

Fungal Succession and Decomposition of *Acacia mangium* Leaf Litters in Health and *Ganoderma* Attacked Standings

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Leaf litters of *Acacia mangium* play an important functional role in ecosystem, producing sources of nutrients and giving diversity of microorganisms. Understanding the variation in fungal populations in *A. mangium* forest is important due to the roles of fungi in regulating populations of other organisms and ecosystem processes. For these purposes, the tests were conducted under two years old of health standing (2S) and *Ganoderma* attacked standing (2G) using litterbag method. Litter weight loss and lignin, cellulose, C, N contents were measured each month during eight months of decomposition, as well as fungal community involved was observed. Litter weight loss and lignin, cellulose, C, N contents were measured each month during eight months of decomposition, as well as fungal community involved was observed. After eight months of decomposition, litter weight losses were low up to 34.61% ($k = 0.7/\text{year}$) in 2S and 30.64% ($k = 0.51/\text{year}$) in 2G, as well as lignin weight losses were low up to 20.05% in 2S and 13.87% in 2G. However, cellulose weight losses were 16.34% in 2S and 14.71% in 2G. In both standings, the numbers of fungal species were 21 and 20 respectively, while the total of fungal populations tends to increase after one month of decomposition and tend to decrease in the last three months. In the first and second months of decomposition fungal species were dominated by genera of *Penicillium* and *Aspergillus* and the last three months by *Trichoderma*, *Phialophora*, and *Pythium*.

Key words: fungal succession, decomposition, leaf litters, *A. mangium*

INTRODUCTION

Chemical contents of leaf litters consist of structural component of plant cell wall as hemicelluloses, celluloses and lignin, therefore carbon contents are greater than other nutrients. During decomposition process, the organic materials flow to soil subsystem in the forest ecosystem. Rates of litter decomposition depend on many factors, for example decomposer populations, environmental conditions, and quality of decomposed materials. The last factor supposed to be a primary determinant of decay rate (Sariyildiz 2003). The differences of climate and litter quality result in differences of decomposition time, and also fungal communities. Decomposition in tropical region is faster than temperate one, for example decomposition of *Pheonix hanceana* takes 12 month, *Saccarum officinarum* 14 month and *Ananas comasus* two years, whereas in temperate one takes 13 years for *Fagus crenata* with 50% of weight losses and 11 to 23 years for *Pteridium aquilinum* with 90% of weight losses (Tang *et al.* 2005).

Schmit *et al.* (1999) observed for three years in oak forest and they found 177 fungal species with 30 species from litters, 79 grown on wood, 36 as ectomycorrhizae and 29 species nonmycorrhizae. Osono and Takeda (2002) observed the ability of 79 fungal isolates on litter decomposition of deciduous forest in cool temperate in Japan, and they found

6 species of Basidiomycetes causing 15.10 to 57.67% of weight losses, 14 species of *Xylaria* and *Geniculosporium* causing weight losses 4.00 to 14.4%. Another isolates from Ascomycetes and Zygomycetes gave the low weight losses. They also observed that Basidiomycetes and *Xylaria* had bleaching activities on the litters and causing lignin and carbohydrate decomposition.

Acacia mangium leaf litters contain high lignin and cellulose because their leaves are philodium. In the forest floor, fungal communities tend to change during litter decomposition. The earliest period, only sugar fungi or pioneer colonizer will be able to colonize the litters, and than colonization followed by survivors that could utilize the more enduring cellulose and lignin (Dix & Webster 1995). The researchs of *A. mangium* were limited mainly in management aspect of plantation (Siregar *et al.* 1999; Hardiyanto *et al.* 2004). Setiawan (1993), studied decomposition process of *A. mangium* leaf litters but no data of fungal decomposer. In the preliminary study, we had isolated fungi from *A. mangium* leaf litters and identified as *Curvularia* sp., *Cladosporium* sp., *Trichoderma* sp., *Phaecilomyces* sp., *Diamargaris* sp., and *Botrytis* sp. (Samingan & Sudirman 2007, unpublished data).

These research results would give basic information of fungal succession and decomposition of *A. mangium* leaf litters in health and *Ganoderma* attacked standings (areas) and whether *Ganoderma* (basal stem rot pathogen) could survive on those litters. Diversity of fungi including antagonistic fungi on both areas might be correlated with the disease development.

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MATERIALS AND METHODS

Study Sites. The study was conducted at Trial Research and Development area of *A. mangium* industrial forest, PT Riau Andalan Pulp and Paper (RAPP) Riau, at Baserah sector, Kuantan Hilir Subdistrict, Kuantan Singingi District, since March to November 2007. *A. mangium* trees at study sites were two years old.

Decomposition of Leaf Litters. The experiment carried out using litterbag method (White & Haines 1988; Sariyildiz 2003). Freshly fallen leaf litters of *A. mangium* were collected about 10 kg, and then dried under sunlight to give the dry weight of 50%. The litters were cutting of ca 3 x 5 cm² and 50 gram of those put in a litterbag (20 x 20 cm²) made of nylon net with a mesh size of 1 x 1 mm². Decomposition experiment was carried out in forest floor of 2 years old of *A. mangium* at health (2S) and *Ganoderma* attacked standing (2G) in J.007 compartment (Longitude E 101° 47' 32.1" Latitude S 00° 20' 48.2"). Litterbags were placed within plot of 6 x 15 m² (3 rows x 7 columns of trees). Eleven litterbags were placed randomly in each of three replicated plots and late them for eight months of observation. Total of litterbags were 66 for both treatments (2S and 2G). Each litterbag was placed between F and H litter layers (7-10 cm of the top soil) and then fixed with bamboo stick. Litter weight losses, cellulose, lignin, C, and N contents were measured each month for eight months, as well as, fungi were isolated from all litter samples. The treatment consisted of three replications for both 2S and 2G.

Litter Analysis. Weight losses of litters were measured by Andersson method (2005), cellulose and lignin contents determined by Van Soest method. Organic-C contents measured by titration method and N contents measured by Kjeldahl. The analysis conducted at Laboratory of Animal Husbandry Research Bureau, Department of Agriculture at Ciawi Bogor and at Laboratory Research Centre for Bioresources and Biotechnology, IPB, Bogor.

Isolation of Fungi. Fungal isolation of each litter samples was conducted by dilution method according to Osono and Takeda (2002). Litter samples were cut ca. 0.5 cm and 10 gram of samples added to 90 ml distilled sterile water. Sample suspensions were shaken in vortex for three minutes to release fungal spores and mycelia. Suspensions were diluted in order to get a dilution of 10³ and 10⁴ and 1 ml of each dilution was pured into Petri dish. Then malt extract agar (MEA) with 250 mg/l chloramphenicol (temperature ca. 40 °C) was poured into Petri dish and it was swirled gently with a rotary motion in order to get the homogeneous mixture. The work was repeated twice. Fungal population Colony Forming Unit (CFU) was counted after 24 hours of incubation at room temperature (± 28 °C). The counting of colonies were continued until no colonies appeared anymore. Populations of each species were calculated as well. Each colony was isolated and identified by observing the texture characteristics of colonies, and microscopic characters i.e. hyphae, spore, and others structures (Watanabe 2002).

Data Analysis. Decomposition rate of *A. mangium* leaf litters was measured using Olson model (Takeda *et al.* 1984):

$$x_t/x_0 = e^{-kt}$$

where: x_t = the mass of litters at t, x_0 = the initial mass of litters, e = natural logarithm (2.7183), k = decomposition rate per month, t = the time of observation.

For data analysis, that model was transformed to linier regression:

$$Y = bx$$

where: $Y = \log x_t/x_0$; $b = -k \log e$; $x = t$

Diversity indices of fungi was calculated by *Shannon's diversity Index* (Magurran 1988):

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

$$p_i = \frac{n_i}{N}$$

where: H' = diversity index, s = number of species, p_i = propotion of individuals found in the i^{th} species, n_i = number of species to i , N = total of species.

Evenness index of fungi was determine using equation $E = 1/\ln(S)$

where: H' = diversity index, S = number of species (Atlas & Bartha 1993).

RESULTS

Decomposition of Leaf Litters. Eight months after decomposition of *A. mangium* leaf litters were placed under canopy of 2S and 2G standings, the results showed that remained litters were of 65.39% (weight losses of 34.61%) and 69.36% (weight losses of 30.64%), respectively (Figure 1). There were significant differences in percentages of litter losses for both standings ($P = 0.009$). Lignin contents of litters in 2S and 2G standings were decrease after eight months of decomposition from 51.34 to 31.29% (losses of 20.05%) and 51.52 to 37.65% (losses of 13.87%), respectively (Figure 2a). Likewise cellulose contents of litters were decreased from 34.16 to 17.82% (losses of 16.34%) in 2S standing and 34.39 to 19.68% (losses of 14.71%) in 2G standing (Figure 2b). There were significant differences in percentage of lignin and cellulose losses for both standings ($P = 0.03$ and $P = 0.02$

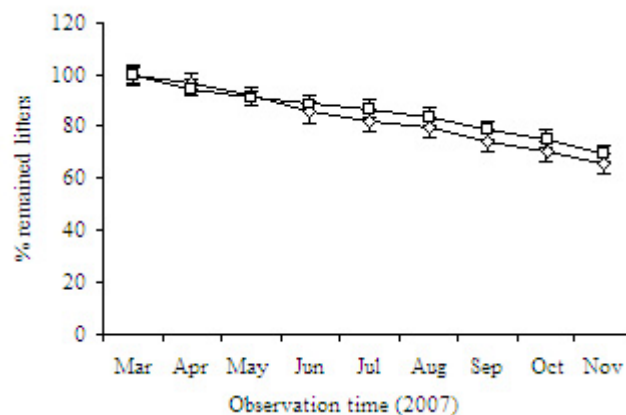


Figure 1. Percentage of remained litters of *A. mangium* during decomposition in two years' old standings. (◇) Health standing, (□) *Ganoderma* attacked standing.

respectively). Decomposition rates of litters placed under canopy of 2S and 2G standings were 0.7 and 0.51 year⁻¹ respectively, decomposition rates of lignin were 0.59 and 0.36 year⁻¹ respectively, and decomposition rates of cellulose were 0.99 and 0.81 year⁻¹ respectively (Table 1).

During decomposition process, organic-C contents tend to decrease from 38.18 to 25.91% in 2S standing and 40.07 to 24.34% in 2G standing, but N contents tend to increase from 1.43 to 2.27% on 2S standing and 1.43 to 2.20% on 2G standing (Figure 3).

Fungal Population. The number of species isolated from leaf litters placed under 2S and 2G standings were relatively resemblance, that were 21 and 20 respectively with similar patterns of species number during eight months for both standings (Table 2). The number of species during eight months for both standings was in a range of 4 to 9 and 3 to 8

respectively. The highest number of species on June and July for 2S standing, but only on June for 2G standing. Average total populations of 2S and 2G standings were in range 36 to 202 x 10³ and 40 to 269 x 10³ CFU/ml respectively. The highest total populations were found on March for 2S standing that were dominated by *Penicillium minioluteum* with relative frequency (RF) of 11.76% and on March and April for 2G standing that were dominated by *Aspergillus flavus* (RF of 10.64%) and *Aspergillus* sp5 (RF of 6.39%). Based on these results, *Aspergillus* sp5 had high population but low in relative frequency. The highest diversity index was discovered on July for 2S standing and on November for 2G standing, while the lowest of evenness index (indication of dominant species) in 2S and 2G standings were happened on October and April, respectively.

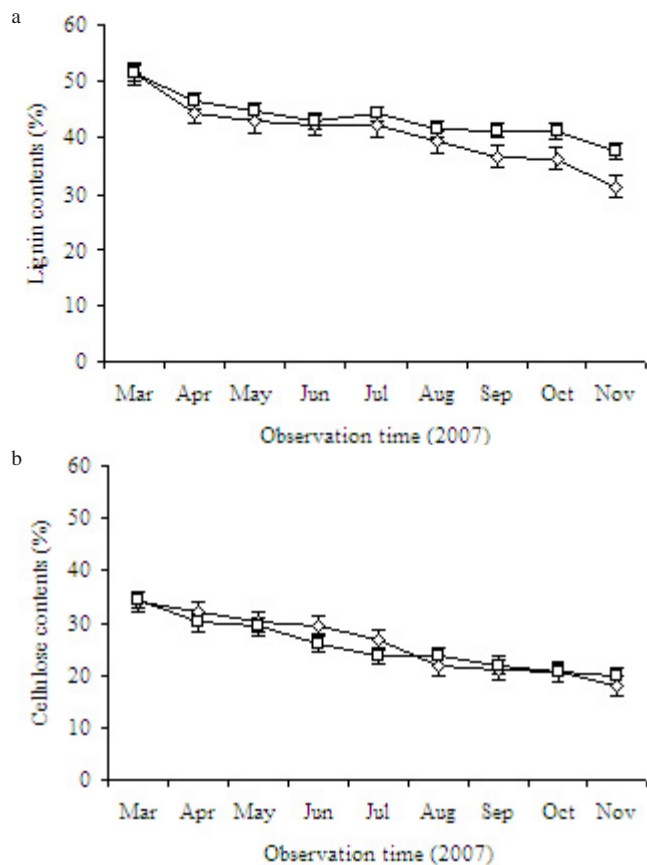


Figure 2. Lignin and cellulose contents of *A. mangium* leaf litters during decomposition in two years old standings. a. Lignin contents, b. Cellulose contents. (◇) Health standing, (□) *Ganoderma* attacked standing.

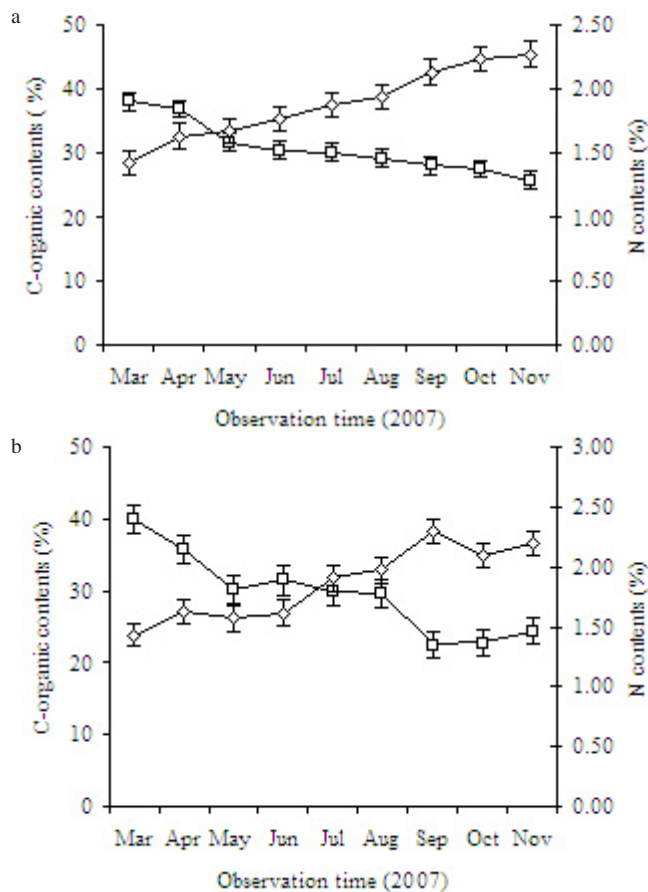


Figure 3. N and C contents of *A. mangium* leaf litters during decomposition in two years old standings. a. Health standing, b. *Ganoderma* attacked standing. (◇) N, (□) C.

Table 1. Decomposition rate of litters, cellulose, and lignin in two years old of *A. mangium*s standings

Standing condition		Decomposition rate	Lignin decomposition	Cellulose decomposition
Health standing	k	0.70	0.59	0.99
	R ²	0.99	0.92	0.97
<i>Ganoderma</i> attacked standing	k	0.51	0.36	0.81
	R ²	0.98	0.87	0.97

k = decomposition rate per year, R² = coefficient of determination.

Table 2. Fungal diversity during decomposition of *A. mangium* litters in two years old standings

Observation time (2007)	Total species (S)/months	Mean of total population (CFU x10 ³)/ml	H'	E	Fungal species with highest population	RF
Health standing (21 species)						
March	5	202	1.371	0.852	<i>Penicillium minioluteum</i>	11.76
April	5	142	1.097	0.682	Sp 28D	7.84
May	6	88	1.200	0.696	<i>Penicillium</i> sp2	9.80
June	7	147	1.645	0.845	<i>Aspergillus fumigatus</i>	5.88
July	9	58	1.788	0.814	Sp 6D	7.84
Augusts	5	88	1.206	0.749	<i>Trichoderma</i> sp2	5.88
September	4	75	1.342	0.968	<i>Mortierella exigua</i>	1.96
October	5	137	0.807	0.501	<i>Aspergillus parasiticus</i>	3.92
November	5	36	0.974	0.703	<i>Pythium splendens</i>	3.92
Attacked <i>Ganoderma</i> standing (20 species)						
March	5	269	1.462	0.908	<i>Aspergillus</i> sp5	6.39
April	6	160	0.902	0.560	<i>Aspergillus flavus</i>	10.64
May	5	122	1.252	0.778	<i>Penicillium minioluteum</i>	12.77
June	8	123	1.481	0.712	<i>Penicillium minioluteum</i>	12.77
July	5	71	1.449	0.900	<i>Aspergillus fumigatus</i>	8.51
Augusts	3	62	0.790	0.719	<i>Phialophora richardsiae</i>	2.13
September	5	99	1.361	0.846	<i>Mortierella exigua</i>	4.26
October 2007	5	119	1.164	0.723	<i>Aspergillus parasiticus</i>	2.13
November 2007	5	40	1.551	0.964	Sp 40 D	2.13

Explanation: H' = Diversity index, E = Evenness index, RF = Relative frequency.

DISCUSSION

Litter weight losses, lignin and cellulose contents were significant among 2S and 2G standings during litter decomposition. Decomposition process in 2S was more rapidly than 2G. Climate condition as well as litters water content might affect the decomposition process. In this study, litters water content in 2G standing were lower than 2S (Figure 4), and physically the litter conditions under 2G standing were more dry than 2S. This condition could affect the growth of decomposer especially fungal community. Water existence in the substrates is an important factor that affected the fungal activities especially for *Trichoderma* strains (Kredics *et al.* 2003). In this study, litter weight losses after eight months decomposition i.e. 34.61% in 2S and 30.64% in 2G were lower than previous studies i.e. 61.07% after 4.5 month (Siregar *et al.* 1999) and 55.8% after 12 month (Hardiyanto *et al.* 2004). These results obtained by using litterbag with a mesh size bigger than 1 mm². By using litterbag with a mesh size of 1 mm², litters could not contact with worms and another soil animals which affected decomposition process, therefore litters decomposition was conducted only by soil microbes mainly by fungi and bacteria (Dickinson & Pugh 1974).

Litter decomposition rates of *A. mangium* in this study were 0.7 year⁻¹ in 2S standing and 0.51 year⁻¹ in 2G standing. These results were lower than previous study i.e. 0.84 year⁻¹ (Hardiyanto *et al.* 2004). As well as cellulose decomposition rate was higher than lignin, because during litter decomposition process, fungi will degrade simple substances than the complex ones (Deka & Mishra 1982). Basically, carbohydrates such as celluloses and hemicelluloses are more degradable, however, lignin is resistant to most microorganisms due to its structure of phenylpropane units and the recalcitrant linkages between them (Schmidt 2006).

During decomposition process, organic-C content tend to decrease and the other hand N content tend to increase.

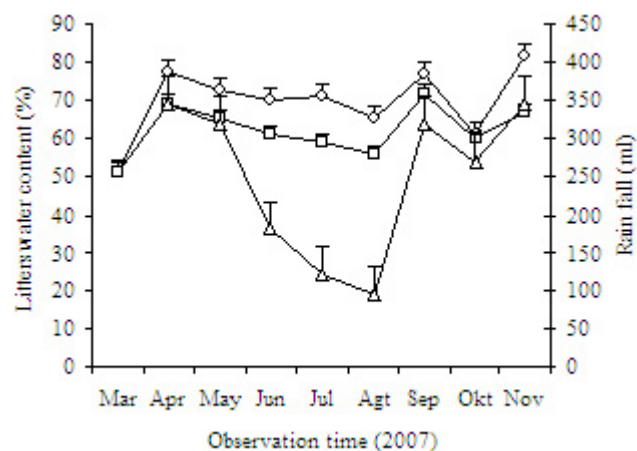


Figure 4. Litters water content and rain fall during during decomposition of *A. mangium* leaf litters in two years old standings. (○) Health standing, (□) *Ganoderma* attacked standing, (△) Rain fall.

This results had similar patterns with previous study that done by Setiawan (1993). Increasing of N content in remained litters came from cell protein of leaf litters degraded by fungi, and also from mobile nitrogen in fungal hyphae and immobile nitrogen of enzymes. In addition, mycelia grown in leaf litters contributed in nitrogen concentration (Miyamoto & Hiura 2008).

The number of species in 2S and 2G standings during eight months of leaf litters decomposition had resemble patterns. They were low during the first period (March and April) and then tend to increase on May, June, and July (second period), afterwards they tend to decrease on August to November (third period). Species grown on both standings were different. High number of fungal species in second period related to high in diversity and evenness indices. It indicated that there were no dominant species in communities. Low evenness indices or low dominant species were occurred only

on October and April for 2S and 2G respectively. This result was similar to Atlas and Bartha (1993) that during early stages of community succession, the number of species tend to increase and peak of species diversity was probably during early or middle period of succession and gradually decline in the stable community.

Fungal succession occurred on leaf litters of *A. mangium* during eight months of decomposition in 2S and 2G standings (Figure 5). In the first to second period of decomposition, the fungal species were dominated by genera of *Penicillium* and *Aspergillus* as sugar fungi and soil inhabiting fungi while the third period by *Trichoderma* (as cellulose consumer), *Phialophora*, *Mortierella*, and *Pythium* as secondary sugar fungi. This pattern of succession was similar to the pattern explained by Dix and Webster (1995). Earlier fungal colonizer could consume cellulose in the litters other than sugar,

therefore they are able to hydrolyze carbohydrates and holocelluloses (Osono 2005).

The presence of *Penicillium*, *Aspergillus*, and *Trichoderma* was useful to reduce basal stem rot incidence of 5 to 40% in oil-palm seedlings (Sariah & Zakaria 2000). Based on this research results, *Trichoderma* was found on third period (August to November). Thus, the application of *Trichoderma* in *A. mangium* plantation is suggested after five months of leaf litter decomposition. *Trichoderma* was highly antagonistic to *Ganoderma*, the pathogen of basal stem rot diseases (Harjono & Widyastuti 2001).

In this study, *Ganoderma* and other Basidiomycetes were not found during eight months of decomposition. In general, these fungi could degrade leaf litters of *A. mangium* which contain high lignin and cellulose contents. These results were correlated to the lower losses of litter weight, lignin, and cellulose contents. The other hand, the ability of many Agarics (Basidiomycetes) to degrade lignin and cellulose tested on *Fagus sylvatica* leaf litters and straw of *Glyceria maxima* was high with lignin losses of 32.3 to 76.5% on *Fagus sylvatica* and 41 to 77% on *Glyceria maxima*. Cellulose losses was 6.4 to 73.9% on *Fagus sylvatica* and 35.4 to 84.0% in *Glyceria maxima* (Dix & Webster 1995).

Future studies should be undertaken to observe the decomposition of *A. mangium* leaf litters in long period more than eight months, in order to find fungal species including *Ganoderma* with might be completed the decomposition. In addition, the research of fungal succession on leaf litters of *A. mangium* in older standings should be observed in the future in order to completed basic information of leaf litter degradation.

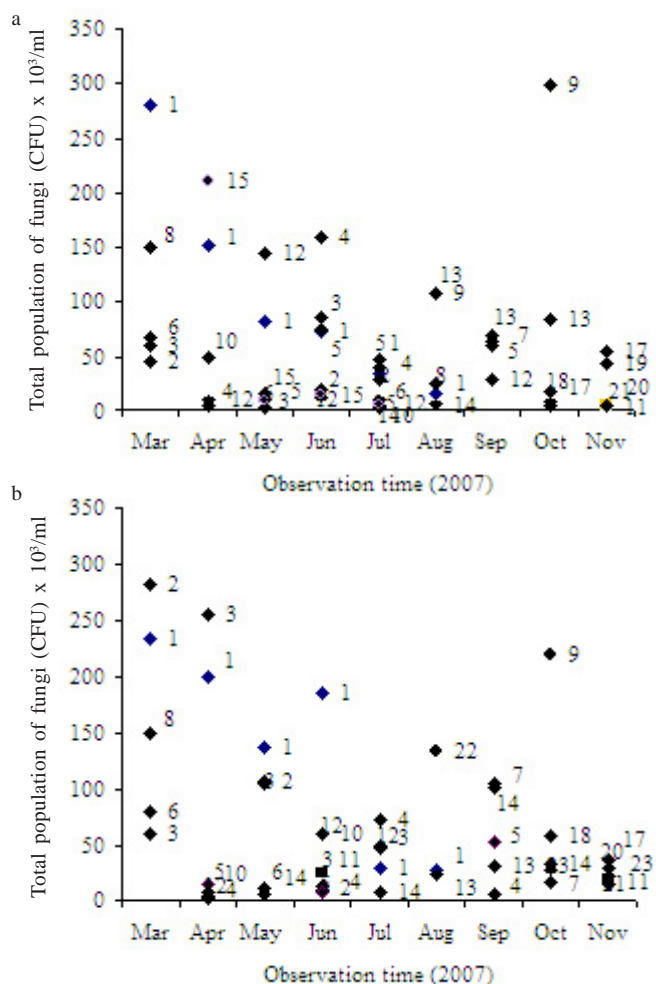


Figure 5. Population of fungi during decomposition of *A. mangium* leaf litters in two years old standings. a. Health standing, b. *Ganoderma* attacked standing. 1. *Penicillium minioluteum*, 2. *Aspergillus* sp5, 3. *Aspergillus flavus*, 4. *Aspergillus fumigatus*, 5. Sp 6D, 6. *Aspergillus* sp3, 7. *Mortierella exigua*, 8. *Aspergillus* sp6, 9. *Aspergillus parasiticus*, 10. *Aspergillus* sp7, 11. Sp 16D, 12. *Penicillium* sp2, 13. *Trichoderma* sp2, 14. *Pythium intermedium*, 15. Sp 28D, 16. *Pythium* sp1, 17. *Pythium splendens*, 18. *Trichoderma koningi*, 19. *Pythium elongatum*, 20. Sp 40 D, 21. Sp 41D, 22. *Phialophora richardisiae*, 23. *Trichoderma* sp3.

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