Hendersonia GanoEF1 GRANULES FOR THE CONTROL OF *Ganoderma boninense* IN OIL PALM

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MPOB INFORMATION SERIES • ISSN 1511-7871 • JUNE 2011 MPOB TT No. 483

Advances in biotechnology have led to a significant increase in the use of microbes as biological control agents for plant pathogens. The biological properties of several antagonistic fungi, mainly *Aspergillus* (Shukla and Uniyal, 1989), *Penicillium* (Dharmaputra *et al.*, 1989) and *Trichoderma* (Sariah and Zakaria, 2000; Izzati and Abdullah, 2008; Shamala and Idris, 2009), as well as endophytic bacteria (Sapak *et al.*, 2008), have been studied and proven to be antagonistic against *Ganoderma boninense*. Relatively few of these microbes have been commercialised as bio-control agents due to their inconsistent performance in the field, interactions with non-target organisms, varying rhizosphere or soil colonisation by the bio-control agent, problems in sustaining initial population levels, and genetic diversity of the target pathogens (Meyer and Roberts, 2002).

Nursery trials have shown that a pure culture of endophytic fungus GanoEF1 has potential in controlling *Ganoderma* disease infection in oil palm seedlings (Idris *et al.*, 2010). A further study has been conducted to develop *Hendersonia* GanoEF1 into a granular formulation. The efficacy of the *Hendersonia* GanoEF1 granules against *G. boninense* was investigated through *in vitro* and nursery trials.

PREPARATION OF *Hendersonia* GanoEF1 GRANULES

Formulation of the *Hendersonia* GanoEF1 granules was conducted based on the method described by Ramle *et al.* (2009) with modifications. Fungal spores were propagated in liquid media consisting of palm oil mill effluent (POME) and jaggery. The concentration of spores was determined with a haemocytometer prior to granule preparation. Alginate solution was prepared by dissolving sodium alginate in absolute alcohol. The fungal mycelia, spores and sterilised kaolin were added into the alginate solution and mixed until a homogenous mixture was formed.

The granulation process was performed by dropping the final mixture containing *Hendersonia* GanoEF1, alginate, kaolin and nutrient substrate into a calcium chloride ($CaCl_2$) solution. The granules formed were washed in distilled water and dried at room temperature (*Figure 1*).

Figure 1. Hendersonia *GanoEF1 granules.*

The random amplification polymophism DNA-polymerase chain reaction (RAPD-PCR) is the standard method for DNA fingerprinting, and was used to determine the DNA profile of the fungus. Using RAPD-PCR analysis and 20 random primers (OP1 to OP20), the DNA profile of *Hendersonia* GanoEF1 was examined. The patterns for primers of OP8, OP9 and OP10 were produced repeatedly. Thus, these three random primers can be used in the DNA profiling of *Hendersonia* GanoEF1.

QUALITY AND STABILITY OF *Hendersonia* GanoEF1 GRANULES

The quality of *Hendersonia* GanoEF1 granules was reported in terms of population as colony-forming units (CFU) per gramme (CFU g^{-1}) of granule. A viability test of *Hendersonia* GanoEF1 in the granular formulation during storage was

ISSN 1511-7871
9 771511 787001

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conducted over a nine-month storage period. The initial population was at 10^8 CFU g^{-1}. After one month's storage, the population remained at 10^8 CFU g^{-1}. After six months of storage at room temperature, the viability of *Hendersonia* GanoEF1 fungi in the granular formulation declined to 10^6 CFU g^{-1}. Viability was even lower after nine months of storage at room temperature with a value of 10^4 CFU g^{-1}.

The efficacy of the viable spores of *Hendersonia* GanoEF1 surviving in the formulation during storage was assessed based on the percentage of *Hendersonia* GanoEF1 colonies capable of reducing the mycelial growth of *G. boninense* in a dual culture plate. After one month of storage, *Hendersonia* GanoEF1 granules gave a percentage inhibition of radial growth (PIRG) value of more than 60% against *G. boninense*. After six and nine months in storage, the efficacy of *Hendersonia* GanoEF1 granules was reduced to 50% and 45%, respectively.

BENEFITS OF *Hendersonia* GanoEF1 GRANULAR FORMULATION

- Effective in controlling *Ganoderma* disease in oil palm.
- Easy to apply for the end user, and does not require any equipment.
- Has extended shelf-life as the microbial cells continue to grow and multiply because the required nutrients are maintained.
- Easy to store.

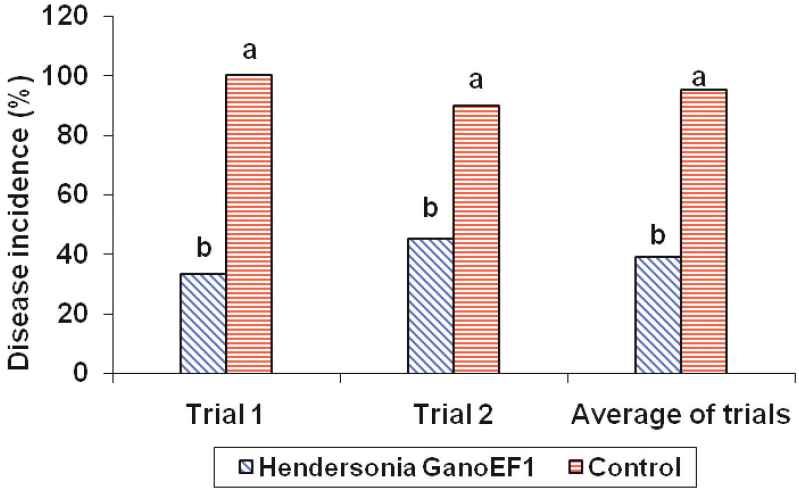
NURSERY EVALUATION OF *Hendersonia* GanoEF1 GRANULES AGAINST *G. boninense*

The effectiveness of *Hendersonia* GanoEF1 granules in controlling *Ganoderma* disease was based on two nursery trials conducted over 18 months. At six months after treatment, seedlings treated with *Hendersonia* GanoEF1 granules showed a significantly ($p<0.05$) lower percentage of disease incidence (DI) at an average of 39.2% compared with the untreated seedlings having DI of 95% (*Figure 2).*

Disease development was also monitored based on the percentage of severity of foliar symptoms (SFS). The average percentage SFS of seedlings treated with *Hendersonia* GanoEF1 granules (54.5%) was significantly ($p<0.05$) lower compared with the untreated seedlings (90.5%) (*Figure 3*).

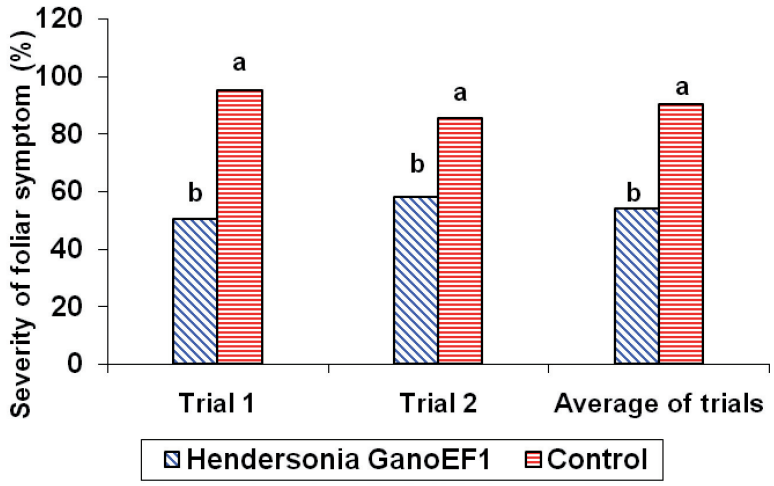
The average percentage of dead seedlings in the untreated was significantly ($p<0.05$) higher at 80.8% as compared with the seedlings treated with *Hendersonia* GanoEF1 granules at 34.2% (*Figure 4*).

The effectiveness of *Hendersonia* GanoEF1 granules in controlling basal stem rot (BSR) is shown in *Table 1*. The seedlings treated with *Hendersonia* GanoEF1 granules gave lower areas under the disease progress curves (AUDPC) of 86.6 and 89.0 in Trials 1 and 2, respectively, compared with the untreated seedlings with AUDPC of 193.3 and 190.0 in Trials 1 and 2, respectively. An average



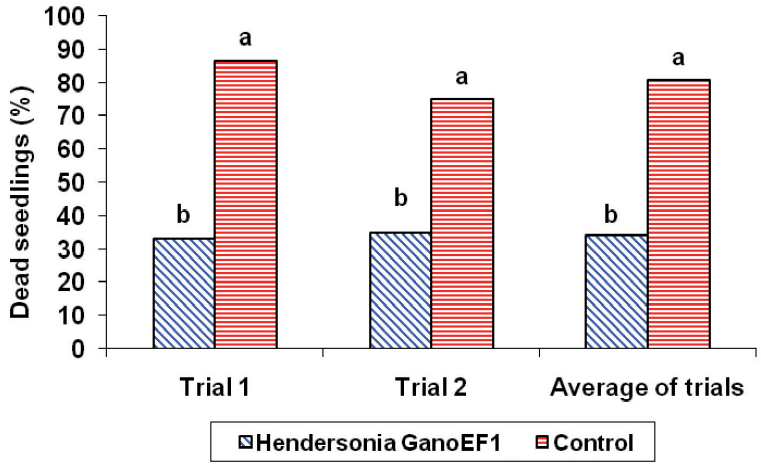
Note: Means within a group with the same letter are not significantly different from one another according to the t-test at $p<0.05$.

Figure 2. Disease incidence in seedlings due to Ganoderma boninense *infection at six months after treatment.*



Note: Means within a group with the same letter are not significantly different from one another according to the t-test at $p<0.05$.

Figure 3. Severity of foliar symptoms in seedlings due to Ganoderma boninense *infection at six months after treatment.*



Note: Means within a group with the same letter are not significantly different from one another according to the t-test at $p<0.05$.

Figure 4. Dead seedlings due to Ganoderma boninense *infection at six months after treatment.*

reduction of 54.16% in BSR disease incidence was observed in seedlings treated with *Hendersonia* GanoEF1 granules.

ECONOMIC ANALYSIS

The fixed cost for a pilot plant producing *Hendersonia* GanoEF1 granules is RM 5 million. The payback period is six years with an internal rate of return (IRR) of 14%. The net present value (NPV) at 10% discount rate is RM 716 529.00, with a benefit:cost ratio (B:C) of 1.33. As B:C is >1, NPV is positive and IRR is greater than the opportunity cost of capital; thus, the investment is financially feasible.

CONCLUSION

Hendersonia GanoEF1 granules have the capability of inhibiting the growth of *G. boninense in vitro*, and are effective for controlling *Ganoderma boninense* in oil palm. The nursery trials have been repeated twice for confirmation of the consistent efficacy of the *Hendersonia* GanoEF1 granules in controlling *Ganoderma* disease in oil palm.

TABLE 1. EFFECT OF *Hendersonia* GanoEF1 GRANULES ON BASAL STEM ROT DISEASE DEVELOPMENT IN OIL PALM SEEDLINGS AT SIX MONTHS AFTER TREATMENT

Treatments	Trial 1		Trial 2	
	AUDPC*	DR** (%)	AUDPC*	DR** (%)
Seedlings untreated with *Hendersonia* GanoEF1 granules and inoculated with *G. boninense* (as control, C1)	193.34	-	190.00	-
Seedlings treated with *Hendersonia* GanoEF1 granules and inoculated with *G. boninense* (T1)	86.67	55.17	89.00	53.15

Note: * Area under disease progress curve (AUDPC). ** Disease reduction (DR).
Average disease reduction (DR) = 54.16%.

REFERENCES

DHARMAPUTRA, O S; TJITROSOMO, H S and ABADI, A I (1989). Antagonistic effect of four fungal isolates to *Ganoderma boninense*, the causal agent of basal stem rot of oil palm. *Biotropia*, *3*: 41-49.

IDRIS, A S (2009). Basal stem rot in Malaysia – Biology, epidemiology, economic importance, detection and control. *Proc. of the International Workshop on Awareness, Detection and Control of Oil Palm Devastating Diseases* (Kushairi, A; Idris, A S and Norman, K, eds.), MPOB, Bangi, Selangor. p. 13-57.

IDRIS, A S; NOOR HAIDA, S and NUR RASHYEDA, R. (2010). GanoEF1-A fungal biocontrol agent for *Ganoderma* in oil palm. *MPOB Information Series No. 501*.

IZZATI, M Z and ABDULLAH, F (2008). Disease suppression in *Ganoderma*-infected oil palm seedlings treated with *Trichoderma harzianum*. *Plant Protect. Sci.*, *44(3)*: 101-107.

MEYER, S L F and ROBERTS, D P (2002). Combinations of bio-control agents for management of plant-parasitic nematodes and soil borne plant-pathogen fungi. *J. Nematology*, *34*: 1-8.

RAMLE, M; NORMAN, K and MOHD BASRI, W (2009). Pathogeniciy of granule formulations of *Metarhizium anisopliae* against the larvae of the oil palm rhinoceros beetle, *Oryctes rhinoceros* (L.). *J. Oil Palm Research*, *21*: 602-612.

SARIAH, M and ZAKARIA, H (2000). The use of soil amendments for the control of basal stem rot of oil palm seedlings. In *Ganoderma* Diseases of Perennial Crops (Flood, J; Bridge, P D and Holderness, M, eds.). CABI Publishing, U K. p. 89-100.

SAPAK, Z; SARIAH, M and AHMAD, Z A M (2008). Effect of endophytic bacteria on growth and suppression of *Ganoderma* infection in oil palm. *International J. Agriculture & Biology*, *10*: 127-32.

SHAMALA, S and IDRIS, A S (2009). *Trichoderma* as a biological control agents against *Ganoderma* in oil palm. *MPOB Information Series No. 463*.

SHUKLA, A N and UNIYAL, K (1989). Antagonistic interactions of *Ganoderma lucidum* (lyss) Karst. against some soil microorganisms. *J. of Current Science*, *58*: 265-267.

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