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FUNGICIDAL ACTIVITY OF GARLIC (*Allium sativum*) BULBS EXTRACTS AGAINST PLANTS PATHOGENIC FUNGI

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ABSTRACT

Fungicidal activity of garlic* (Allium sativum) *Bulbs extracts against plants pathogenic fungi. Garlic *(Allium sativum* Linn.) has been known containing organosulphur compounds. These compounds are convinced to possess antibacterial, antifungal, and anticancer activities. The aim of this study was to determine fungicidal activity of garlic bulb extracts against some plants phatogenic fungi. The paper disc agar difussion technique was used to determine fungicidal activity of garlic bulbs extracts. The results showed that in general garlic bulbs extracts provides fungicidal activities. Calculated EC_{50} values indicated that ethyl acetate garlic bulbs extract was most active against *Colletotrichum capsici, Fusarium oxysporum* f. sp.*capsici*, and *Sclerotium rolfsii* by EC_{50} values of 48,6; 50,3; and 51.3% respectively. Meanwhile methanolic garlic bulbs extract was the most active against *S. rolfsii* with EC_{50} values of 24,3%.

Key words: *Allium sativum*, *Colletotrichum capsici, Fusarium oxysporum* f. sp. *capsici*, phyto-fungicide, plants phatogenic fungi, *Sclerotium rolfsii*

INTRODUCTION

Chili (*Capsicum annuum* L.) is one of vegetable commodity cultivated in Indonesia due to its high economic value (Kardinan, 2002). The Directorate General of Horticulture Indonesia mentioned that in 2017, the productivity of *C. annuum* reached 8.4 tons per Ha, this is not much higher than the productivity of chili in 2013 which reached 8.16 tons per Ha (Direktorat Jenderal Hortikultura, 2013; 2017). The problem is caused by pests and diseases (Bosland & Votana, 2012). The common pests attacking on the chili plants are mostly caused by *Colletotrichum capsici* (Al-Askar, 2012; Alwathnani & Perveen, 2012), *Fusarium oxysporum* f. sp. *capsici* (Shafique *et al.,* 2015), and *Sclerotium rolfsii* (Abdel-Fattah *et al.,* 2011). Anthracnose plant disease on chili mostly caused by the fungus *C. capsici*, while wilt and necrosis disease on chili plant usually are caused by the fungus *F. oxysporum* f. sp. *capsici*. Anthracnose disease in chili causes decreased production of almost 60% (Duriat *et al.,* 2007; Setiyawati *et al.,* 2007). According to Semangun (2007), the early symptoms caused by the fungus *F. oxysporum* is the leaf become pale mainly on the top of leaf, followed duck down of the stem, and eventually the plants becomes whiter. Abdullah *et al.* (2015) mentioned that *S. rolfsii* damping-off disease caused loss of almost 80% of seedbed of chili plants, and when an environmental conditions suitable for the development of this disease, the loss could reach 100%.

Due to high economic values of chili, the farmers tend to use synthetic or commercial pesticides in efforts to control of pests and diseases such as phenylamide and metalaxyl (Basuki, 1988; Bi, 2018). The excessive use of synthetic fungicides and uncontrolled can cause health problems, environmental pollution, kill non-target organisms and resistant to fungicides (Prapagdee *et al.,* 2008; Untung, 1996). To avoid this situation, the use of biofungicide is very potential to develop due to ecofriendly, environmentally safe, and does not cause phytotoxicity (Sigee, 1993; Boonsang *et al.,* 2014). Some extracts from the plant are reported effectively used as biofungicide, such as essential oils of *Rutaceae*, *Eucalyptus globulus* and *Thymus vulgaris* are known to effectively inhibit the growth of fungi *Pythium* spp., *Rhizoctonia solani* (Katooli *et al.,* 2011) and *Colletotrichum gloeosporioides* (Hur *et al.,* 2000). Nosrati *et al.* (2011) reported that the essential oil of

Mentha spicata can be used to control the fungus *F. oxysporum* f. sp. *radicis-cucumerinum* on cucumber. Pawar & Thaker (2007) also reported that the essential oil of citronella, clove, cinnamon bark and leaves have fungicidal activity against *Alternaria porri* and *Fusarium oxysporum* f. sp. *Cicer*. *Rhizoctonia solani* (Katooli *et al.,* 2011) and *Colletotrichum gloeosporioides* (Hur *et al.,* 2000).

Aqueous garlic extract is reported to have activity against *Botrytis cinerea*, *Penicillum expansum*, and *Neofabraea alba*. Some garlic extracts have been also reported to have antifungal effect on the fungus *Fusarium oxysporum* f. sp. *phaseoli* and *Penicillium digitatum* (Obagwu & Korsten, 2003). Antimicrobial activity of garlic is apparently due to the high content of organosulfur compounds (Khadri *et al.,* 2011; Ankri & Mirelman, 1999) such as allycin or diallyl thiosulfinate (Block, 2010), allyl ethyl trisulfides, dithiins, ajoen, diallyl sulfides, diallyl disulfides, allyl propyl disulfides, diallyl trisulfides, and diallyl tetrasulfides (Amagase, 2006; Chekki *et al.,* 2016). The purpose of this study was to determine the fungicidal activity of some garlic extracts on plant pathogenic fungi such as *C. capsici*, *F. capsici*, and *S. rolfsii*.

MATERIALS AND METHODS

Research Site. The research was conducted at the Laboratory of Plant Diseases, Department of Plant Diseases, Faculty of Agriculture, University of Syiah Kuala from January 2018 to June 2018.

Preparation of Simplicial Garlic Bulb. Garlic bulbs used in this study was obtained from the traditional market of Lambaro, Aceh Besar District. Criteria samples taken are garlic bulbs with cloves of ± 2 cm size. Garlic bulb were hand-cut and then exhaustively dried at room temperature (25–29°C) for 4 weeks to produce a simplicial of garlic bulb. This simplicia was then used for the preparation of extracts of garlic bulbs extracts.

Preparation of Garlic Bulb Extracts. Preparation of garlic bulb extracts were performed according to the method developed by Anief (2010) with slight modification. A 500 g of simplicial garlic bulb were macerated with 3 L of *n*-hexane at room temperature for 7 days with occasionally shake, and the macerate of *n*-hexane than filtered. The residue was macerated again with 2 L of *n*-hexane for next 3 days and filtered. The macerates were than combined and evaporated *in vacuo* to afford a crude extract of *n*-hexane. Using the same procedure, the total garlic residue of *n*-hexane was extracted respectively with cyclohexane, ethyl acetate, and methanol to obtain the extracts of cyclohexane, ethyl acetate, and methanol.

Phytochemicals Screening of Garlic Extracts. The methods described by Harborne (1980) were used for the phytochemicals analysis. The phytochemicals tests of garlic extracts in this study included alkaloids, flavonoids, saponins, and tannins.

Fungicidal Activity. The antifungal activity of garlic extracts was conducted by a disc diffusion (Kirby-Bauer) method. Potato dextrose agar (PDA) was poured into sterile petri dishes and allowed to solidify. The isolates of *C. capsici*, *F. oxysporum*, and *S. rolfsii* in separate of sterile petri dishes were spread all over the surface of solidified PDA using a sterile cotton bud. The paper disc with a diameter of approximately 6 mm was placed on the surface of the agar medium. Each disc was filled with 20ml of garlic extracts with the concentration of 10, 25, 50, 75, and 100%. In this assay, a paper disc of nystatin was used as a positive control. The plates were incubated at 37°C for 48 hours, and the diameter of inhibition zones surrounding the agar disc was measured in millimeter using a ruler (WHO, 2009).

The EC_{50} values. The EC_{50} values were calculated with sigmoidal curve by using the OriginPro 7.5 software using Boltzmann sigmoidal curve.

RESULTS AND DISCUSSION

Phytochemical Screening. The phytochemicals screening of garlic extracts is presented in Table 1. It shows the results of the preliminary phytochemicals analysis. The results showed that alkaloids were present in all of garlic extracts used in this study. These results are consistent with studies that have been conducted by Safithri (2004), Rustama (2005), Sovia *et al.,* (2011), and Divya *et al.,* (2017) which stated that an extract of *n*-hexane, ethyl acetate, and methanol garlic contains alkaloids. Meriga *et al.,* (2012), also mentions that the *n*-hexane extract of garlic bulbs contain alkaloids. According to Sadikin (2002), garlic is a *Liliaceae* plant and rich of alkaloid content. Alkaloids are semi-polar compounds which soluble in semi-polar and polar solvents (Harborne, 1987), and non-polar solvent (Baht *et al.,* 2006).

Table 1 also reveals that the ethyl acetate and methanolic garlic extracts containing the metabolite of

saponins. These results are consistent with studies that have been conducted by Sovia *et al.,* (2011) and Lanzotti *et al.* (2012) which mentioned that ethyl acetate and methanol garlic extracts are positive containing of saponins. Shah & Seth (2010), also stated that garlic contains metabolites alkaloids and saponins. According to Gholkar *et al.* (2013), a compound that plays a role in the antioxidant activity of garlic are phenolic compounds, steroids, alkaloids and saponins.

Fungicidal Activity. The fungicidal activity of garlic extracts (*n*-hexane, cyclohexane, ethyl acetate, and methanol garlic extracts) against plant pathogenic fungi *C. capsici*, *F. oxysporum*, and *S. rolfsii* obtained is presented in Table 2. The result showed that the fungicidal activities of garlic extracts were appeared at concentration above of 20%. Table 2 shows that the diameter inhibition zone of *n*-hexane garlic extract (HGE) on plant pathogenic fungi ranged between 14–24 mm, with the highest inhibition on *C. capsici* and *S. rolfsii*. Meanwhile, the highest fungicidal activity of cyclohexane garlic extract (CGE) against plant pathogenic fungi was on *S. rolfsii* at concentration of 50%. The results also mentioned that ethyl acetate garlic extract (EAGE) has a strongest activity against all plant pathogenic fungi at all concentrations tested with ranged of diameter inhibition zone between 27–46 mm.

HGE (hexane garlic extract); CGE (cyclohexane garlic extract); EAGE (ethyl acetate garlic extract); and MGE (methanolic garlic extract); (C^+) control positive; *C. capsici*); *F. oxysporum* and *S. rolfsii*). The diameters inhibition zone were determined in triplicate. Methanol garlic extract (MGE) has higher fungicidal activity against *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp.*capsici* with a diameter of inhibition zone ranged from 10–30 mm (Table 2).

Interestingly, the results presented that the higher concentration of garlic extracts have lower fungicidal activity. This is presumably due to lower solubility with the result that the distribution of active metabolite from garlic extract to the cell membrane of fungi is limited so that the antifungal activity becomes low.

According to Morales *et al.* (2003) the antifungal activity of plant or material extract can be determined by diameters of inhibition zone (Table 3).

The antifungal activity of garlic extracts (HGE, CGE, EAGE, and MGE) are expected due to organosulfur compounds and active metabolite such as alkaloids and saponins contained in garlic extracts. The phytochemicals screening results presented in Table 1 indicate that all garlic extracts positively contain alkaloid. Aniszewki (2007) and Amagase *et al.* (2001) reported that the mechanisms of alkaloids on fungi with inhibiting the process of cell respiration. Tariq *et al.* (1988), stated that allycin (diallyl-dithiosulfinate) is the class of alkaloids contained in garlic very important role in the activity of garlic. Allycin is organosulfur compounds that believed have antifungal activity (Lawson *et al.,* 1991).

The results also show that ethyl acetate and methanol garlic extracts have stronger fungicidal activity against all plant pathogenic fungi compared with *n*-hexane and cyclohexane garlic extracts. These activities were due to contains secondary metabolite of saponins. Saponins, are non-organosulfur compound which have hydrophilic glycoside group (Tariq *et al.,* 1988). This compound is believed to have properties as antibacterial, antimicrobial, and antiinflamantori (Harmatha, 2000). According to Cowan (1999) and Nuria (2009), saponins are polar compounds that can attract water molecules and dissolve of lipids. Thereby these compounds can destabilizing the membrane of fungi cells by decreased of surface tension of the cell membrane in the results that lysis of the cell, and eventually caused death cells. Saponins that contained in garlic is saponins in the group of erubocide-B. This group of saponins is plays very important role in antifungal activity of garlic (Tariq *et al.,* 1988), acts as antifungal activity against several plant pathogenic fungi (Morrissey & Osburn, 1999) antibacterial and antifungal activity (Turk, 2006). According to Obagwu & Korsten (2003), the

Table 1. Phytochemicals profile of garlic extracts (*Allium sativum*)

Secondary metabolites	Phytochemical constituents			
	HGE	CGE	EAGE	MGE
Alkaloids	+	+	+	+
Flavonoids	-	-	-	-
Saponins	-	-	+	+
Tannins	-	-	-	-

HGE (hexane garlic extract); CGE (cyclohexane garlic extract); EAGE (ethyl acetate garlic extract); and MGE (methanolic garlic extract); (+) present; (-) absent.

mechanism action of saponins is by forming a complex reaction with the cell membrane sterols in fungi. These complex reactions caused the porosity of fungal cell membrane and consequently fungal cell membrane integrity over time damage. In this study, we used nystatin as a positive control. Nystatin (also called micostatin), is an antifungal from the class of polyene (Macesic & Wingard, 2018). The mechanism action of nystatin as antifungal is by destroying the fungal cell wall to form a channel, so that the cells lose electrolyte or ion channels such as K^+ ions. These conditions causes the gradient proton inside the cell is disturbing, and eventually causes death cell (Bhanderi *et al.,* 2009). Several researchers reported that besides sterols, ergosterols is also as main target of nystatin as antifungal so that this compound is often used as a positive control for antifungal (Ridawati *et al.,* 2011).

Table 2. The fungicidal activity of garlic extracts against plant pathogenic fungi

Extract	Concentration (%)	Diameters of inhibition zone (mm)		
		Plant pathogenic fungi		
		C. capsici	*F. oxysporum*	*S. rolfsii*
HGE	C^+	24.0	23.0	9.0
	10	0	0	0
	25	20.0	19.5	15.5
	50	24.0	20.5	24.0
	75	27.0	19.6	15.3
	100	19.0	14.7	15.0
CGE	C^+	24.0	23.0	9.0
	10	0	0	0
	25	32.0	19.3	21.5
	50	30.5	23.0	38.0
	75	27.0	27.0	21.0
	100	28.0	28.5	20.0
EAGE	C^+	24.0	23.0	9.0
	10	0	0	0
	25	27.0	20.7	22.2
	50	39.0	23.3	26.5
	75	46.0	27.6	44.3
	100	42.0	27.0	33.5
MGE	C^+	24.0	23.0	9.0
	10	0	0	0
	25	15.0	13.0	10.0
	50	27.5	19.0	30.2
	75	20.7	19.0	21.0
	100	21.6	30.3	23.0

HGE (hexane garlic extract); CGE (cyclohexane garlic extract); EAGE (ethyl acetate garlic extract); MGE (methanolic garlic extract); (C^+) positive control; The diameters of inhibition zone were determined in triplicate.

Table 3. Determination of antifungal activity by diameters of inhibition zone according to Morales *et al.* (2003)

Diameters of inhibition zone (mm)	Antifungal activity
< 6	No active
6-10	Less active
10-20	Active
>20	Strong active

In addition, table shows that nystatin has lowest activity against *S. rolfsii* with the averages of diameters inhibition zone is 9 mm. Fichtner (2005), states that the fungus *S. rolfsii* has the ability to survive and thrive in a variety of environmental conditions. Haas & Defago (2005) also stated that the ability of *S. rolfsii* against environmental conditions due to this fungi has higher virulence (degree of pathogenicity) compared with other plant pathogenic fungi.

Due to higher fungicidal activity of ethyl acetate garlic extract (EAGE) compared with other garlic extracts, we isolated the EAGE with column chromatography using chloroform: ethanol: water with a ratio of 6: 4: 1 (v/v) as eluent system. The results produced two dominant isolates of EAGE namely isolates A and B, with the Rf value of each isolate are 0.8 and 0.6 respectively. To each isolate was then determined the fungicidal activity against plant pathogenic fungi such as *C. capsici*, *F. oxysporum*, and *S. rolfsii*. The fungicidal activity of the isolates is presented in Table 4.

Table 4 shows that the two isolates of the ethyl acetate extract of garlic did not show any fungicidal activity against *C. capsici*, *F. oxysporum*, and *S. rolfsii*. These results indicated that the fungicidal activity of EAGE acts synergy between organosulfur compounds and secondary metabolites of alkaloids and saponins in inhibiting of plant pathogenic fungi.

EC_{50} Value. Half maximal effective concentration (EC_{50}) refers to the concentration of a drug, antibody or toxicant that induces a response halfway between the baseline and the maximum after a specified exposure time. The EC_{50} values of garlic extracts on plant pathogenic fungi obtained by sigmoidal curve at position x at y 50 using software OriginPro 7.5 software are listed in Table 5 and the sigmoidal curves are presented in Figure 1.

Table 5 shows that overall EAGE have a lower EC_{50} values compared with HGE, CGE, and MGE. Meanwhile, MGE have a lowest EC_{50} values on *S. rolfsii* with the EC_{50} values is 24.3%, this result indicated that EAGE have a higher activity against all plant pathogenic fungi. Additionally, MGE showed strongest fungicidal activity against *S. rolfsii*. Saxena *et al.* (2013) mentioned that the extract with lower EC_{50} values indicates a higher activity.

Table 4. The fungicidal activity of isolates A and B against plant pathogenic fungi

Isolate	Diameters of inhibition zone (mm)		
	Plant pathogenic fungi		
	C.capsici	*F.oxysporum*	*S.rolfsii*
Nystatin (C^+)	24.0	23.0	9.0
EAGE (100%)	42.0	27.0	33.5
Isolate A	0	0	0
Isolate B	0	0	0

EAGE (ethyl acetate garlic extract); (C^+) positive control. The diameters of inhibition zone were determined in triplicate.

Table 5. The EC50 values of garlic extracts against plant pathogenic fungi using OriginPro 7.5 software

Extracts	EC_{50} values (%)		
	C. capsici	*F. oxysporum*	*S. rolfsii*
HGE	75.4	92.2	79.9
CGE	50.5	55.6	73.7
EAGE	48.6	50.3	51.3
MGE	75.6	96.0	24.3

EAGE (ethyl acetate garlic extract); (C^+) control positive. The diameters inhibition zone were determined in triplicate.

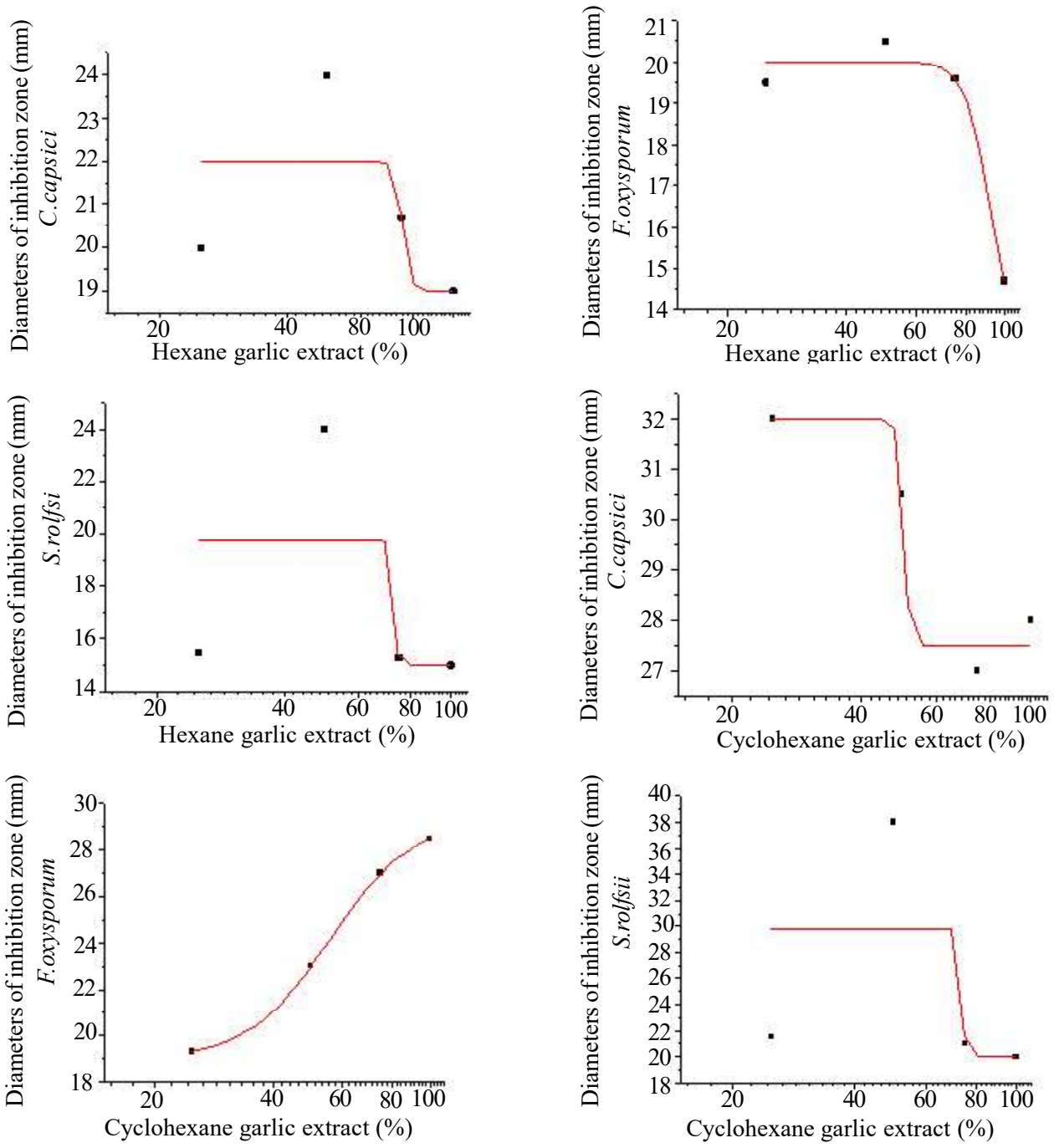

Figure 1. The sigmoidal curve EC_{50} of garlic extracts on several plant pathogenic fungi. The EC_{50} values were fitted based on sigmoidal Boltzmann curves-fitting using OriginPro 7.5 software

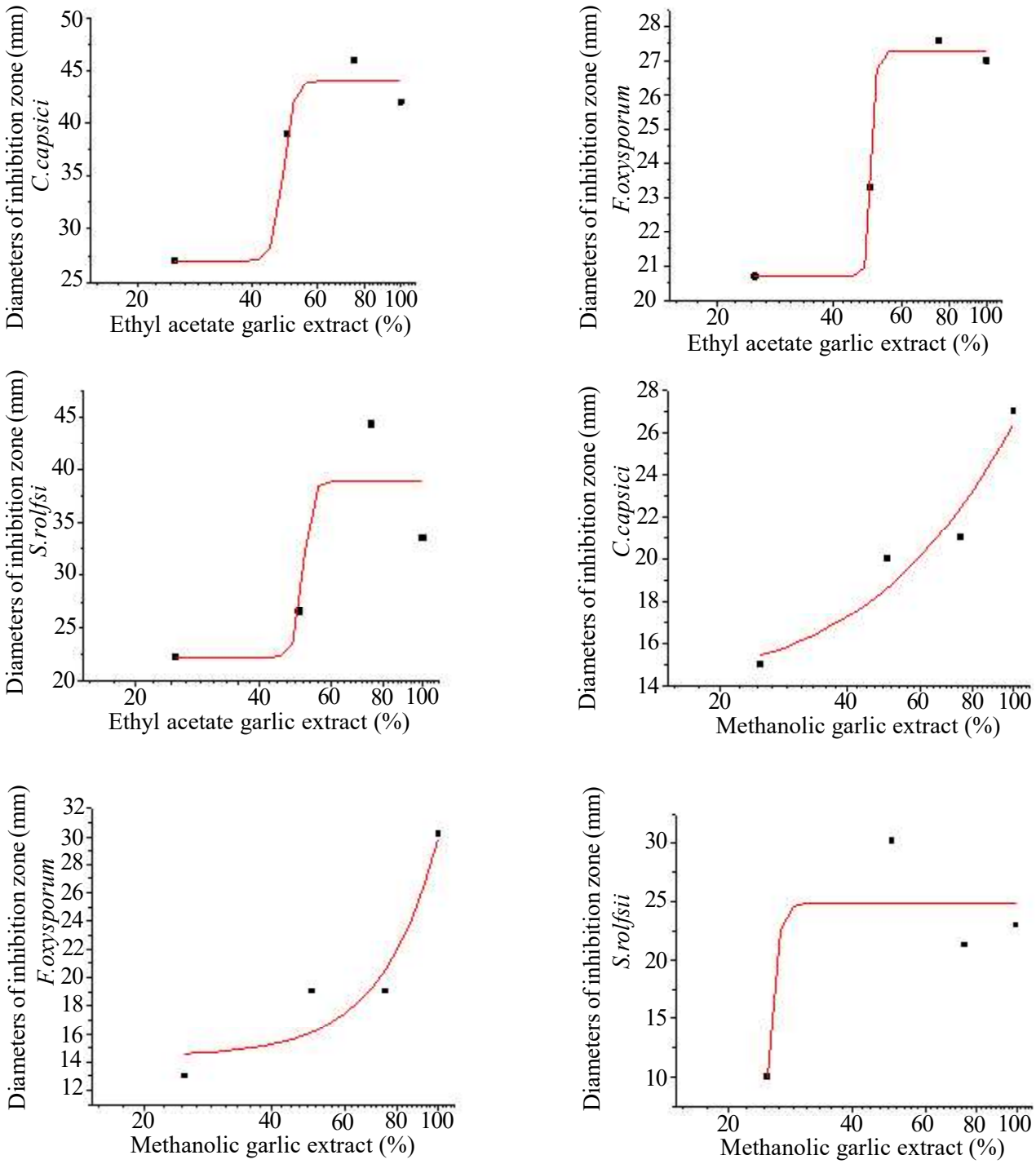

Figure 1. The sigmoidal curve EC_{50} of garlic extracts on several plant pathogenic fungi. The EC_{50} values were fitted based on sigmoidal Boltzmann curves-fitting using OriginPro 7.5 software (continued).

CONCLUSION

Phytochemical screening test showed that the garlic extracts of *n*-hexane, cyclohexane, ethyl acetate, and methanol positively containing alkaloids, while ethyl acetate, and methanol garlic extract also contain saponins.

The ethyl acetate extract of garlic has the highest activity against fungi *C. capsici*, *F. oxysporum* f. sp. *capsici*, and *S. rolfsii*, while the methanol extract of garlic has the highest activity against fungi *S. rolfsii*.

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