

Research Article

Application of organic amendment and *Trichoderma* sp. to control basal *Sclerotium rolfsii* on peanut grown on partially degraded land

H. Suheri, M. Isnaini, A.Rohyadi

Faculty of Agriculture, University of Mataram, Jln. Majapahit 62, Mataram 83125, Indonesia

Abstract: An experiment involving the application of organic matters, either fresh or composted, application of bioagent *Trichoderma* sp. was conducted to suppress basal stem rot caused by *S. rolfsii* on peanut. The research was conducted at dry land in Amor-Amor North Lombok District with the pump water well system. The experiments were arranged as split plot design with the main plot was pathogen inoculation and non pathogen inoculation. As sub-plot were the addition of organic matters and or addition of bioagent *Trichoderma* sp. plus two control treatments. Pathogen *S. rolfsii* applied as fragment mycelia of 1×10^6 fragment/ mL with the number of 10 L/plot. Inoculation of the pathogen was conducted when seedlings were about one month old. The bioagent *Trichoderma* sp. was applied 50 g/plot as powder in the same time of organic matters application (before planting the seeds). The results showed that application of composted organic matters reduced disease incidence 6-10%, while application as fresh organic matters reduced 1-6%. Combination between application of bioagent *Trichoderma* sp. with fresh and or composted organic matters suppressed disease incidence 8-10% and 8-12% respectively. Application of bioagent alone on planting medium reduced disease incidence 7.5%. Application of composted organic matters with bioagent *Trichoderma* sp. increased the number of fresh plant biomass 4.93-11.78 kg.

Keywords: organic amendments, peanut, *Sclerotium rolfsii*, *Trichoderma* sp.

Introduction

The fungus of *S. rolfsii* is one of the important soil borne pathogen which causes diseases on many different crops species (Mordue, 1974). According to Punja (1985), the fungus *S. rolfsii* is an endemic soil-borne pathogen which attacks a wide host range hosts including cultivated crops and even wild plants in the tropical and sub-tropical areas of the world. It has been reported to cause damages on seedlings and crops at vegetative, as well as generative stages. The main inoculum of the pathogen exists in the form of sclerotia, a structure by which the pathogen survives under unfavorable environment. These structures are formed in large numbers on the surface of infected host or in the soil surrounding infected hosts. With this structure the fungus survives for long period of time in the absence of host, and is ready for infection when susceptible host are present and favorable environment prevails. Incidence of peanut disease in Lombok,

Indonesia caused by the pathogen reached 10-50% (Isnaini and Krisnayanti, 2003) (Figure 1).

Due to this nature, the pathogen has been difficult to control with commonly practiced cultural technique such as crop rotation. Fungicide application to control the pathogen tends to be ineffective and expensive. Because the fungicides have negative impact to non pathogenic microorganisms in the soil and their toxic residue can cause contamination to ground water which lead to dangerous to human and also other organisms.

Therefore, a more environmentally friendly management strategy is required to control the disease. One of the methods currently being promoted is the use of biocontrol agent such as *Trichoderma* sp. Isnaini *et al.* (2006) has shown that locally isolated strains of *Trichoderma* sp. provided an effective means of controlling basal stem rot disease of vanilla caused by *S. rolfsii* under greenhouse conditions. Similar results showing the success of the use of biocontrol agent

Trichoderma sp. have been reported by many authors on different pathogens such as *Rhizoctonia solani* (Lumsden and Locke, 1989; Howel and Stipanovic, 1995), *Sclerotinia sclerotiorum* and *S. minor* (Tu, 1980; Isnaini, 2000).



Figure 1. Symptom of Disease Incidence of Basal Stem Rot caused by *S. rolfsii* on Peanut in West Lombok

Another promising method is the application of organic matters. Incorporated composted organic matters and or fresh organic matters in soil will not only increase soil fertility but also reduce disease incidence caused by many soil borne pathogens (Hoitink, 1980). Composted bark wood suppressed *R. solani* due to increasing the number of *T. harzianum* in soil (Nelson, *at al.*, 1983). Increased population of *T. hamatum* in planting medium due to incorporation of natural composted agricultural waste and man made compost into soil has been reported by Kuter *et al.*, (1983). The use of growing media amended with composts resulted in better growth of peanut plant compared to that without compost (Isnaini and Krisnayanti, 2003).

The use of organic matters as plant residues such as maize plants and rice hulls, are the wise way to not only to enrich soil fertility but also to give a positive effect on plant rhizosphere. Addition of organic matters would also create a better environment for the growth of microorganisms as biocontrol agents. Combinations of application biocontrol agent *Trichoderma* sp. and organic matters in soil to suppress basal stem rot pathogen were the promising alternative methods. The research presented the results of different application of organic matters and application of biocontrol agent *Trichoderma* sp. on growing medium in

reduction of disease incidence on peanut caused by *S. rolfsii*.

Materials and Methods

Preparation of organic amendments

Organic matters used in this experiment were rice hull and residue of maize plants. These materials were prepared as composted and as fresh materials. For the preparation of composted material, a commercial bioactivator labeled as PROMI obtained from Research Center of Estate Crop and Biotechnology, Bogor was used. The bioactivator is indicated to contain a range of cellulose degrading fungi and *Trichoderma* sp. The composting process took about six weeks, while the un-composted material were prepared separately. Composting process of selected crop residues (which included maize residue and rice husks) was carried out as follows. Stalks of maize residue was initially soaked in water for at least 12 hr, drained to remove excess water, and rewetted with the suspension of PROMI (1 kg in 100 L water) to reach approximately 40% water content in the materials. The stalks were then arranged in layers of approximately 10 cm thick to form a large mound of approximately 1 m height. The mound was covered with a large plastic tarpaulin to keep the moisture in place and was kept out of direct sunrays by putting bags of un-composted rice husks on top of the mound (Figure 2).



Figure 2. Composted process organic matters: Rice Hull and Maize Residues with a Comercial Bio Activator "Promi

Preparation of biological agent *Trichoderma* sp. and inoculum of pathogen *S. rolfsii*

Biological agent (*Trichoderma* sp.) was isolated soil from peanut rhizospheres collected from peanut growing areas indicated to have a severe

infection by *S. rolfsii* in Central Lombok District. The agent was prepared as solid state medium on a substrate composed of powdered maize cobs, rice bran and coarse sand (4:4:2 v/v) according a method developed by Isnaini *et al.*, (2006). The inoculum (*S. rolfsii*) was prepared by initially growing the pathogen for 7 days on potato dextrose agar (PDA) medium amended with an antibiotic Streptomycin sulphate at 50 µg/L and was prepared as liquid suspension by blending the actively growing mycelia of the fungus on its agar medium. The inoculum suspension was calibrated to contain approximately 10⁶ CFU/mL before application.

Preparation and design of the experimental plots

Experimental site was located on a dry land of Amor-Amor village in North Lombok whose

irrigation was supplied using deep well irrigation systems. The experiments consisted of a series of three sub experiments involving three consecutive growing seasons. The overall sets of experimental series were prepared according to a split plot design. The experiment involved the application of organic amendments to suppress inoculum of the pathogen and to reduce the incidence of basal stem rot disease on peanut. The experiment was prepared based on a split-plot design with the main plot being with pathogen or no-pathogen treatments. Sub-plots comprising the application of different composition organic matters, with or without the amendment of the bioagent *Trichoderma* sp. was arranged randomly within the main plots to form a set of 14 sub plot treatments as presented in Table 1.

Table 1. Sub plot treatments of the study

No	Treatment description
1	Fresh rice husks without the bioagent <i>Trichoderma</i>
2	Fresh rice husks with the bioagent <i>Trichoderma</i>
3	Composted rice husks without the bioagent <i>Trichoderma</i>
4	Composted rice husks with the bioagent <i>Trichoderma</i>
5	Fresh maize residue without the bioagent <i>Trichoderma</i>
6	Fresh maize residue with the bioagent <i>Trichoderma</i>
7	Composted maize residue without the bioagent <i>Trichoderma</i>
8	Composted maize residue with the bioagent <i>Trichoderma</i>
9	Fresh rice husks + fresh maize residue, without the bioagent <i>Trichoderma</i>
10	Fresh rice husks + fresh maize residue, with the bioagent <i>Trichoderma</i>
11	Composted rice husks + maize residue, without the bioagent <i>Trichoderma</i>
12	Composted rice husks + maize residue, with the bioagent <i>Trichoderma</i>
13	No organic amendment or no bioagent <i>Trichoderma</i>
14	No organic amendment or with bioagent <i>Trichoderma</i>

Plant cultivation and crop management

Each treatment was set up on a bed of 1 m wide by 10 m long with the spaces of 0.5 m between beds and 1 m between main plots. Base fertilizers which consisted Urea (@50kg/Ha), super phosphate (SP36 – @75kg/Ha) and potassium chloride (KCl – @75 kg/Ha), were applied together with a granular insecticide (carbofuran – Furadan 3G®) prior to planting, after which the beds was furrow irrigated to field capacity.

Local variety of peanut, known to be susceptible to basal stem rot disease, was planted as seeds at an inter-row spacing of 40 cm and within-row spacing of 20 cm. The seeds were placed in planting holes of approximately 3 cm deep (one seed per planting hole). Organic amendments at the rate of approximately 5

tons/Ha were then applied on beds according to treatments after the planting of the crop.

Regular crop management including irrigation, weed and pest control, and fertilisation were applied as required. Inoculation of the pathogen was conducted when the plant were about one month old. Inoculation substrate containing approximately the bioagent *Trichoderma* sp. was applied 50 g/plot in the same time of organic matters application (before planting the seeds). with 10 L/plot.

Results

The results showed that disease incidence with the application of organic matters reduced the number of diseased plants from total unit treatments of

population number. The addition of composted organic matters suppressed disease incidence even though plants inoculated with pathogen *S. rolfsii*. The lowest disease incidence occurred on plants applied with composted maize residues either added as single or combination with composted rice hull (Table 1). There was no significant difference disease incidence between application of fresh rice hull and disease incidence applied without organic matters (control treatment).

Treatment of organic matters application as fresh rice hull and fresh maize residues and treated with *Trichoderma sp.* did not have effect on fresh plant biomass. The effect of fresh biomass showed significantly when organic matters applied as compost materials. However, the addition of *Trichoderma sp.* on planting medium had greater effect on fresh plant biomass compared to treatment of application fresh organic matters with or without *Trichoderma sp.* or compared to the treatment without organic

matters and without *Trichoderma sp.* There was a correlation between fresh plant biomass and disease incidence. The higher of percentage disease incidence caused the lower of fresh plant biomass. In general, the addition of composted organic matters reduced disease incidence 6-10%, mean while the addition of fresh organic matters reduced disease incidence 1-6% compared to control treatment (Table 2). Combination treatments between addition of biological agent and composted organic matters reduced disease incidence 8-12%. The addition of biological agent in combination with fresh organic matters reduced disease incidence 8-10% compared to control treatment. In this experiment was also found that plants were not inoculated with pathogen *S. rolfsii* had disease incidence (Table 2). Disease incidence was as high as 1.95% mean while plants were added with *Trichoderma sp.* reduced disease incidence up to 0.9% compared to control treatment.

Tabel 2. Disease Incidence of Sclerotium Basal Stem Rot on Peanut Treated with or without *Trichoderma sp.* and Inoculated or Uninoculated with the *S. rolfsii* pathogen

Treatment	Without <i>Trichoderma sp.</i>		With <i>Trichoderma sp.</i>	
Inoculation with <i>S. rolfsii</i>				
Fresh rice hull	13,05%	(1,86%)	4,50%	(0,71%)
Fresh maize residue	9,15%	(1,48%)	5,85%	(1,13%)
(Maize residue + Rice hull) Fresh	8,10%	(1,16%)	4,65%	(1,21%)
Composted Rice hull	7,65%	(1,70%)	6,30%	(0,90%)
Composted Maize residue	4,50%	(0,52%)	4,05%	(0,45%)
(Maize residue + Rice hull) Composted	4,05%	(0,86%)	2,55%	(0,57%)
Control	14,55%	(1,74%)	7,35%	(0,62%)
Without inoculation of <i>S. rolfsii</i>				
Fresh rice hull	1,05%	(0,38%)	0,90%	(0,17%)
Fresh maize residue	1,05%	(0,15%)	1,05%	(0,38%)
(Maize residue + Rice hull) Fresh	1,35%	(0,38%)	0,90%	(0,17%)
Composted Rice hull	1,20%	(0,24%)	1,05%	(0,15%)
Composted Maize residue	1,05%	(0,15%)	0,90%	(0,17%)
(Maize residue + Rice hull) Composted	1,05%	(0,15%)	0,75%	(0,29%)
Control	1,95%	(0,45%)	0,90%	(0,30%)

The number in the brackets are Standard Error (SE) from the mean data

The lower disease incidence on the treatment of application composted organic matters was found the higher of the number of total microorganisms at the end of experiment. Total microorganisms at final counting at the end of experiment was 1.67-2.76 x 10⁴ CFU/g soil on the treatment without addition of *Trichoderma sp.*, and 1.33-7.33 x 10⁴ CFU/g soil with the addition of *Trichoderma sp.* (Table 3). The high number of microorganism population can be seen on the treatment of *Trichoderma sp.* addition on fresh rice hull organic

matters (4 x 10⁴ CFU/g soil) and 7.33 x 10⁴ CFU/g soil on composted rice hull organic matters. In general, on planting medium that did not inoculated with pathogen *S. rolfsii* the total number of microorganisms were higher than on the planting medium that were inoculated with the pathogen and did not apply with *Trichoderma sp.* Mean while the number of total microorganisms on the planting medium added with *Trichoderma sp.* did not show their consistency.

Table 3. Population of Microorganism ($\times 10^4$ CFU/g soil) in the Field Planting Medium Treated with or without *Trichoderma* sp. and with or without Inoculation of Pathogen *S. rolfsii*

Treatment	Without <i>Trichoderma</i> sp.		With <i>Trichoderma</i> sp.	
Inoculation with <i>S. rolfsii</i>				
Fresh rice hull	1,67	(0,88)	4,00	(1,00)
Fresh maize residue	2,00	(1,15)	2,67	(0,67)
(Maize residue + Rice hull) Fresh	2,00	(0,58)	2,33	(1,86)
Composted Rice hull	1,67	(0,88)	7,33	(1,67)
Composted Maize residue	0,67	(0,33)	2,33	(1,20)
(Maize residue + Rice hull) Composted	2,67	(0,67)	1,33	(0,67)
Control	2,33	(0,88)	2,00	(0,58)
Without inoculation of <i>S. rolfsii</i>				
Fresh rice hull	11,00	(2,08)	4,00	(1,00)
Fresh maize residue	3,33	(0,88)	1,33	(0,67)
(Maize residue + Rice hull) Fresh	3,67	(0,88)	3,33	(0,33)
Composted Rice hull	2,67	(0,67)	2,67	(0,33)
Composted Maize residue	2,00	(0,58)	2,67	(1,20)
(Maize residue + Rice hull) Composted	1,67	(0,67)	5,33	(1,20)
Control	0,67	(0,33)	2,33	(0,67)

The number in the brackets are Stadar Error (SE) from the mean data.

Discussion

Application of composted organic matters especially maize residues combination with bioagent *Trichoderma* sp. in the soil was a promising treatment to reduce disease incidence of basal stem rot on peanut caused by *S. rolfsii*. Combination between application of *Trichoderma* sp. and composted maize residues increased double on fresh plant biomass compared to treatments without addition of neither organic matters nor bioagent. The addition of bioagent *Trichoderma* sp. increased the weight of fresh plant biomass compared to the addition of fresh organic matters with or without bioagent. This appeared that the environment was favorable for the growth and proliferated of bioagent in soil. The number of unit fungal colony also increased in planting medium added with composted organic matters. The used of microorganisms from commercial product for composting might contribute to the increasing number of total microorganisms in planting medium. Bio-decomposers of Promi commercial were *Aspergillus*, *Trichoderma* and white rotting fungi. It seems that those organisms appeared a lot on media when used as counting medium for total microorganisms.

The reduction of disease incidence on plant treated with both fresh and or composted organic matters supported by the high number of microorganisms in the planting medium. The

number of unit colony were $1.67-2.76 \times 10^4$ CFU/g soil and $1.33-7.33 \times 10^4$ CFU/g soil on planting medium treated without the addition of *Trichoderma* sp. and treated with the addition of bioagent *Trichoderma* sp. respectively. The high number of total microorganism can be seen on the planting medium treated with the addition of *Trichoderma* sp. on fresh and on composted rice hulls i.e. 4×10^4 CFU/g soil and 7.33×10^4 CFU/g soil respectively.

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