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Research Article


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# Effect of mixed cropping of water yam (*Dioscorea alata*) on *Ganoderma* disease of oil palm

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## ABSTRACT

Basal stem rot caused by a soil-borne fungus, *Ganoderma boninense*, is one of the most severe diseases in monoculture oil palm plantations. The effect of mixed cropping with water yam on infection and inoculum potential of *Ganoderma boninense* on oil palm seedlings was assessed under a pot experiment. Plant inoculation with rubber woodblock (RWB)-colonized *Ganoderma* induced disease on both mixed cropped hosts (multiple host infection), but with less severe root necrotic and less plant mortality relative to inoculation on a single oil palm host. Inoculum potential of the pathogen was reduced under mixed cropping, as shown by more considerable RWB decay than a single host, and the fungal survival was suppressed to be 55% for 12 months trial. Water yam mixed cropping did not interfere with the growth of the inoculated oil palm seedling. This study revealed the multiple host infection under mixed cropping with water yam suppressed damage caused by *Ganoderma boninense* to oil palm as the primary host.

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**KEYWORDS:** Basal stem rot, *Ganoderma boninense*, mixed cropping, water yam

## INTRODUCTION

Oil palm cultivation in Southeast Asia has severe problems with basal stem rot (BSR) disease caused by fungi of the genus *Ganoderma* (Siddiqui *et al*., 2021). *Ganoderma boninense* has been the most identified species associated with the BSR (Midot *et al*., 2019). BSR fungi decay the xylem tissue, stopping the translocation of water and nutrients and resulting in plant death (Rees *et al*., 2009). In the endemic area of Sumatra, Indonesia, the disease caused plant mortality and reduced plantation stand by 31-67% (Pujianto *et al*., 2016; Riyanto *et al*., 2020). Both direct loss of plantation stands and reduced yield of diseased palms contribute to the yield loss caused by the disease. BSR disease was predicted to cause losses of oil palm yields by 68% in an endemic area (Kamu *et al*., 2021).

Cultural practices and mechanical and chemical treatment have been introduced and applied as control measures for BSR control but have not proved satisfactory (Siddiqui *et al*., 2021). Oil palm plantations commonly operate large-scale monoculture cropping practices for 25-30 years of a single generation plantation. A long-term monoculture practice of genetically homogenous populations is vulnerable to increasing infection pressure by soil-borne fungal pathogens (Yang *et al*., 2020). The marked increase in BSR infestation over the generation of monoculture oil palm plantations has been widely reported in Indonesia (Priwiratama *et al*., 2020) and Malaysia (Kamu *et al*., 2021).

Mixed cropping practices have long been known to contribute to the control of soil-borne diseases (Yang *et al*., 2014; Gibson & Nguyen, 2021). Mixed cropping with understory herbaceous plants has suppressed the white root disease of rubber trees caused by a polypore fungus, *Rigidoporus microporus* (Situmorang *et al*., 2007; Silva *et al*., 2014). Water yam (*Dioscorea alata*) is a perennial herbaceous plant usually grown for its tuber as an understory of smallholder plantation crops, including rubber (Yulianti *et al*., 2017) and oil palm (Teuscher *et al*., 2016). Under pot experiments, water yam could reduce the mycelium growth and viability *of R. microporus* and accelerate the decaying of rubberwood colonized by the white root fungus (Yulianti *et al*., 2017). This study aimed to determine the effects of mixed cropping with water yam on the infection and survival of *Ganoderma boninense* on oil palm seedlings.

## MATERIALS AND METHODS

### Plant Materials and Cropping System

Germinated oil palm seeds of crossed D×P were obtained from the Indonesian Oil Palm Research Institute (IOPRI). The sprouted seeds were sown in trays containing sand media for three months until having 2 to 3 leaves. Water yam tubers were collected from a farmer's garden in Muara Kuang, South Sumatra. The tubers were then cut into squares and sown in a tray with sand media for two months until having a vine of 10 cm in length. The homogenous seedlings of oil palm and water yam were then transplanted into a 5 L-pot filled with a sand and field soil mixture (ratio 1:1) as a planting medium. Plants were planted in a pot under three cropping systems: 1) mixed cropping of oil palm and water yam, 2) single cropping of oil palm, and 3) single cropping of water yam. Each cropping system consisted of 20 plants, and every ten plants were inoculated with or without *Ganoderma* inoculation. For mixed cropping, plants were planted 10 cm apart. The pots were laid under the greenhouse with 50 cm space and watered daily. Plants were fertilized monthly with 0.5% NPK 16-16-16.

### Inoculum and Inoculation

A virulent isolate of *G. boninense* (GBA) was used throughout the study. The fungus was propagated on a rubber woodblock (RWB) (5×12 cm in size). RWB was prepared by soaking in water for 24 hours, arranged in a heat-resistant plastic bag, and sterilized using an autoclave at 121°C 1.5 atm for 2 hours. The autoclaved WRB was added with 5 ml of 2% malt extract liquid media and then re-autoclaved. An MEA culture (5-day-old) of *G. boninense* was then transferred to RWB, and the culture was incubated in the dark for two months until the fungus mycelium covered the entire RWB. The plant inoculation was done by binding the fully colonized RWB with the primary root of the test plants, burying it under the basal stem, and covering it with the planting medium.

### Evaluation of Disease, Inoculum Potential, and Plant Growth

The disease was evaluated monthly based on symptoms, plant mortality, foliar disease severity, percentage number of root necrosis, and length of root necrosis. Symptoms and plant mortality were observed monthly. Foliar disease severity was recorded at three-month intervals using a 0-5 severity scale (Naidu *et al*., 2017). The percentage number and length of root necrosis were assessed at 3- and 12-months post inoculation (mpi). Root infection on necrotic root was confirmed after recovery of *Ganoderma* mycelium on the Ganoderma selective medium (GSM) (Ariffin & Seman, 1991).

Inoculum potential on RWB was assessed after 12 mpi based on dry weight loss of RWB and mycelial viability. Dry weight loss of *Ganoderma*-colonized RWB was calculated by reducing RWB dry weight after burial for 12 months relative to the initial RWB dry weight. Mycelial viability was calculated by the percentage number of RWB sections colonized by *Ganoderma* mycelium out of 20 plated sections on the GSM. Growth oil palm was measured monthly as plant height and leaf area.

## DATA ANALYSIS

A univariate ANOVA test and the post hoc Tukey HSD test were performed to compare means variables of plant disease or plant growth. To meet the ANOVA assumption for normality and variance homogeneity, the Shapiro-Wilk normality test and the Levene variance homogeneity test data were tested before ANOVA. Analysis was carried out using the RStudio, PBC.

## RESULTS

### Infection and Disease Under Mixed Cropping

*Ganoderma* inoculation induced disease on oil palm seedlings and water yam, either planted singly or mixed cropped. Symptoms were earlier produced on water yam (at 3 mpi) than those on oil palm (at 4 mpi). The initial symptom of oil palm was observed in lower leaves that were turned yellowish, wilted, and dry. Once two basal leaves become dead and dry, the symptoms progress toward the upper leaves within one month. Diseased plants experienced stunted growth and died within two months from the first symptoms. The infection originated from the inoculated root, as shown by grayish tissue necrosis on the primary root. Root infection then developed in the basal stem causing a dark color of tissue necrosis. Fruiting bodies are formed at the base of diseased seedlings (Figure 1a).

Inoculated yam showed a sudden wilting on some or all vines, and then a rapid death occurred on the affected vines (Figure 1b). A total vine death was found at 6 mpi. Fruiting bodies grew on the diseased yam plant (Figure 1b). Infection caused partial dark tissue necrosis on the tuber tissue and did not kill the tuber bud. Almost infected yams with a total vine death (10 of 15 plants) sprouted to produce a new vine after 11 mpi (Figure 1c). The typical mycelium of *G. boninense* was confirmed to colonize diseased tubers after being grown on GSM media.

Under mixed cropping, a delay of one month in the first symptoms was observed on oil palm seedlings. *Ganoderma* infection caused plant mortality that gradually increased from 5 to 9 mpi (Figure 1d). After 5-6 mpi, oil palm mortality increased by 4 of 10 plants on single cropping, but the mortality was lower (2 of 10 plants) on mixed cropping. In contrast to oil palm, *Ganoderma* inoculation caused a more progressively increased water yam vine mortality under mixed cropping or single cropping. However, the fungal infection did not kill the tuber bud of the water yam. The growth of water yam was recovered by sprouting the new vine after 8 and 11 mpi for mixed cropping and single cropping, respectively (Figure 1d), and eventually reduced plant mortality.

Foliar disease severity of oil palm was not significantly affected ($P>0.05$) by mixed cropping. Still, the severity was lower under mixed cropping than single host cropping (Figure 2a). When the tested plants were uprooted, root infection of oil palm as measured by percentage number and length of root necrosis was significantly interfered with and suppressed under mixed cropping. The number of necrosis roots was significantly less (P= 0.0224) at 12 mpi on mixed cropped compared to single cropped oil palm (Figure 2b). Length of root necrosis was significantly lower (P= 0.0085) at 3 mpi on mixed cropped compared to single cropped oil palm (Figure 2c). *Ganoderma* colonization was confirmed after plating necrosis root on the GSM.

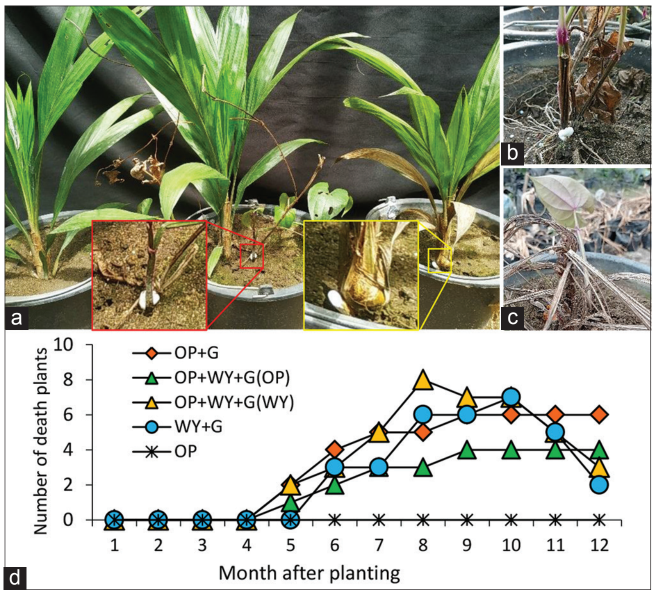


**Figure 1:** Effect of mixed cropping with water yam on symptoms development and plant mortality caused by infection of *Ganoderma boninense*. a) Oil palm seedling after 6 months of inoculation; from left to right, uninoculated control, a healthy mixed cropped oil palm with basidiocarp formation on infected water yam, and diseased single crop oil palm with the formation of basidiocarp at the basal sem. b) Total vine death of infected water yam with the formation of basidiocarp. c) A new vine growth of wilted water yam. d) OP+G: oil palm + Ganoderma; OP+WY+G (OP): Number of dead oil pam on mixed oil palm + water yam + Ganoderma; OP+WY+G (WY): Number of dead water yam on mixed oil palm + water yam + Ganoderma; WY+G: water yam + Ganoderma; and OP: non inoculated control oil palm

## EFFECT ON INOCULUM POTENTIAL AND SURVIVAL

Ganoderma colonization on rubberwood blocks caused losses in dry wood weight due to the decaying of wood materials (lignin and celluloses), which may reduce inoculum potential. *Ganoderma*-colonized RWB buried in contact with oil palm root had significantly less ($P<0.05$) dry weight losses compared to that buried under bare soil or planted with water yam or under mixed cropping of oil palm and water yam (Figure 3a). Burying *Ganoderma*-colonized RWB under mixed cropping with water yam resulted in lower mycelium viability (55%) compared to single cropping oil palm (Figure 3b).

## EFFECTS ON OIL PALM GROWTH

*Ganoderma* inoculation significantly inhibits the growth of oil palm seedlings as measured by plant height and leaf area. Marked inhibition of inoculated oil palm was increased as the plant aged. Mixed cropping with water yam did not affect the growth of oil palm seedlings as there was no significant difference in height and leaf area of uninoculated oil palms with single cropping. Mixed cropping with water yam did not significantly improve inoculated oil palm growth (plant height and leaf area) (Figure 4).

## DISCUSSION

Inoculating *G. boninense* on water yam caused fungal infection at the vine's base, resulting in tissue necrosis and the death of part or all of the vine. The infection caused necrosis at the bottom of the yam tuber but did not damage the dormant buds. *G. boninense* was more pathogenic on the vine of water yam than on oil palm, but the infection did not damage the tuber. This report for the first time that water yam can be infected by *G. boninense*. *Ganoderma* spp. has a wide range of woody plants in the field (Loyd *et al.*, 2018), but under artificial inoculation, the fungus could also infect herbaceous plants such as rhizomatous plants. The severe disease also has been recorded on inoculated galangal (Suwandi *et al.*, 2022).

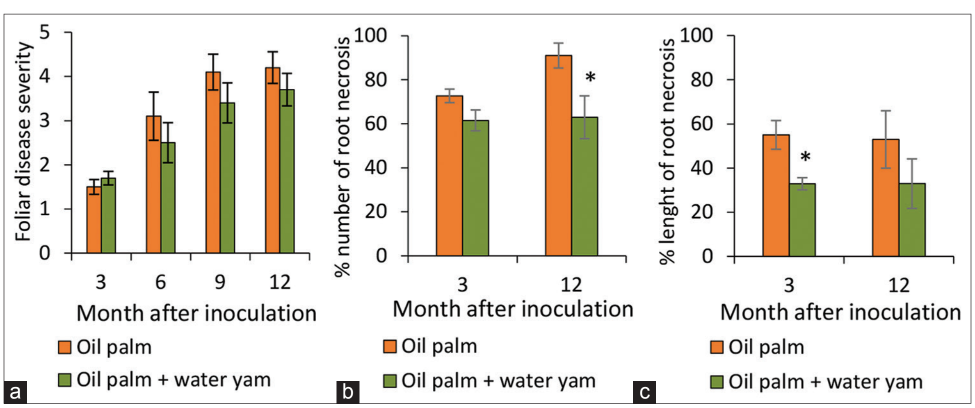


**Figure 2:** Effect of mixed cropping with water yam on a) foliar disease severity, b) number of root necrosis and c) length of root necrosis of oil palm caused by infection of *Ganoderma boninense*. *For each month, *denotes a significant difference (t-test) between the mean values of oil palm in single cropping compared to mixed cropping*

Under mixed cropping, a single mycelium network of *Ganoderma* caused simultaneous infection (multiple host-species infections) on both oil palm and water yam. Similar multiple host-species infection also was revealed in artificial dual inoculating *Ganoderma* in a mixed planting of oil palm and rhizomatous plants (Suwandi *et al.*, 2022). Multiple host species infection by *G. boninense* has not been reported in the field, but in some instances, the infection can co-occur in two individual oil palm trees (Miller *et al.*, 2000; Rees *et al.*, 2012). A natural multiple host species infection has also been reported by Suwandi (2007) on the fungus *Rigidoporus microporus*, where one individual on a rubber stump concurrently infected six different plant species.

*G. boninense* infection in oil palm, as assessed by the number and length of root necrosis, was reduced under multiple hosts in mixed cropping compared to that on a single oil palm host. Delayed oil palm infection progress was also recorded under multiple hosts as a delay in the appearance of first symptoms. During the initial disease progress at 3 mpi, less root necrosis was observed in mixed cropping, suggesting the benefit of water yam mixed cropping in suppressing initial *Ganoderma* infection on oil palm. A subsequent new infection was also suppressed between 3 and 12 mpi in mixed cropping as shown by a reduction in the number of necrosis roots. Ultimately, multiple host infections resulted in lower mortality of oil palm than a single oil palm host. Protection of water yam mixed cropping on initial infection, and further new root infection of oil palm could be responsible for the delay in oil palm disease severity and mortality. Simultaneous infection of oil palm and water yam might lead to the interference of the *Ganoderma* infection on the primary oil palm host. This result showed the benefits of multiple hosts mixed cropping for protecting oil palm as the primary host. Disease protection by water yam under mixed cropping has also been reported on rubber seedlings against rhizomorph development and infection of root rot polypore fungus, *R. microporus* (Yulianti *et al.*, 2017). The infection interference by water yam was likely not associated with the allelochemical as no allelopathic activity of the yam root exudate against *Ganoderma* mycelium (Rahmadhani *et al.*, 2020). Water yam was more susceptible to *Ganoderma* infection. Therefore, the shifting of the infection focus by a single *G. boninense* individual might operate in infection interference by this herbaceous plant. It was likely that water yam act as a trapping crop for *G. boninense* and the host dilution by water yam is attributed to the disease suppression.

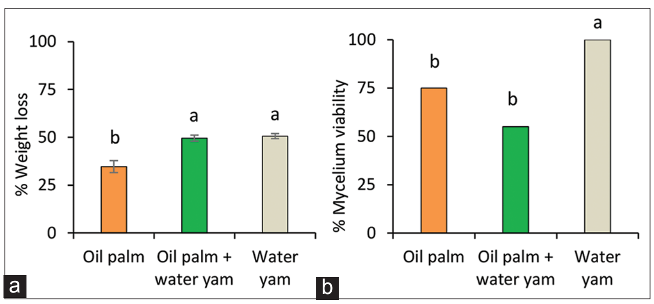


**Figure 3:** Effect of mixed cropping with water yam on a) dry weight losses and b) mycelium viability of *Ganoderma*-colonized rubber woodblock (RWB) after burial for 12 months. Error bars denote standard error of means (SEM) and values followed by different letters are significantly different (HSD test)

Mixed cropping with water yam increased dry weight losses of *Ganoderma*-colonized RWB due to the fungus's decaying of wood materials (lignin and celluloses). *Ganoderma* used a lignin riches material such as wood as a food base to initiate infection (Rees *et al.*, 2007). Consequently, the increase in wood decay might lead to a reduction in the potential inoculum of the pathogen. Mixed cropping with water yam reduces mycelium viability in parallel with wood decay. Increased wood decay of RWB and reduced mycelium survival have also been recorded on other dual susceptible host systems, oil palm and galangal (Suwandi *et al.*, 2022) or oil palm and taro plants (Alesia *et al.*, 2021). In contrast to water yam, mixed cropping with galangal had increased RWB decay but had also induced severe disease in oil palm (Suwandi *et al.*, 2022).

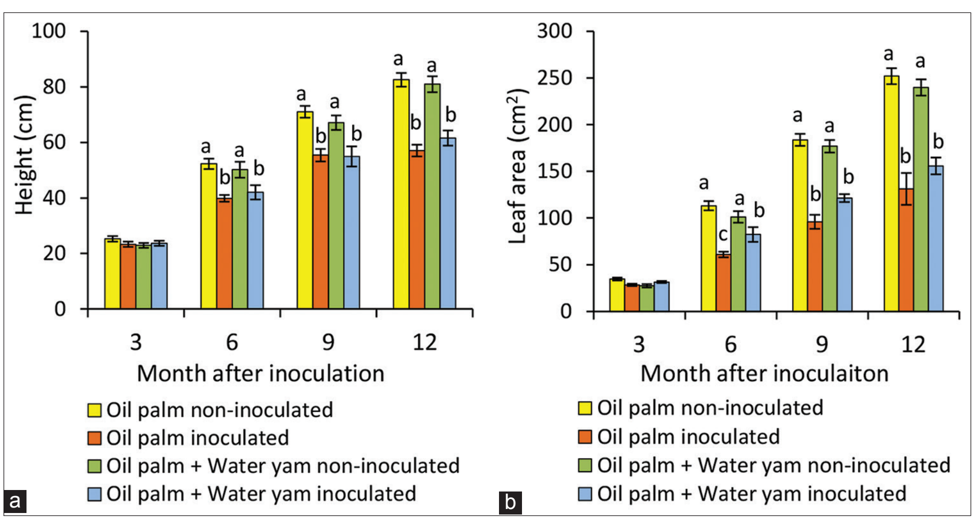


**Figure 4:** Effect of mixed cropping with water yam on the a) plant height and b) leaf area of infected and non-infected oil palm seedlings. Error bars denote standard error of means (SEM). For each month, values followed by different letters are significantly different (HSD test)

Mixed cropping with water yam in a small 5-liter pot did not affect the growth of oil palm seedlings suggesting the yam has no allelopathic against oil palm. A similar result of minor growth interference on oil palm growth has been recorded on mixed cropping with water yam using a large 40-liter pot (Rahmadhani *et al*., 2020). This study shows the crop protection benefit of oil palm mixed cropping with understory herbaceous plants, and further field trials need to be applied.

## CONCLUSION

*Ganoderma boninense* inoculation under mixed cropping with water yam induced disease on both diverse hosts (multiple host infection), but with less severe root necrotic and less plant mortality relative to inoculation on a single oil palm host. The inoculum potential of the pathogen as measured by wood decay and mycelium survival was reduced under mixed cropping. Water yam mixed cropping did not interfere with the growth of the inoculated oil palm seedling.

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