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The abilities of spent mushroom substrate to suppress basal rot disease (*Fusarium oxysporum* f.sp *cepae*) in shallot

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Abstract

Basal rot disease caused by *Fusarium oxysporum* f.sp *cepae* is an important disease in shallot. One of control measure safe to the environment is the use of organic matters including spent mushroom substrate (SMS). The aims of this research were to examine the potential and application method of spent substrate of oyster mushroom (*Pleurotus ostreatus*), straw mushroom (*Volvariella volvaceae*) and shiitake (*Lentinula edodes*) to control basal rot disease caused by *F. oxysporum* f.sp *cepae* in shallot. In this study three kinds of SMS were applied in planting site and or drenching their water extract every two weeks. The results showed that the SMS of oyster mushroom, straw mushroom and shiitake reduced the intensity of basal rot disease in shallot by 44-76,8%. The treatment that showed highest disease reduction and shallot growth was the application of *V. volvaceae* SMS in the planting site and drenching its water extract every two weeks.

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Introduction

Shallot is one of important horticultural crops in Indonesia. Problems that are often faced during shallot cultivation include the occurrence of plant diseases. One of important shallot diseases in Indonesia is basal rot disease, caused by *Fusarium oxysporum* f.sp. *cepae* (Harz.) Snyd.& Hans. The shoot of infected plants become yellowing and twisted and easily uprooted as the roots are disrupted and even rotted (Udiarto *et al*., 2005). In Indonesia, this disease can result in yield lost up to 50% (Wiyatiningsih, 2007).

The disease control is still emphasized on the use of pesticides. However, the use of pesticides does not always result in satisfactory results, but it can even cause adverse environmental impacts. Therefore, environmentally safe control measures are needed to be developed, such as the use of organic matters. One of organic matters potential for plant diseases control is spent mushroom substrate (SMS).

Substrates after mushroom cultivation still contain sufficient nutrients and hence can be used as organic amendment for improving soil structure and nutrients (Maher *et al*., 2000; Ahlawat and Sagar, 2007; Ahlawat *et al*., 2011; Uzun, 2014; Hanafi *et al*, 2018). In addition, it is also potential for controlling plant diseases. The abilities of Spent Mushroom Substrate (SMS) to control soil borne disease have been reported (ie. Romaine and Holcomb, 2000; El-Fallal and Mousa, 2008; Ahlawat *et al*., 2011; Zeeshan *et al*., 2016). The mechanisms of SMS in controlling plant diseases include the microbial activities antagonistic to the pathogens (Ahlawat and Sagar, 2007; Goonani *et al*., 2011; Adedeji and Modupe, 2016). The left over substrates after mushroom harvesting can also contain antimicrobial substances produced during the development of the mushrooms (Kwak *et al*., 2007; Kang *et al*, 2017) or produced by the microbes in the spent substrate (Cronin *et al*., 1996). The mushroom commonly cultivated in Indonesia is oyster mushroom (*Pleurotus ostreatus*), straw mushroom (*Volvariella volvaceae*) and shiitake (*Lentinula edodes*). The main components in media for cultivating *P. Ostreatus* and *L. edodes* used in this study were sawdust and rice bran, while for *V. Volvaceae* were composted paddy straw and cotton waste.The SMS can be applied as solid substrate or as water extract (Istifadah and Sianipar, 2015; Zeeshan *et al*., 2016). This aims of study were to examine the potentials and application method of spent substrates of *P. ostreatus, V. volvaceae* and *L. edodes* to control bottom rot disease in shallot.

Material and methods

The spent mushroom substrate used

Spent mushroom substrate (SMS) used in the experiments were spent substrate of oyster mushroom (*P. ostreatus*), shiitake (*L. edodes*) and straw mushroom (*V. volvaceae*). The spent substrate of *Pleurotus* sp., *L. edodes* were obtained from mushrooms farm in Lembang, West Java, Indonesia, while *V. volvaceae* spent substrate was obtained from Subang, West Java, Indonesia. The spent mushrooms used were the spent substrate that had been weathered for about 5 months. The C/N ratio of the substrates of *P. ostreatus*, *L. edodes,* and *V. volvaceae* was 33.98, 17.39 and 12.36 respectively.

The treatments and application of SMS

The experiment was arranged in Randomized block design with 12 treatments and three replications. The treatments were untreated plants (control), fungicide (with active ingredient Difenoconazole), farmer's practice (fungicide and synthetic fertilizer), three kinds of SMS applied as solid substrate or their water extract solely or in combination. The planting medium used was pasteurized soil (Andosol type) placed in a plastic container (60 x 40 cm x 10 cm). Each container was planted with 15 shallot seed bulbs (Bima Brebes variety) with plant spacing of 10 x 15 cm. To find out the effects of SMS on shallot growth, in almost treatments, except in the treatment as farmer's practice, the soil was not mixed with other organic matters or the synthetic fertilizers.

The water extract of SMS was prepared by soaking the SMS in water (20%, v/v) for one week in a close lid container. The solid substrate was applied in planting hole (20 g per plant), while the water extract was applied by drenching the plant (50 ml per plant) every two weeks.The drenching was stopped two weeks before the last observation. In the fungicide treatment and famer's practice treatment, the fungicide (active ingredient: difenoconazole) was applied by spraying the shallot plants every two weeks.

The pathogen inoculum preparation and inoculation

The pathogen *F. oxysporum* f.sp. *cepae,* was mass cultured in rice medium. The medium was prepared by soaking the rice overnight then it was drained and sterilized with autoclave at 121^{o}C for 30 minutes. The medium was inoculated with plug of the pathogen culture (7 days old culture on Potato Dextrose Agar) as much as 4 pieces (0.8 cm in diameter) per 100 g medium, then the culture was incubated for two weeks. The pathogen mass culture was inoculated at the time of planting,10 g per plant.

The variables observed

The disease intensity was observed every two weeks until the plants on the control are infected up to> 80%. The disease intensity was calculated based on the formulae: Disease Intensity (%) = ((Σ(n.v)) / (N.V))100% in which n : number of leaves in each category, v: disease index or score, N: numbers of leaves observed, and V: the highest score determined. The disease scoring or disease index used was: 0: no disease symptom; 1: the symptom was 0 <x ≤ 20% of the leaf area; 2: the symptom was 20%<x ≤ 40% of the leaf area; 3: the symptom was 40%<x ≤ 60% of the leaf area; 4: the symptom was 60%<x ≤ 80% of the leaf area; 5: the symptom was 80 % <x ≤ 100% of the leaf area (Juwanda *et al.*, 2016). The disease intensity data were used to calculate Area Under Disease Progress Curve (AUDPC) (Madden *et al.*, 2007), which is reflected the development of disease during the experiment. Growth variables observed were plant height, number of leaves, fresh weight of shoot and underground part as well as air-dried weigh of the shallot bulbs. At the end of experiments, microbial population in shallot rhizosphere was observed by serial dilution and total planting count methods (Dhingra and Sinclair, 1995).

Results

Effect of the SMS on basal rot disease in shallot

The results showed that application of spent substrate of *P. ostreatus*, *L. edodes* and *V. volvaceae* reduced intensity of basal rot disease in shallot. However, the disease suppression was started to appear four weeks after planting. Up to second weeks after planting the disease intensities in all treatment were not significantly different (Table 1).

Table 1. Intensity of basal rot disease on shallot on various treatments.

Treatments	Disease Intensity (%)			
	2 WAT	4 WAT	6 WAT	8 WAT
A. Oyster mushroom SMS	36.7 a	37.3 bc	35.8 a	29.7 a
B. Straw mushroom SMS	26.0 a	24.0 ab	23.1 a	18.3 a
C. Shiitake mushroom SMS	33.0 a	23.7 ab	23.9 a	20.0 a
D. Oyster mushroom SMS + drenching its water extract every 2 weeks	13.7 a	19.3 a	21.1 a	18.7 a
E. Straw mushroom SMS + drenching its water extract every 2 weeks	10.3 a	18.3 a	14.3 a	13.7 a
F. Shiitake SMS + drenching its water extract every 2 weeks	12.3 a	21.0 a	21 a	14.7 a
G. Drenching water extract of oyster mushroom every 2 weeks	24.7 a	18.0 a	20.7 a	19.0 a
H. Drenching water extract of shiitake mushroom every 2 weeks	18.7 a	23.3 a	23.9 a	17.3 a
I. Drenching water extract of oyster mushroom every 2 weeks	20.0 a	28.0 ab	21.4 a	20.7 a
J. Farmerpractice (fertilizers and pesticides)	8.3 a	17.0 a	25.1 a	36.0 ab
K. Fungicide (Difenoconazole)	20.7 a	37.7 bc	43.9 b	45.3 b
L. Check	36.0 a	48.3 c	83.3 c	85.0 c

Note: The average value in the columns followed by the same letter is not significantly different according to the Tuckey HSD Test (p<00.5).

The kinds of mushroom substrate and the application method did not influence the efficacy of the SMS. Based on the AUDPC value, which reflects the total of disease progress during the observation, it showed that all kinds of tested SMS significantly suppress bottom rot disease in shallot by 43.7 – 76.3 % (Table 2). The highest suppression was found in the application of the straw mushroom spent substrate in the planting holes and drenching its water extract every two weeks.

The application of *P. ostreatus* at planting site was relatively least effective in controlling the disease (only 43.7%) than other SMS treatments. When this application was followed by drenching its water extract every two weeks, the disease control was improved (the disease reduction become 68.7%).

Table 2. AUDPC value and percentage of inhibition of basal rot shallots on various treatments.

Treatments	AUDPC Value	Level of inhibition (%)
A. Oyster mushroom SMS	1506.3 b	43.7
B. Straw mushroom SMS	1006.0 ab	62.4
C. Shiitake mushroom SMS	1138.3 ab	57.5
D. Oyster mushroom SMS + drenching its water extract every 2 weeks	837.3 ab	68.7
E. Straw mushroom SMS + drenching its water extract every 2 weeks	633.3 a	76.3
F. Shiitake SMS + drenching its water extract every 2 weeks	788.3 ab	70.5
G. Drenching water extract of oyster mushroom every 2 weeks	917.0 ab	65.7
H. Drenching water extract of shiitake mushroom every 2 weeks	936.3 ab	65.0
I. Drenching water extract of straw mushroom every 2 weeks	914.3 ab	65.8
J. Farmer's practice (fertilizers and pesticides)	878.3 ab	67.2
K. Fungicide (Difenoconazole)	1531.7 b	42.8
L. Check	2676.0 c	0

Note: The average value in the columns followed by the same letter is not significantly different according to the Tuckey HSD Test (p<00.5).

In this study, application of fungicides (without fertilizer application) reduced the basal rot disease by 42.8%. However, when it was combined with application of synthetic fertilizer (as practiced by farmers) the disease reduction was 67.2%. The isolation of microbes from the rhizosphere at the end of the experiment showed that the population microbes in the SMS-treated rhizosphere were relatively higher than the untreated plants. The highest population of microbes was found in the treatment that showed highest reduction of the bottom rot disease which was straw-mushroom spent substrate applied in planting site and drenching its water extract every two weeks (Table 3).

Table 3. Population of microbes in the shallot rhizosphere from various treatments (9 Weeks After Planting).

Treatments	Population of bacteria (cfu/g sampel)	Population of fungi (cfu/g sampel)
A. Oyster mushroom SMS	$5{,}1 \times 10^8$	$9{,}0 \times 10^5$
B. Straw mushroom SMS	$9{,}7 \times 10^7$	$4{,}6 \times 10^4$
C. Shiitake mushroom SMS	$2{,}0 \times 10^7$	$5{,}0 \times 10^4$
D. Oyster mushroom SMS + drenching its water extract every 2 weeks	$3{,}2 \times 10^8$	$7{,}0 \times 10^3$
E. Straw mushroom SMS + drenching its water extract every 2 weeks	$1{,}0 \times 10^9$	$7{,}9 \times 10^5$
F. Shiitake SMS + drenching its water extract every 2 weeks	$5{,}1 \times 10^8$	$8{,}1 \times 10^4$
G. Drenching water extract of oyster mushroom every 2 weeks	$5{,}0 \times 10^8$	$2{,}7 \times 10^4$
H. Drenching water extract of shiitake mushroom every 2 weeks	$1{,}3 \times 10^8$	$9{,}7 \times 10^3$
I. Drenching water extract of straw mushroom every 2 weeks	$1{,}1 \times 10^7$	$5{,}2 \times 10^4$
J. Farmerpractice (fertilizers and pesticides)	$7{,}5 \times 10^8$	$1{,}5 \times 10^4$
K. Fungicide (Difenoconazole)	$6{,}5 \times 10^6$	$9{,}5 \times 10^3$
L. Check	$3{,}5 \times 10^6$	$9{,}5 \times 10^3$

The microbes isolated were bacteria, yeast and fungi. The fungal isolates found belonged to genera *Trichoderma, Aspergillus* and *Penicillium*. However, the most common fungal isolate found was *Trichoderma* sp.

The effect of treatment on shallot growth

Application of SMS did not significantly enhance the shallot growth at 2 and 4 weeks after planting. Based on the plant height and the numbers of leaves, the significant increase was found only at 8 weeks after planting particularly in the treatments of spent substrate of straw mushroom and shiitake that were applied in planting site and or/ drenching its water extract every two weeks (Table 4).

Table 4. The effect of SMS on shallot growth (plant height and numbers of leaves).

Treatment	Plant height (cm)				Number of leaf			
	2 WAP	4 WAP	6 WAP	8 WAP	2 WAP	4 WAP	6 WAP	8 WAP
Oyster mushroom SMS	11.7 ab	27.0 bc	39.0 a	47.0 b	4.0 ab	14.3 a	20.3 ab	18.7 ab
Straw mushroom SMS	13.3 ab	23.7 abc	41.7 a	45.0 ab	3.3 ab	14.3 a	23.3 b	28.3 b
Shiitake SMS	12.0 ab	19.7 abc	41.3 a	50.0 b	3.7 ab	15.7 a	21.0 ab	20.0 ab
Oyster mushroom SMS + drenching its water extract	13.0 ab	27.3 bc	40.3 a	44.0 ab	4.7 ab	15.7 a	21.7 ab	28.3 b
Straw mushroom SMS + drenching its water extract	15.7 b	28.7 bc	37.0 a	46.7 b	4.0 ab	17.7 a	25.3 b	29.7 b
Shiitake SMS + drenching its water extractevery 2 weeks	11.3 ab	17.0 ab	38.3 a	54.3 b	5.3 ab	10.7 a	21.0 ab	27.3 b
Drenching water extract of oyster mushroomevery 2 weeks	12.0 ab	28.3 bc	39.7 a	45.3 ab	4.7 ab	15.3 a	22.8 b	29.7 b
Drenching water extract of shiitake every 2 weeks	13.3 ab	31.3 c	40.7 a	44.0 ab	4.3 ab	15.7 a	18.3 ab	27.3 b
Drenching water extract of straw mushroomevery 2 weeks	13.0 ab	27.7 bc	41.7 a	46.7 b	4.0 ab	15.3 a	23.3 b	30.7 b
Farmerpractice (fertilizers and pesticides)	10.7 a	33.3 abc	34.7 a	42.7 ab	2.7 a	14.7 a	30.0 ab	20.3 ab
Fungicide (Difenoconazole)	10.0 a	12.7 a	35.0 a	44.3 ab	3.33 ab	11.33 a	19.3 ab	10.67 a
Check	10.7 a	22.3 abc	30.0 a	32.7 a	3.00 a	12.00 a	11.67 a	9.33 a

Note: The data in a column followed by the same letter is not significantly different according to the Tuckey HSD Test ($p<00.5$).

In these treatments the shallot growth was not significantly different to the plants treated with synthetic fertilizer and fungicide.

Based on fresh weight of shoot and underground part (roots and bulb) of shallot it was found that the SMS did not significantly enhance the shallot shoot. However, the SMS application tended to enhance the development of roots and shallot bulb. The significant increase ($p<0.05$) on roots and shallot bulb was found in the application of straw mushroom substrate in planting hole and drenching its water extract every two weeks (Table 5).

Discussion

In this study, spent substrate of oyster mushroom (*P. ostreatus*), straw mushroom (*V. volvaceae*) and shiitake (*L. edodes*) reduced the intensity of basal rot disease in shallot. The disease suppression, however, started to appear at 4 weeks after planting. This delay on the suppressive effect was perhaps related to the mechanism of the SMS in controlling the pathogen. In many studies, the mechanisms of SMS in inhibiting the pathogen are mainly because of the activities of antagonistic microbes in the SMS (Ahlawat and Sagar, 2007; Adedeji and Modupe, 2016). Adedeji and Modupe (2016) found that unsterilized water extract of SMS inhibited *F. oxysporum* f.sp. *lycopersici* better than the unsterilized extract, indicating that the microbes in the SMS water extract played important role in the pathogen inhibition. Istifadah and Sianipar (2015) also found that water

extract of non-sterile SMS of oyster and shiitake mushrooms inhibited *Ralstonia solanacearum* better than the sterile ones.

The SMS of oyster mushroom, straw mushroom and shiitake contained microbes antagonistic to *F. oxysporum* f.sp. *cepae* (unpublished data). Concerning that the microbes in SMS play an important role, the delay of disease reduction probably because the microbes had to adapt and establish before providing significant inhibition to the pathogen.

Table 5. The effects of SMS on shallot growth (weight of the shoot and underground parts).

Treatments	Shoot fresh weight (g)	Fresh weight of underground part (g)	Air-dried weight of the bulbs (g)
A. Oyster mushroom SMS	147.3 a	134.0 ab	68,0 ab
B. Straw mushroom SMS	162. 3 a	280.0 ab	183,3 ab
C. Shiitake mushroom SMS	194.3 a	176.7 ab	101,0 ab
D. Oyster mushroom SMS + drenching its water extract every 2 weeks	228.0 a	207.3 ab	120,3 ab
E. Straw mushroom SMS + drenching its water extract every 2 weeks	227.3 a	323.7 b	232,7 b
F. Shiitake SMS + drenching its water extract every 2 weeks	225.3 a	238.7 ab	162,0 ab
G. Drenching water extract of oyster mushroom every 2 weeks	226.0 a	264.0 ab	180,7 ab
H. Drenching water extract of shiitake mushroom every 2 weeks	220.0 a	172.0 ab	118,7 ab
I. Drenching water extract of straw mushroom every 2 weeks	263.7 a	274.3 ab	170,7 ab
J. Farmer's practice (fertilizers and pesticides)	178.3 a	162.3 ab	76,3 ab
K. Fungicide (Difenoconazole)	133.7 a	122.0 a	86,7 ab
L. Check plants	175.7 a	104.7 a	45,0 a

Note: The data in a column followed by the same letter is not significantly different according to the Tuckey HSD Test (p<00.5).

The repetition of SMS application tended to increase the effectiveness of SMS particularly in the treatment of SMS of oyster and straw mushrooms. The mechanisms of SMS in controlling the disease seems to involve the antagonistic activities of the microbes and hence several applications allowed the accumulation of the microbes in the planting site that lead to better control of the disease. Bonanomi *et al.* (2017) found that frequent application of organic matters enhanced the microbial activities in the soil. Istifadah and Sianipar (2015) also reported that the application of SMS in planting media and drenching its water extract every week inhibited the development of bacterial wilt disease in potato.

The assumption that the effectiveness of SMS in inhibiting the disease is due to the activities of antagonistic microbes was supported by the result of the isolation of microbes from rhizosphere of shallot treated with SMS. The highest microbe population was found in the treatment that also showed highest reduction of the bottom rot disease.

The most common microbes found in SMS were *Trichoderma* spp. Ahlawat and Sagar (2007) pointed out that the *Trichoderma* spp. dominated spent substrate of various mushrooms. The genera *Trichoderma* is known as biological control agent that can inhibit various soil-borne pathogens (Cumagun, 2012; Saba *et al.,* 2014).

The application of SMS also supported the shallot growth, but the significant effects were started at 6 weeks after planting. This delay effect was probably related to the slow release of nutrients from SMS as stated by Uzun (2014). In this study, the tested SMS did not significantly enhance the shallot shoot but tended to enhance the development of roots and shallot bulb. It seems that the increased of plant height and leaves of the SMS-treated shallot at 6 and 8 weeks after planting did not lead to significant increase of the shoot weight.

The abilities of the SMS to support the development of bulb may be related to the nutrients availability particularly P and K. Spent Mushroom Substrate contains sufficient P and K (Uzun, 2014) and its application enhanced the P and K level in the soil (Maher *et al*., 2000). The phosphorus and potassium nutrients are essential for the development of onion roots and bulbs, therefore their sufficient availabilities increased the shallot yield (Napitupulu and Winarto, 2009; Anisyah *et al*., 2014).

In this study, the shallot growth was not only affected by the nutrients availability, but it was also affected by the basal rot disease. The shallot treated with the SMS did not significantly different to the shallot that received NPK fertilizer and treated with pesticide as the farmer's practices. It means that the SMS of *Pleurotus* sp., *V. volvaceae* and *L. edodes* can be used for fertilization and plant disease control supporting sustainable agriculture.

Conclusion

Based on the results of this study it can be concluded that spent substrate of *Pleurotus* sp., *V. volvaceae* and *L. edodes* reduced the bottom rot disease caused by *F. oxysporum*f.sp. *cepae* and improved the shallot growth. The spent substrate of straw mushroom (*V. volvaceae*) applied in planting hole followed by drenching its water extract every two weeks provided the best disease control and the shallot growth.

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