Preliminary research of pathogenic components from *Ganoderma* isolates, in relation to their aggressiveness after artificial inoculation of oil palm seedlings

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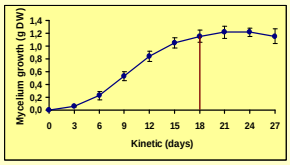
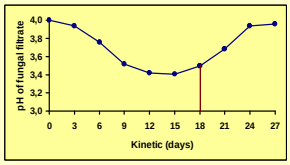
I- SUMMARY

The objective of this research was to investigate the relationship between the aggressiveness, assessed by nursery screening tests, of *Ganoderma* with a pathogenic factor which is quantifiable in the laboratory. This should lead to a rapid test to screen for isolate aggressiveness. A Czapek modified liquid medium was first improved for the culturing of *Ganoderma boninense*. The culture filtrate was used to study metabolites secreted by the fungus which could be involved in pathogenicity, as the production of toxin(s). As a first step a wilting leaf bioassay, using the fungus filtrate, was set up to develop this approach. This tool was used in another pathosystem (Breton *et al.,* 2000) and allowed a quantitative estimate of the production of toxin(s) which was estimated by water loss from leaves (wilting). In parallel, the ability of isolates to degrade rubber wood blocks (RWB) was studied *in vitro*. Preliminary results led to the hypothesis that (i) there is no correlation between RWB degradation by isolates with their natural aggressiveness and (ii) *Ganoderma* isolates seem to produce toxic compound(s) which lead to leaf wilting *in vitro*. The chemical nature of these compounds is unknown and it is too early to assert whether (i) there is a role of these compounds in *Ganoderma* pathogenicity and (ii) there is a positive correlation between the production of toxic compound(s) with isolate aggressiveness observed in the nursery. However, preliminary results indicate that such relationships may exist.

Keywords : Liquid medium, toxic effect, rubber wood block, degradation, *Ganoderma*, oil palm

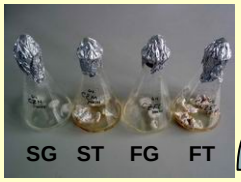


Rapid acidification of the medium during the growth stage



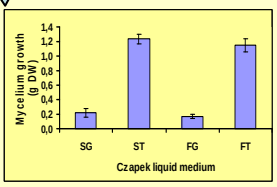
II- CULTIVATION OF *GANODERMA* IN MODIFIED CZAPEK LIQUID MEDIUM

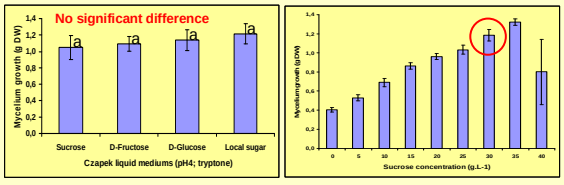
One hundred millilitres of liquid medium was placed in 500mL flasks. Each flask was inoculated with 3 mycelial plugs (25mm²) from a 14-day-old culture of *Ganoderma*. Liquid cultures were incubated without agitation at 27°C.



Ganoderma is able to use sucrose and *D*-fructose as its carbone source. Concerning to nitrogen sources, tryptone appears more efficient than *L*-glutamic acid which seems not to be used by the fungus.

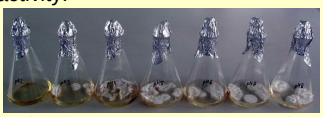
SG : Sucrose/*L*-glutamic acid
ST : Sucrose/Tryptone
FG : *D*-Fructose/ *L*-glutamic acid
FT : *D*-Fructose/Tryptone



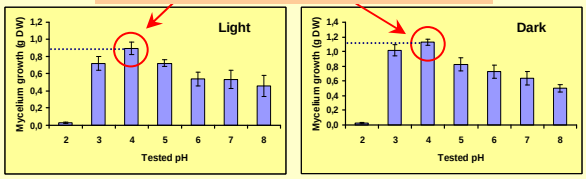


Sucrose —(**Invertase**)→ **Glucose + Fructose**

Results are contrary to those of Ho & Nawawi (1991) who demonstrated a significant difference between sucrose, D-fructose and D-glucose, leading to the hypothesis that *Ganoderma* has a low invertase activity.



Best *Ganoderma* growth in dark at acid pH with a maximum at pH4



A modified Czapek liquid medium pH4 was improved for *Ganoderma* cultivation. Eighteen days after incubation (in continuous light), the surface of the medium was completely covered by the fungus and the cultures were then filtered.

III- PREPARATION OF FUNGUS FILTRATE

Fungus culture → Vacuum filtration → Concentration → Dialysis → Concentration

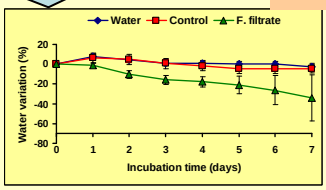
Pellets are dissolved in distilled water (concentration *in fine* x100) → **Fungus Filtrate (FF)** → **Leaf wilting bioassay**

IV- LEAF-WILTING BIOASSAY

The leaflets of a BLRS oil palm clone (BL001.2A) from frond N°1 were used for the biotest. The leaflet was excised (30cm from the end), immediately weighed (FW) and transferred to a glass tube containing fungus filtrate previously diluted in 7 mL of distilled water. The leaflets were incubated at 27°C in continuous light. The toxic activity caused wilting of the oil palm leaflets. The wilting intensity was estimated by the percentage of water loss from the leaflet after incubation in relation to the initial water content of the leaflet. Tukey test was used for statistic analysis of data.

Only two days after incubation, a significant toxic effect from fungus filtrate can be observed. The toxic effect increases in relation to the incubation period of leaflets; therefore, high variability between toxic effects was recorded.

Treatments	Mean	Groups
Water	-2.391	A
Control	-4.642	A
F. filtrate	-34.157	B



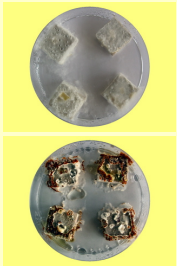
Internal water variation

Isolates	Mean	Groups
D	-11.133	A
9810511	-16.256	AB
GI3	-19.101	ABC
A	-21.909	BC
J	-25.802	C
GI2	-26.107	C

Fungus filtrates from Isolates GI2, J and A resulted in greater wilting (water loss) probably because of more toxic effect.

V- DEGRADATION OF RWBs

Four inoculated RWBs (4cm³) per Petri dish were incubated for 12 weeks in the dark at 27°C.The intensity of wood degradation was estimated as a percentage of dry weight loss.



Isolates	Mean	Groups
D	26.124	A
J	25.761	A
A	23.857	AB
GI2	20.837	AB
9810511	18.134	B

No correlation was found between the capacity of isolates to degrade RWB with the toxic effect observed in their culture filtrate respectively (see table §IV isolates/toxic effect).

VI- ISOLATES AGGRESSIVENESS

Germinated seeds were inoculated by pre-infected RWBs the aggressiveness of isolates was estimated by scoring symptom intensity.

Isolates	Mean	Groups
J	4.1750	A
GI2	4.0000	A
A	2.5500	AB
9810511	0.7750	BC
D	0.0750	C

J, GI2 and A are the most aggressive isolates. Moreover, the FF of these isolates was characterised by a high toxic effect (see table §IV). In contrast, D was a non aggressive isolate and its FF resulted in low wilting.

VII- CONCLUSION

This preliminary study has demonstrated the presence of a toxic effect in fungus filtrate which was estimated by a wilting bioassay. However, the origin of this toxic effect was still unknown (toxin…?). Moreover, the FF from aggressive isolates is characterised by an important toxic effect and inversely for the non-aggressive isolate. However, It is too early to make any conclusions about a correlation between these two factors.

VIII- REFERENCES

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