

Original Research Article

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Impact of Pesticides on the Diversity of Fungi at Local Shallot in Palu, Indonesia

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ABSTRACT

Intensive pesticide application caused damaged of natural balance in agricultural land. Purpose of the study is to evaluate frequency of pesticides application and their impact presence of fungi at local shallot plants in Oloboju village, Sigi Regency, Central Sulawesi. Almost all of local shallot farmers was used synthetic pesticides controlled pests and diseases with high, medium and low of their frequency applications. By comparing final application of pesticides, the results was showed increasing pesticides application can reduced fungi index diversity in rhizosphere and phylosphere by approximately 0.5623 and 0.6002, respectively. It compared with frequency of pesticides application in low and medium with a diversity index value about 1.332 and 1.696 in rhizosphere, different in phylosphere result at 1.56 and 1.889. The similar application was showed the evenness index and species richness index of rhizosphere and phylosphere. The impact application of pesticides was showed a resistant fungi species such as *Fusarium* sp. as a pathogenic fungi and found in all of frequencies pesticide application. The presence of *Trichoderma* spp. and *Aspergillus niger* as endophytic fungi at all of frequencies pesticide application, giving important information and very useful for this study. Antagonistic fungi and their existence in local shallot field can be developed to be biological agents, because they have survival properties in environments with varying levels of pesticide residues.

Keywords

Rhizosphere,
phylosphere,
Fusarium sp.,
Trichoderma spp.,
Aspergillus niger

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Introduction

Shallot (*Allium cepa* var. *aggregatum*) a famous local varieties from Palu valley are one of the important commercial vegetable crops in Central Sulawesi, Indonesia. These commodities are quite well known as source of raw materials in manufactures of fried shallot products that savory taste and delicious. Based morphology performance such as bulbs rather pink and white color, the typical of local shallot from Palu valley almost similar in general, but they are small size.

Average harvest of local shallot still lower, accompanied by high demand and good prices. This is main reason farmers continue planting these commodities on their land every years (Maskar *et al.*, 1999; Shahabuddin *et al.*, 2012 and Saidah *et al.*, 2014). The low harvest of local shallot production because application of cultivation techniques less supportive beside presence plant pests and diseases.

Commonly few pests found in the field such as: mole cricket (*Grylotalpa* sp.), army worm (*Spodoptera litura* L.), onion caterpillar

(*Spodoptera exigua*), leafminers (*Liriomyza chinensis*) and thrips (*Thrips tabaci*). The important diseases on shallot including: *Fusarium* wilt (*Fusarium oxysporum*), downy mildew (*Peronospora destructor*), antraknose (*Colletotrichum gloeosporioides*), leaf spots (*Cercospora duddiae*). The dominant diseases in the field are purple spot (*Alternaria porrii*) (Semangun, 2006; Ratnawati and Idris, 2010; Rusdam *et al.*, 2013; Marlitasasri *et al.*, 2016).

The economic implications of the damage and loss of shallot harvest based on the presence of plant pests and diseases encourage farmers to use high frequency pesticides application. Pesticides are still considered by shallot farmers in the Palu valley as a savior of production from pest attacks (Jaya *et al.*, 2015). The increasing of total concentration by farmers used reaching approximately 150 - 200% higher than the recommended level by mixing two or three types of pesticides at once application (Basuki, 2011; Waryanto *et al.*, 2014). This intensive of pesticides application resulted in several ecological consequences for the environment such as resistance to pests and diseases, potential decreases in soil quality, negative impacts on other creatures, including antagonistic fungi and high chemical residues in shallot bulbs (Basuki, 2011; Nelly *et al.*, 2015 and Joko *et al.*, 2017).

The use of microorganisms as biological control agents needs to be pursued continuously and ideally using the potential of local natural enemies. These microorganisms will work more effectively and supported by appropriate environmental factors. They keep ecosystem changes and cheaper formulations for farmers. According to Cook and Baker (1983), efforts to control plant diseases by biological means have a fairly bright opportunity because the microorganisms are available in nature and their activities can be stimulated by

environmental and host modifications. The group of fungi microorganisms that potentially suppress pathogens, especially in the family Moniliales, such as *Verticillium* sp., *Trichoderma* spp. and *Gliocladium* sp. Genus *Trichoderma* spp. has several species parasitized other fungi and very potential as biological control agents (Santoso *et al.*, 2007). However, disturbances in the existence, diversity and abundance were affected ecosystems including soil fertility, microorganism populations and populations of plant pests and diseases.

Based on the risk of pesticides application on local shallot in Palu valley, the purpose of the study is to evaluate pesticides application by farmers, to determine the diversity, wealth and abundance of phosphorus fungi and zyzosphere as an impact of pesticides. The data collected from the study will be useful in designing Integrated Pest Management (IPM) on local shallot in Palu valley.

Materials and Methods

The research was conducted from January to June 2018 located in Oloboju Village, Seromiromaru, Sigi district. The place is the center of local shallot plantations in Palu valley, Central Sulawesi. The other treatment was continued at Laboratory of Disease Sciences, Faculty of Agriculture, Hasanuddin University Makassar.

Survey and sampling of local shallot farmers

The survey was conducted in interview purpose to determine the level of pesticides application of local shallot farmers in Palu valley. According to previous observational data and interviews with local agricultural extension workers, there are around 300 shallot farm families in the village. Approximately ten percent of the population (30

farmers) as samples for interviews about the landas on the shallot land area owned, the frequency of pesticide use per season is around 60 days, and the type of pesticide used. From interviews it was described 3 farmers only used herbicides and natural pesticides, 13 farmers used pesticides less than ten times, and 14 farmers applied more than ten times per season.

The frequency of application of pesticides to local shallot was categorized as each application is low, medium, and high. The data was added observation sample rhizosphere and phylosphere. The local shallot samples are taken from the farmers' field. Sampling was used purposive sampling method, which is taking samples of onion plants at ten points diagonally at each stage with several categories level pesticides application. The local shallot was removed and put into plastic bag clip, labeled and stored in the cooler box for laboratory activities.

Fungi Isolation

Fungi from similar field with local shallot was isolated based Nirwanto (2007). In laboratory, each soil sample from rhizosphere weighed 10 gr, then dilution to concentration 10^{-4} cfu. Each of fungi sample measure 0.1 ml was cultured in Potato Dextrose Agar (PDA) and incubated at room temperature 3 - 14 days. Different colonies of fungi that grew on PDA was counted, isolated and purified as a stock culture. The fungi from the rhizosphere was labeled. The phylosphere sample was cutting long 1 cm then surface sterilization using 0.5% NaOCl, 70% ethanol and distilled water for 1 minute, respectively. Samples then dried by clamping between sterile tissue paper. The sample was cultured in PDA then incubated at room temperature for 3-14 days. The growing of colonies fungi from phylosphere was counted, isolated and purified.

Shannon-Wiener Index Diversity

Fungi diversity index from rhizosphere and phylosphere was calculated by Shannon-Wiener index diversity (H') (Magurran, 1983).

$$H' = - \sum_{i=1}^s p_i \ln p_i$$

Variables:

H' = Shannon - Wiener diversity index.

S = number of species in the community (richness).

P_i = the proportion first species in the total individuals in the sample.

\ln = natural logarithm.

Index of Individual Abundance

Index of individual abundance was calculated by Simpson index diversity (D) (Magurran, 1983)

$$D = - \sum_{i=1} (n_i(n_i - 1) / (N - 1))$$

Variables:

n_i = individual species number.

N = total individual number

Margalef species richness indeks (Ludwig dan Reynolds, 1988)

Fungi species richness index was calculated by Margalef index (R):

$$R = \frac{S - 1}{\ln(n)}$$

Variables:

- R = species richness index
- S = measurement species number
- N = Total individual number
- Ln = natural logarithm

Results and Discussion

Pesticides application by farmers for local shallot

Oloboju village as the study site in Sigi Regency known as the Palu valley. The majority of the population is local shallot farmers with approximately 300 family farm in areas 286 ha. Local shallots was planted continuously in four planting season per year. The methods controlling pests and diseases was showed about 46.7% farmers used synthetic pesticides with more than ten times the frequency of application per planting season; 43.3% farmers was used less than ten times pesticides; only 10.0% use natural pesticides, respectively (Table 1 and 2). About 10% farmers was used herbicides controlled weeds and natural pesticides from neem controlled pests and diseases.

The type of pesticides including fungicides and insecticides was showed in Table 3. The fungicides was used are generally classified as pesticides that do not cause acute harm in regular use (U), while insecticides used are

generally classified as quite dangerous (II), and one of them is very dangerous (Ib). All these pesticides was controlled pests and diseases of local shallot.

Pesticides Impact on Fungi

The pesticides application by farmers on local shallot has impact the presence of fungi in field. The result of fungi identification and the number of isolates are shown in Table 4.

Table 4 was showed more isolates were found in high frequency of pesticide application, but generally unidentified isolates with relative low diversity index, in rhizosphere and phylosphere. Fungi index diversity including abundance and species richness of fungi in plantation of local shallot farmer in Oloboju village (Table 5).

Increasing demand the local shallot from Palu valley as raw material for Palu's typical fried shallot in Central Sulawesi, caused farmers planting throughout the year. This activities caused increasing pest attacked plant in Oloboju village as the main source of local shallots. Effort controlled presence of pests was identified approximately 90% farmers used synthetic pesticides. Pesticides not used based recommended dosage at application in every week. This is potential caused outbreak of secondary pests, killed natural enemies and resistance diseases on the field.

Table.1 Indicator of Shannon-Wiener Indeks Diversity (H')

H'<1.0	1.0<H'<3,322	H'>3,322
* species diversity is classified as lower;	* species diversity is classified as moderate;	* species diversity is classified as higher;
* Poor (very low productivity) as an indication of heavy ecological pressure;	*medium productivity; *The condition of the ecosystem is quite balanced;	*stability of the ecosystem; *high productivity
*Unstable ecosystem	*medium ecosystem pressure;	

Table.2 Frequency of pesticide application in level of onion farmers

Number of respondent	Frequency of pesticide application	Percentage	Level
14	≥10 times	46.7	High
13	< 10 times	43.3	Medium
3	once (herbicide)	10	Low
30		100	

Table.3 Pesticides Active Compound was used by Local Shallot Farmers

Type of Pesticides	Active Compound	Group	Class
Fungicide	Ziram	Dithiocarbamate	III
Fungicide	Mancozeb	Dithiocarbamate	U
Fungicide	Propineb	Dithiocarbamate	U
Fungicide	Carbendazim	Benzimidazole	U
Fungicide	Phosphorous acid	Phosphonate	U
Fungicide	Thiophanate-methyl	Benzimidazole	U
Fungicide	Prochloraz	Imidazole	III
Fungicide	Iprodione	Dicarboximide	U
Insecticide	Chlorpirifos	Organophosphate	II
Insecticide	Alpha-cypermethrin	Botanical	II
Insecticide	BPMC	Carbamate	II
Insecticide	Methomyl	Carbamate	Ib
Insecticide	Chlorantraniliprole	Antranilicdinamide	U
Insecticide	EmamectinBenzoat	Avermectin	U
Insecticide	Chlorfenapyr	Pyrrrole	II
Insecticide	Carbosulfan	Carbamate	II
Herbicide	Paraquat	Piridin	II

Ia = Extremely hazardous; Ib = Highly hazardous; II = Moderately hazardous; III = slightly hazardous; U = Unlikely to present acute hazard in normal use; FM =Fumigant, not classified; O = Obsolute as pesticide, not classified.

Table.4 Fungi Identification and Number of Isolates

No.	Fungi Species	Frequently of Pesticide Application						Status
		Low		Medium		High		
		Rhizo sphere	Phylo sphere	Rhizo sphere	Phylo sphere	Rhizo sphere	Phylo sphere	
1.	<i>Aspergillus niger</i>	2	1	1	1	-	2	Endophyte
2	<i>Aspergillus flavus</i>	-	2	1	1	-	-	Endophyte
3	<i>Fusarium</i> sp.	-	1	3	2	-	1	Pathogen
4	<i>Gliocladium</i> sp.	-	-	-	1	-	-	Endophyte
5	<i>Penicillium</i> sp.	1	2	1	1	-	-	Endophyte
6.	<i>Trichoderma</i> sp.	1	-	2	2	1	-	Endophyte
7	Unidentified	1	2	2	1	3	9	-
Total number		5	8	10	9	4	11	

Table.5 Fungi Index Diversity Analysis on the Local Shallot at Oloboju Village

Index diversity analysis	Frequently of Pesticide Application					
	Lower		Medium		High	
	Rhizo sphere	Phylo sphere	Rhizo Sphere	Phylo sphere	Rhizo Sphere	Phylo sphere
Evenness index	0.720	0.781	0.800	0.839	0.375	0.314
Diversity index	1.332	1.560	1.696	1.889	0.562	0.600
Species richness index	1.864	1.924	2.171	2.731	0.721	0.834
Total individual number	5	8	10	9	4	11

Commonly typical of pesticide was applied in local shallot field divide into two form: insecticides and fungicides. Insecticides consist of eight group divided into seven group was classified as II and remain as Ib. The II is quite dangerous and Ib is very dangerous (WHO, 2008). Insecticides has impact directly and indirectly, they are very dangerous for natural enemies surrounding the field (Cloyd, 2006). The direct effects was associated with pests death or their offspring survival over a period of time, 24 to 96 hours (Stapel *et al.*, 2000). The indirect effects was associated with physiological disorders and behavior of natural enemies (Desneux *et al.*, 2007). Fungicides consist from five groups and four groups including class U and III. Nevertheless, fungicides very important because they are widely used as control tools in agricultural and horticultural production system (Wright and Verker, 1995).

Comparing the number of isolates found in differences frequency application of pesticides was showed influence on the type and number of isolates fungi. The frequency of low pesticide application into rhizosphere, there are all endophytic fungi such as *A. niger*, *A. flavus*, *Gliocladium* sp. and *Penicillium* sp., only *Fusarium* sp. as pathogenic fungi. *Trichoderma* spp. as endophytic fungi was found on field in moderate pesticide application. Local shallot field with high pesticide application, dominant species were identified with low

fungi diversity (diversity index <1). Nirwanto and Mujoka (2009) stated that diversity of phylospheric saprobial of fungi species in shallot plantations was relatively low due to the use of pesticides. *A. flavus* and *Penicillium* sp. as endophytic fungi were not found in field with low and medium of pesticides application. *Fusarium* sp. is a pathogenic fungi found in the atmosphere at all field of pesticide application. This indicate *Fusarium* sp. have been resistance to the pesticide application in the s study area. According to Christanti *et al.*, (2013); Paramita *et al.*, (2014) and Rao *et al.*, (2015) state that the continuous of fungicides application, especially systemic fungicides will product new strains target to resistance. This is caused contain variety of chemicals has a systemic effect.

Khan (2003) state that presence of *Trichoderma* spp. and *A. niger* as endophytic fungi on all fields applied by pesticides is important to develop as biological agents. The reason is fungi has a life-resistant nature with varying levels of pesticide residues. This is opportunity working more effectively and supported by appropriate environmental factors and not causing ecosystem changes and cheaper formulated for farmers. Madigan and Martinko (2006) report that *A. niger* from Ascomycetes found abundantly in nature. Usually *A. niger* collected and isolated from soil, plant residues and in the room. *Trichoderma* spp. is a type of fungi has same

class with *A. niger* working as antifungal activities, commonly found in woody substrates on forest and agricultural land (Samuels, 2006). Santoso *et al.*, (2007) mentioning that *Trichoderma* spp. very potential to be used as biological control for future management diseases in local shallot in Palu valley.

The continuous pesticides application to local shallot plants in Palu valley caused negative impact on antagonistic fungi presence by reducing their abundance, diversity and evenness. The most phenomenal of these impacts is the emergence of resistant fungi such as *Fusarium* sp. as pathogenic fungi in all frequency of pesticide application. However, the emergence of *Trichoderma* spp. and *A. niger* as endophytic fungi on all fields applied by different pesticides, gave important meaning in this study. Their existence could be developed into biological agents, because the fungi has a life-resistant nature with a variety of pesticide residues.

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