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Growth responses of oil palm seedling inoculated with *Ganoderma boninense* under competition with edible herbaceous plants

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ABSTRACT

Basal stem rot (BSR) caused by long-term survival fungus *Ganoderma boninense* is the most important of oil palm disease that difficult to be controlled. Perennial herbaceous species are potentially developed for long-term BSR control and applied as mixed planting with oil palm. This research was aimed to study the competition effects of mixed planting with edible herbaceous perennial plants (edible canna (*Canna indica*), arrowroot (*Maranta arundinacea*), cocoyam (*Xanthosoma sagittifolium*), and water yam (*Dioscorea alata*) on growth of oil palm seedlings. Two competition trials (non-inoculated and *Ganoderma*-inoculated trial) were conducted. The herbaceous plants were grown together with oil palm seedling in a polybag filled with 40 L field soil. Mixed planting of *Ganoderma*-inoculated oil palm and herbaceous plants and grown on a large soil volume resulted in a minor infection with a high variation in severity leading to insignificant effect of herbaceous competition on *Ganoderma* infection. Herbaceous competition significantly suppressed oil palm seedling growth under both inoculated with *Ganoderma* and without inoculation. Arrowroot exhibited as the most competitive herbaceous species and followed by canna and cocoyam. Water yam showed a weak competitive against oil palm seedling since only a minor interference in all observed growth characteristics.

KEYWORDS: Herbaceous competition, oil palm seedling, *Ganoderma boninense*, *Canna indica*, *Maranta arundinacea*, *Xanthosoma sagittifolium*, *Dioscorea alata*.

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INTRODUCTION

Oil palm is the most economically important industrial crop and now cultivated for more than 14 million hectares in Indonesia. Basal stem rot (BSR) caused by wood decay fungus *Ganoderma boninense* Pat. is the most serious and widespread disease on oil palm plantations in Indonesia [1]. BSR causes direct economically losses on due to both direct mortality and decreasing in fresh fruit bunch yield of infected palm [2]. BSR is more prevalence as increasing of plant generations. In North Sumatra, disease prevalence of immature palm was 0, 4, 7, and 11% in first, second, third and fourth generation plantings, respectively. Disease incidence of mature palm at 1% can lead to an estimated USD 38 million loss to the Indonesian economy [3]. *G. boninense* has long-term survival on diseased root debris in soil and infects roots of the new establishing palms during replanting [4]. Oil palm harbors abundant small roots and difficult to be fully cleaned leading to failure of disease control through common field sanitizing procedures.

Oil palm plantations in Indonesia commonly implements a standard monoculture planting procedure, although some smallholding farmers apply a mixed crops planting. There was no report about BSR infestations on the mixed crop plantations of small farmer. Potential use of mixed cropping with perennial herbaceous plants against long-term survival wood decay fungus *Rigidoporus microporus* has been evaluated in rubber plantation [5]. Mixed planting with herbaceous plants such as edible canna (*Canna indica*), arrowroot (*Maranta arundinacea*), and cocoyam (*Xanthosoma sagittifolium*), and water yam (*Dioscorea alata*) resulted in reduced mycelial growth and inoculum viability of *R. microporus* [6]. Silva *et al.*[7] demonstrated that aqueous rhizome extracts of *Curcuma domestica* (turmeric) and *Alpinia galanga* (galangale) showed an allelopathic inhibition on *R. microporus* both on agar and soil medium.

Edible herbaceous perennial plants are commonly grown in Indonesia for sources of starches and medicinal herbs as understory home garden plants. The plants produce rhizome and corm and undergo perennial life cycle. The plants have a robust growth and produce an extensive root system and therefore can either potentially influence survival of soil pathogens or interfere the growth of main crop. This study was aimed to determined interference competition of herbaceous plants on infection of *G. boninense* and growth of oil palm as main crop.

MATERIALS AND METHODS

Competition Experiments

Two competition trials between edible herbaceous perennial plants and oil palm seedling were conducted, *viz.* non-inoculated and *Ganoderma*-inoculated trial. Each trial consisted of mixed planting between single herbaceous plant and single oil palm seedling and the plant combinations were grown on ten polybags as replication. Single oil palm seedling was used for non-competition control. The herbaceous plants used in this study were edible canna (*Canna indica*), arrowroot (*Maranta arundinacea*), cocoyam (*Xanthosoma sagittifolium*), and water yam (*Dioscorea alata*). Rhizomes or corms of the herbaceous were collected from home gardens in South Sumatra, Indonesia. Soil collected from experimental field of Faculty of Agriculture Sriwijaya University filled to 40-L volume within 60 × 60 cm black polybag (68 L) was used as planting medium throughout study. Sprouting rhizomes or corms with homogenous in sized and growth were selected and grown at 15-cm spaced with 3-month-old palm seedling. Polybags were arranged in green house allowing 90-cm spacing between oil palm seedling. Plant were monthly fertilized with 10 g NPK 15-15-15 per polybag and allowed to growth for one months prior to inoculation with *Ganoderma boninense*. For trial with inoculation, *G. boninense* colonizing a cylindrical rubber wood block (RWB, 5 cm diam. × 5 cm length) incubated for 6 months was used as pathogen inoculum. Inoculation was performed by burying RWB in contact with root of oil palm in depth 15 cm from the soil surface.

Effects on *Ganoderma* Infection

Infection of *G. boninense* was measured at the end of trial (7 month after mixed planting or 6 months post-inoculation) by counting percentage number of infected main root (number of main roots showing typical necrotic symptoms / total number of main roots of individual palm × 100%) and length of necrotic main root (total length of necrotic lesion of main roots / total length of main roots of individual palm × 100%). Colonization of inoculated *G. boninense* on the necrotic lesion was confirmed by re-isolation of *G. boninense* from surface sterilized necrotic root on a semi-selective *Ganoderma* medium [8] and followed by microscopic examination. Disease symptoms were also examined for necrotic lesion on bole (swollen stem base) of oil palm by cutting the bole longitudinally at the end of trial.

Effect of herbaceous competition on wood decay activity of *G. boninense* was measured as decay of *Ganoderma*-colonized RWB. Wood decay activity of RWB was estimated based on reduction in dry weight of RWB at 6 months after inoculation relative to initial dry weight at inoculation (dry weigh of inoculated RWB / initial dry weight of RWB × 100%). Initial dry weight of RWB was predicted by measuring 5 representing wood block samples after drying at 105 °C for 24 hours.

Effects on Oil Palm Growth

Number of leaves, plant height, and stem diameter of oil palm seedling were measured at 1, 2, 4, and 7 months after mixed planting. Number and length of main roots, and fresh weight of total roots of individual palm was quantified immediately after uprooting at the end of experiment (7 months after mixed planting).

Data Analyses

Disease and plant growth variables between different mixed planting were analyzed using one-way analyses of variance and compared using Tukey's honestly significant test. Percentage number of infected main root and length of necrotic main root data were square root transformed to meet assumptions of normality or homogeneity of variance and data shown are untransformed means.

Analyses were applied using the packages Rcmdr and Agricolae for R version 3.6.2 (R Foundation for Statistical Computing).

RESULTS

Effects on *Ganoderma* Infection

Inoculation of *G. boninense* using *Ganoderma*-colonized RWB and grown on a large volume (40 L) of field soil resulted in a minor infection with a high variation in severity on roots of oil palm seedling as shown by a small portion of necrotic roots. Infection was confirmed by successful re-isolation of *G. boninense* from surface sterilized necrotic root on a semi-selective *Ganoderma* medium and microscopic examination. Mixed planting with herbaceous plants did not significantly ($P \geq 0.05$) affected both the percentage number of infected main root and length of necrotic main root. Mixed planting with arrowroot had a tendency to induce more *Ganoderma* infection as shown by a relative higher number and proportion of infected main root (Table 1). Insignificant effect was associated with a high variation between replication due to no infection on some palms for 6 months post-inoculation. BSR main symptom as either a decay of the bole (swollen stem base) or secondary leaf symptom was not developed when grown on a large volume of soil as conducted in this study. Decay activity of *Ganoderma*-colonized RWB as assessed by reduction in dry weight was not significantly affected by mixed planting with herbaceous plant. Planting with cocoyam resulted in a slightly lower decay activity (statistically non-significant) and had similar decay of the RWB buried in soil without plant (38.9%) (Table 1).

Effects on Oil Palm Growth

Competition of oil palm seedling with herbaceous plants as studied in mixed planting in non-inoculated trial did not significantly affect oil palm leaf number, except for planting with canna and arrowroot that significantly had lower leaf number compared to control. In *Ganoderma*-inoculated trial, planting with arrowroot and cocoyam significantly reduced leaf number of oil palm at 2 and 4 months after competition, respectively. Oil palm seedlings in *Ganoderma*-inoculated trial produced a slightly higher number of leaves compared to that of non-inoculated trial (Table 2).

Stem diameter of oil palm seedling was negatively affected when grown in competition with herbaceous plants. In non-inoculated trial, effect of herbaceous competition was evident over 7 months after mixed planting as significantly reduced stem size of oil palm in mixed planting with cocoyam. Markedly reduced in stem diameter of oil palm was shown in *Ganoderma*-inoculated trial. Planting with arrowroot started to reduce stem growth at 1 month after mixed planting and then continue to the end of trial. Competition with canna and cocoyam showed negative effect on stem growth by significantly reduced stem diameter at 4- and 7-months post-inoculation, respectively. Mixed planting with water yam had a non-significant effect on stem growth of the palm seedling compared to control as observed in both trials (Table 3; Figure 1). Oil palm seedlings under competition with cocoyam and water yam in *Ganoderma*-inoculated trial had a slightly wider stem compared to that of non-inoculated trial (Table 3).

In contrast to the negative effect of herbaceous competition on stem diameter, planting with the herbaceous did not significantly reduce height of oil palm seedling. Significant retardation of plant height was observed only on arrowroot at 7 months after mixed planting in *Ganoderma*-inoculated trial (Table 4, Figure 1). Oil palm seedlings under competition with cocoyam and water yam in *Ganoderma*-inoculated trial had a slightly higher plant height compared to that of non-inoculated trial (Table 4).

Root growth oil palm seedling as measured by number of primary roots, total length of primary root and fresh weight of total roots were severely inhibited particularly when the palms was inoculated with *G. boninense*. In competition trial without inoculation, only fresh weight of total roots was significantly interfered by the herbaceous plants. Arrowroot competition resulted in reduced the primary root number and length, but mixed planting with water yam showed non-significant effect on those variables of root growth. Competition with all herbaceous plants in both trials significantly reduced total root fresh weight of oil palm seedling. Similar to the growth responses of upperparts, root of oil palm seedlings under competition with cocoyam and water yam in *Ganoderma*-inoculated trial grew slightly better compared to that of non-inoculated trial (Table 5).

Table 1: Effect of mixed planting for 7 months with herbaceous plants on root infection by *Ganoderma boninense* and decay of *Ganoderma*-colonized rubber wood block (RWB)

Competition treatment	% number of infected main root	% length of necrotic main root	RWB decay (%)
Oil palm (control)	18.8	7.1	47.2
Edible canna+oil palm	18.4	7.1	43.6
Arrowroot+oil palm	47.7	17.7	43.7
Cocoyam+oil palm	29.9	10.2	42.9
Water yam+oil palm	26.0	9.4	38.2
F statistic	1.90^{ns}	1.38^{ns}	1.16^{ns}
P	0.126	0.256	0.340

Table 2: Effect of competition with herbaceous plants on leaf number of oil palm seedling

Competition treatment	Month after mixed planting			
	1	2	4	7
Non-inoculated trial				
Oil palm (control)	7.2	7.2	8.7^{a}	10.9
Edible canna+oil palm	6.3	7.2	8.3^{b}	10.0
Arrowroot+oil palm	6.3	6.3	7.2^{b}	9.7
Cocoyam+oil palm	6.6	7.1	$7.8a^{b}$	10.4
Water yam+oil palm	6.5	7.3	8.7^{a}	10.7
F statistic	1.64^{ns}	2.0^{ns}	4.47*	0.95^{ns}
P	0.179	0.110	0.039	0.446
Ganoderma-inoculated trial				
Oil palm (control)	7.7	7.8^{a}	9.5^{a}	11.8
Edible canna +oil palm	6.9	7.1^{ab}	8.5^{ab}	11.0
Arrowroot+oil palm	6.5	6.6^{b}	8.2^{ab}	10.2
Cocoyam+oil palm	6.6	6.9^{ab}	7.9^{b}	10.4
Water yam+oil palm	7.4	7.5^{ab}	8.8^{ab}	10.9
F statistic	2.48^{ns}	2.97*	2.68*	2.40^{ns}
P	0.056	0.029	0.043	0.063

Table 3: Effect of competition with herbaceous plants on stem diameter (mm) of oil palm seedling

Competition treatment	Month after mixed planting			
	1	2	4	7
Non-inoculated trial				
Oil palm (control)	13	15	20	32^{a}
Edible canna+oil palm	10	14	18	26^{ab}
Arrowroot+oil palm	10	14	17	26^{ab}
Cocoyam+oil palm	10	12	19	24^{b}
Water yam+oil palm	11	15	20	27^{ab}
F statistic	1.40^{ns}	1.57^{ns}	1.70^{ns}	3.27*
P	0.240	0.190	0.150	0.020
Ganoderma-inoculated trial				
Oil palm (control)	17^{a}	17^{a}	22^{a}	35^{a}
Edible canna+oil palm	14^{ab}	14^{ab}	$19^{ab}c$	25^{b}
Arrowroot+oil palm	13^{b}	13^{b}	16c	24^{b}
Cocoyam+oil palm	13^{ab}	14^{ab}	$17^{b}c$	26^{b}
Water yam+oil palm	16^{ab}	16^{ab}	21^{ab}	29^{ab}
F statistic	3.60*	3.61*	4.51*	6.96*
P	0.010	0.008	0.003	0.0001

DISCUSSION

Mixed planting of *Ganoderma*-inoculated oil palm and herbaceous plants and grown on a large soil volume resulted in a minor infection with a high variation in severity

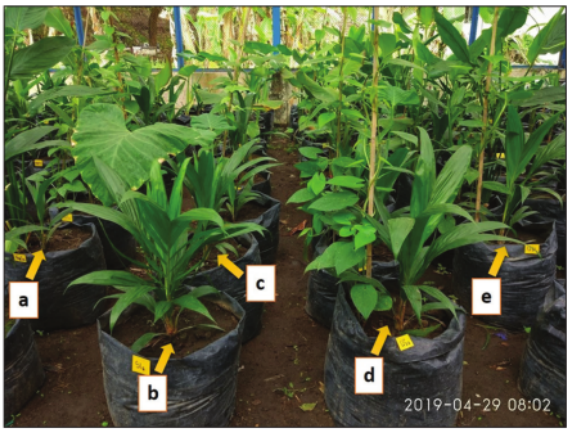

Figure 1: Growth performance of oil palm seedlings under 6-month-competition with herbaceous plants and inoculated for 5 months with *Ganoderma boninense* in greenhouse; a) growth retardation by arrowroot, b) control oil palm inoculated with *G. boninense*, c) a slight growth suppression by cocoyam, and d-e) minor growth interference by water yam.

Table 4: Effect of competition with herbaceous plants on height (cm) of oil palm seedling

Competition treatment	Month after mixed planting			
	1	2	4	7
Non-inoculated trial				
Oil palm (control)	37	42	56	92
Edible canna+oil palm	39	44	57	85
Arrowroot+oil palm	39	42	53	81
Cocoyam+oil palm	34	39	48	71
Water yam+oil palm	39	44	56	89
F statistic	0.86^{ns}	0.80^{ns}	0.98^{ns}	1.44^{ns}
P	0.49	0.53	0.43	0.24
Ganoderma-inoculated trial				
Oil palm (control)	38	43	60	99^{a}
Edible canna+oil palm	36	41	52	78^{ab}
Arrowroot+oil palm	35	38	47	71^{b}
Cocoyam+oil palm	37	41	52	82^{ab}
Water yam+oil palm	42	48	60	92^{ab}
F statistic	1.02^{ns}	1.83^{ns}	2.55^{ns}	2.71*
P	0.36	0.13	0.05	0.04

Table 5: Effect of competition with herbaceous plants for 7 months on root growth of oil palm seedling

Competition treatment	Number of primary roots	Total length (cm) of primary root	Fresh weight of total roots (g)
Non-inoculated trial			
Oil palm (control)	10.7	453	35.5^{a}
Edible canna+oil palm	8.8	411	20.2^{b}
Arrowroot+oil palm	8.5	343	18.9^{b}
Cocoyam+oil palm	8.1	343	15.7^{b}
Water yam+oil palm	8.9	403	20.7^{b}
F statistic	0.90^{ns}	1.05^{ns}	5.27*
P	0.472	0.391	0.001
Ganoderma-inoculated trial			
Oil palm (control)	12.1^{a}	559^{a}	37.9^{a}
Edible canna+oil palm	8.4^{ab}	360^{bc}	20.2^{b}
Arrowroot+oil palm	8.0^{b}	312^{c}	13.9^{b}
Cocoyam+oil palm	10.3^{ab}	474^{ab}	22.8^{ab}
Water yam+oil palm	9.7^{ab}	433^{abc}	22.1^{b}
F statistic	2.62*	5.59*	5.48*
P	0.048	0.001	0.001

leading to insignificant effect of herbaceous competition on *Ganoderma* infection. *G. boninense* is known as a weak pathogen that need a large volume of wood to be successfully initiated an infection. RWB size used in this study was within the range of commonly used for inoculation assays [9, 10], except for large soil volume to accommodate mixed planting of palm and herbaceous plants. Volumetric ratio of RWB inoculum in this study was 0.28% related to soil volume. Breton *et al*. [9] also reported no infection on bole of oil palm over 10 months inoculation when using RWB inoculum at 0.7% soil volume. Lower volumetric inoculation ratio between inoculum and soil might result in lower inoculum potential that delay the pathogenesis. It is well known that disease progress of BSR is low, but its eventually kill the palm once infected. Diseased young palms die within 6–24 months after first symptoms, but need 2–3 years to cause total mortality of mature palms [11].

Mixed planting with herbaceous plants either inoculated with *Ganoderma* or without inoculation resulted in marked growth inhibition of oil palm seedlings suggesting negative interference of herbaceous plant species. Herbaceous vegetation naturally dominates understory habitat of forest tree and is known as a major source of interference with the young woody plants [12]. Amongst the herbaceous tested, arrowroot was showed the most deleterious interference to oil palm seedling. Arrowroot started to reduce stem growth of oil palm at one month after mixed planting and then continue to the end of trial. Canna dan cocoyam also showed a negative interference on growth of oil palm seedling, but the growth inhibition was appeared over 4 months of mixed planting. Water yam was shown to have less aggressive interference to oil palm seedling as mixed planting with this climbing herbaceous resulted in non-significant growth reduction, except for fresh weight of root.

Plant interference typically refers to either competition for resources (mainly light, water and nutrients) or allelopathy [13]. Allelopathy of arrowroot was not reported, although rhizome of *M. arundinacea* contained 49 phytochemical compounds belong to flavonoids, alkaloids, tannins, glycosides, steroids, phenols and saponins [14]. *C. indica* has been reported to secrete a high concentration of organic acid that induced allelopathic effects on paddy weeds (*Echinochloa crusgalli* and *Monocharia vaginalis*) [15] and the blue-green algae *Microcystis aeruginosa* [16]. *X. sagittifolium* has known to contain a high concentration of raphides (needle-shaped calcium oxalate crystals) and cysteine protease [17], which plays defensive roles against herbivores, but not reported to act as allelochemical against other plants. Competition for water was obviously noticed during greenhouse trial, although no detailed measurement of soil water dynamics in response to mixed planting. Soil medium of mixed planting bags (polybag) were easily dried compared to wet appearance of single oil palm soil medium. The most fastest drying in soil medium was shown by mixed planting with arrowroot. Compared to other plants, arrowroot exhibited a robust growth, by actively producing new sucker every week. Among the tested herbaceous plants, formation of new shoot was normally low in water yam. It is like that herbaceous plants that actively produce new shoots are

more competitive for water and could cause severe water leakage on oil palm seedling. Oil palm has been considered to have a high transpiration rate and the transpiration exponentially as increasing of plant age to 5-year-old [18]. Weakly competitive herbaceous plant particularly for soil water should be considered to be further studied as understory crop for BSR control research on young oil palm planting.

Mixed cropping of edible herbaceous species as understory crop of mature oil palm could benefit for food security, biodiversity and ecosystem services. Managed understory vegetations is considered to unlikely to compete for nutrients with 23–29-year-old oil palms, but even to enhance soil biodiversity and decomposition rates [19]. It has been also demonstrated that Leopard Cat habitat use was significantly increased under managed understory vegetation, with potential benefits for rat control [20]. In mature rubber plantation (7–32-year-old), mixed cropping with arrowroot, edible canna, and water yam is being practices as understory plants without any known deleterious interferences for over 7 decades [21].

CONCLUSIONS

Mixed planting of *Ganoderma*-inoculated oil palm and herbaceous plants and grown on a large soil volume resulted in a minor infection with a high variation in severity leading to insignificant effect of herbaceous competition on *Ganoderma* infection. Herbaceous competition significantly suppressed oil palm seedling growth under both inoculated with *Ganoderma* and without inoculation. Arrowroot exhibited as the most competitive herbaceous species and followed by canna and cocoyam. Water yam showed a weak competitive against oil palm seedling since only a minor interference in all observed growth characteristics.

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