than captan (2%). At the highest concentration (80%) of garlic extract, the total inhibition of mycelial growth (100%) of all isolates of *Phomopsis* was higher than those of the highest concentration (80%) of chili and shallot extracts including the positive control (2% captan) as shown in Fig. 2. Among the same concentrations of plants extracts, all concentrations of garlic extract had significantly stronger inhibition of growth than the shallot and chili extracts, respectively, whereas for *Phomopsis* sp. SSK7.1, there was no difference in the inhibition effect of chili or shallot extracts at 20% (Table 2). The lowest concentration (20%) of garlic extract had a higher percentage inhibition of mycelial growth than the chili and shallot extracts. This study clearly indicated that garlic extract (Sisaket variety) had a stronger inhibition of mycelial growth than the shallot and chili extracts (Sisaket variety), respectively. These results were in agreement with the study of five plant extracts against pathogenic plant fungi which revealed that the highest level of percentage inhibition of growth of fungal spores was observed in the case of garlic extract in water (100%) and this percentage inhibition was also higher than that of shallot extracts in water (Noengpa, 2004). Several studies also found that the extract of garlic in water resulted in 100% inhibition of mycelial growth of pathogens of para rubber, *C. gloeosporioides* (Ogbebor et al., 2007). Likewise, the highest mycelial growth inhibition (74.35%) of *C. gloeosporioides*, the causal agent of anthracnose of mango, was observed in the case of garlic extract in water at 70% concentration (Mukherjee et al., 2011). However, there are reports that dried garlic extract in 95% ethanol by maceration had no effect on inhibition of the growth of *C. gloeosporioides*, the causal agent of anthracnose of mango (Sutthisa et al., 2014). Therefore, the efficiency of garlic extract may be due to the methods and solvents used for extraction. Blending fresh plants with water was the process of plant extraction applied in this study. The extracts were directly applied to *Phomopsis* spp. which had been isolated from para rubber. This method using water as solvent for extraction could be applied by farmers, because it is easy and water is an inexpensive solvent.

This study clearly indicated that shallot extracts had higher levels of percentage inhibition of mycelial growth than chili extracts (Table 2). This stronger activity of shallot extracts than chili extracts may have resulted from the higher level of quercetin (35.91 mg/mL) which was only identified in the shallot extracts. Notably, the lowest concentrations (20%) of garlic extracts had a higher percentage inhibition of mycelial growth than the highest concentrations (80%) of chili and shallot extracts. This study also showed that garlic extracts had the lowest composition of polyphenols when compared to chili and shallot extracts. Therefore, it is possible that the effect of the garlic extracts against all isolated *Phomopsis* sp. may have been due to other polyphenols which could not be detected using the current experimental system. Another study reported that garlic and garlic extracts contained allicin substance, which is a physiologically active molecule with many potential health benefits (Lawson and Gardner, 2005). Allicin (diallyl thiosulfinate) is produced when tissue is damaged from the non-proteinogenic amino acid alliin (S-allyl cysteine sulfoxide) in a reaction which is catalyzed by the enzyme alliinase (Miron et al., 2002). Moreover, allicin has a strong antibacterial property by inhibiting pathogenic bacteria and pathogenic fungi (Ankri and Mirelman, 1999). In particular, allicin exhibits antifungal activity both *in vitro* and *in vivo* against many plant-pathogenic fungal species (Borlinghaus et al., 2014). Allicin is not only found in garlic, but is also in shallot (Mikaili et al., 2013). For extraction in the current study, cloves of fresh garlic and shallot were extracted by blending in water. Therefore, it might be assumed that the garlic and shallot extracts may have contained allicin leading to the high activity in the inhibition of mycelial growth of *Phomopsis* spp.

Table 2
Effect of concentrations of plant extracts on mycelial growth of *Phomopsis* spp. incubated at 28 ± 2 °C for 7 d.

Phomopsis sp. isolate	Concentration (%)	Percentage inhibition of mycelial growth		
		Chili extract	Shallot extract	Garlic extract
SSK1.1	NC	0.00 ± 0.00 ^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00 ^{a}
	20	−7.09 ± 1.19^{b,A}	21.65 ± 0.38^{b,B}	89.77 ± 0.63^{b,C}
	40	−6.30 ± 0.73^{b,A}	33.86 ± 0.61^{c,B}	100.00 ± 0.00^{c,C}
	60	−5.12 ± 0.72^{b,A}	40.93 ± 2.55^{d,B}	100.00 ± 0.00^{c,C}
	80	−2.76 ± 0.70^{c,A}	56.69 ± 0.64^{e,B}	100.00 ± 0.00^{c,C}
	Captan (2%)	91.33 ± 0.75^{d}	91.33 ± 0.75^{f}	91.33 ± 0.75^{b}
SSK1.2	NC	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	20	−2.47 ± 2.19^{ac,A}	5.29 ± 1.77^{b,B}	90.20 ± 0.18^{b,C}
	40	−5.32 ± 0.81^{bc,A}	31.01 ± 0.90^{c,B}	89.80 ± 0.69^{b,C}
	60	−1.66 ± 2.57^{ab,A}	33.85 ± 2.26^{c,B}	100.00 ± 0.00^{c,C}
	80	28.55 ± 2.14^{d,A}	71.01 ± 0.98^{d,B}	100.00 ± 0.00^{c,C}
	Captan (2%)	91.43 ± 0.16^{e}	91.43 ± 0.16^{e}	91.43 ± 0.16^{b}
SSK3.1	NC	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	20	7.65 ± 2.11^{b,A}	37.59 ± 0.57^{b,B}	90.14 ± 0.13^{b,C}
	40	9.85 ± 0.99^{bc,A}	63.86 ± 0.46^{c,B}	100.00 ± 0.00^{d,C}
	60	11.67 ± 1.19^{c,A}	74.81 ± 0.32^{d,B}	100.00 ± 0.00^{d,C}
	80	41.97 ± 0.58^{d,A}	88.69 ± 0.57^{e,B}	100.00 ± 0.00^{d,C}
	Captan (2%)	93.07 ± 0.59^{e}	93.07 ± 0.59^{f}	93.07 ± 0.59^{c}
SSK4.1	NC	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	20	3.83 ± 0.62^{b,A}	31.79 ± 1.40^{b,B}	90.80 ± 0.11^{b,C}
	40	4.59 ± 2.26^{b,A}	44.05 ± 1.92^{c,B}	90.80 ± 0.11^{b,C}
	60	8.80 ± 1.66^{c,A}	50.95 ± 1.18^{d,B}	91.18 ± 0.75^{b,C}
	80	35.24 ± 0.99^{d,A}	73.94 ± 0.93^{e,B}	100.00 ± 0.00^{c,C}
	Captan (2%)	90.80 ± 0.11^{e}	90.80 ± 0.11^{f}	90.80 ± 0.11^{b}
SSK5.1	NC	0.00 ± 0.00^{ab}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	20	−1.20 ± 2.05^{b,A}	26.87 ± 0.32^{b,B}	90.51 ± 0.13^{b,C}
	40	0.37 ± 2.48^{b,A}	41.89 ± 0.80^{c,B}	90.11 ± 0.83^{b,C}
	60	−0.40 ± 0.70^{b,A}	62.05 ± 0.52^{d,B}	90.51 ± 0.13^{b,C}
	80	28.06 ± 0.30^{c,A}	86.56 ± 0.61^{e,B}	100.00 ± 0.00^{c,C}
	Captan (2%)	90.51 ± 0.13^{d}	90.51 ± 0.13^{f}	90.51 ± 0.13^{b}
SSK5.2	NC	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	20	0.78 ± 1.36^{a,A}	35.31 ± 1.03^{b,B}	90.08 ± 0.70^{b,C}
	40	3.96 ± 0.64^{b,A}	48.40 ± 1.92^{c,B}	90.08 ± 0.70^{b,C}
	60	8.32 ± 1.96^{c,A}	54.76 ± 0.54^{d,B}	89.28 ± 0.13^{b,C}
	80	32.53 ± 1.44^{d,A}	87.31 ± 0.57^{e,B}	100.00 ± 0.00^{c,C}
	Captan (2%)	90.48 ± 0.11^{e}	90.48 ± 0.11^{f}	90.48 ± 0.11^{b}
SSK7.1	NC	0.00 ± 0.00^{ab}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	20	−0.01 ± 1.91^{ab,A}	0.35 ± 1.67^{a,A}	91.21 ± 0.10^{b,B}
	40	−2.57 ± 1.71^{a,A}	18.31 ± 2.68^{b,B}	100.00 ± 0.00^{d,C}
	60	1.82 ± 2.26^{bc,A}	34.79 ± 1.31^{c,B}	100.00 ± 0.00^{d,C}
	80	24.90 ± 1.03^{d,A}	68.86 ± 0.72^{d,B}	100.00 ± 0.00^{d,C}
	Captan (2%)	92.31 ± 0.08^{e}	92.31 ± 0.08^{e}	92.31 ± 0.08^{b}

NC = negative control. Values are shown as mean ± SD ($n = 3$) and are representative of three independent experiments with similar results. Different lowercase, superscript letters within the same column of each isolates are significantly different at $p < 0.05$. Different capital, superscript letters within the same row are significantly different at $p < 0.05$.

Some fungicides have been reported to inhibit the growth of fungi due to the influence of low pH (Schnürer and Magnusson, 2005). Fatty acids are known to possess antifungal activity and can cause an increase in membrane fluidity, which results in leakage of the intracellular components and cell death (Pohl et al., 2011). Kang et al., 2003 also found that acetic, oxalic, malic and citric acids adjusted to pH 5.2 (weak acid) can inhibit the growth of *C. gloeosporioides*, a phytopathogenic fungus. According to the current study, extracts from chili, shallot and garlic were weak acids with pH 5.34, 5.71 and 5.94, respectively. Notably, increasing the concentration of all plant extracts resulted in a higher percentage of inhibition of mycelial growth of *Phomopsis* sp. In addition, weak acids may have an effect on mycelium growth, by damaging the mycelium. It could be possible that the properties of plant extracts with weak acids and active compounds may have synergistic effects in the inhibition of the mycelial growth of all *Phomopsis* spp.

In conclusion, the current study demonstrated that extracts of local varieties in Sisaket of chili, shallot and garlic possessed antifungal activity against mycelial growth of *Phomopsis* spp. which had infected leaves of the para rubber tree. This study also clearly showed that among these plant extracts, the highest levels of percentage inhibition of mycelial growth occurred using garlic extract followed by shallot and chili extracts, respectively. Some concentrations of chili extract had no effect on the inhibition of the growth of *Phomopsis* spp. (*Phomopsis* sp. SSK1.1, *Phomopsis* sp. SSK1.2, *Phomopsis* sp. SSK5.1 and *Phomopsis* sp. SSK7.1). Characterization of these plant extract constituents in the present study revealed that all plant extracts contained some polyphenols (apigenin, gallic acid, catechin, quercetin, kaempferol and tannic acid) which are well-known compounds that possess antifungal activity. This study also showed that garlic extracts had the lowest composition of polyphenols when compared to chili and shallot extracts. Therefore, it is assumed that the antifungal properties of these plant extracts may be principally due to some polyphenols contained in these plants and also to unknown compounds which could not be analyzed and identified in this study. Taken together, local varieties in Sisaket of shallot and garlic possessed a strong antifungal property and both plants may be further developed for application in agricultural plant protection.

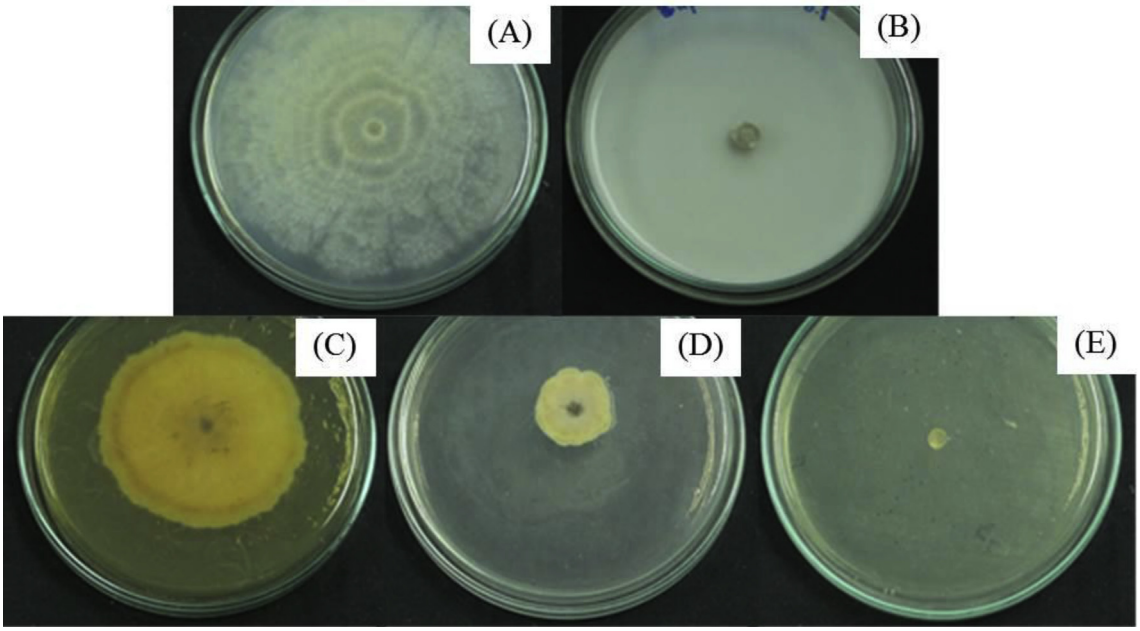

Fig. 2. Inhibition of mycelial growth after incubation at 28 ± 2 °C for 7 d of *Phomopsis* sp. SSK4.1 by: (A) negative control; (B) positive control (2% captan); (C) chili extract (80%); (D) shallot extract (80%); (E) garlic extract (80%).

Conflict of interest

No conflicts of interest influenced this research.

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