# EFFICACY OF *TRICHODERMA ASPERELLUM* OIL FORMULATIONS ON THE CONTROL OF COCOA BLACK POD DISEASE (*PHYTOPHTHORA MEGAKARYA*)

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## SUMMARY

The objective of this study was therefore to develop a formulation of conidia of *T. asperellum* with the aim of improving its efficacy. The formulations developed were oily dispersions. It was a combination of solvents consisting of groundnut oil or palm oil with structural agents and emulsifying-dispersing agents. Emulsification tests were carried out and the stability of the emulsions evaluated. The evaluation of the effect of co-formulants on the growth of conidia of *T. asperellum* was done by reading the optical densities of the formulated samples on multi-plates using a plate reader. The test on detached cocoa pods was done by treating the cocoa pods with selected formulations at $1.10^7$ conidia/ml and inoculation of the treated cocoa pods was done 24 hours later with zoospores of *P. megakarya* at $1.10^5$ zoospores/ml. The growth of necrosis on the fruits was measured daily. The screening of co-formulants and emulsification tests ended up with the selection of two formulations. The first composed of conidia of *T. asperellum*, groundnut oil, Tensiofix NTM® and Tensiofix 869®. The second differed from the first by utilisation of palm oil as the solvent. These formulations proved stable when diluted in water with 1% and 0.5% of sedimentation respectively after 24 hours. The viability test of the conidia indicated that the different formulations selected did not have a fungitoxic effect. The test on detached cocoa pods showed an improved efficacy of *T. asperellum* to control the disease. The growth rates of necrosis were 6.29 mm/day, 7.25 mm/day and 31.6 mm/day for treatment with formulation 1, pure conidia and control treated with water respectively.

**Key words**: Formulation, black pod disease, *Trichoderma asperellum*

## INTRODUCTION

Cocoa black pod disease caused by fungi of the genus *Phytophthora* is one of the major diseases from which this plant suffers. Losses vary from 30 to 40% at global level, but can reach 100% in the absence of control measures (Ndoumbe-Nkeng *et al.*, 2004). The species *Phytophthora megakarya* is the principal pathogenic agent in Cameroon.

The control measures implemented by cocoa farmers to reduce the incidence of black pod disease vary considerably, and are more or less effective depending on the climatic conditions and the virulence of the strains of *P. megakarya*. Chemical control is the most common method.

In southern Cameroon, more than 50% of producers use fungicides to combat cocoa black pod disease. However, the cost and unavailability of fungicides have been cited by producers as the main constraints on the use of fungicides (Sonwa *et al.*, 2008). Chemical control also involves high risks associated with pollution of the environment, the intoxication of operators

and the presence of unwanted residues for consumers. Furthermore, the over-use in Cameroon of metalaxyl and the failure to comply with application doses (Sonwa *et al.*, 2008) raise the problem of the risk of strains of *P. megakarya* appearing that are resistant to these chemical molecules. As for genetic control, no genotype of the cocoa tree that is totally resistant to *P. megakarya* and *P. palmivora* (Nyasse *et al.*, 2007) has yet been identified. Certain clones show a potential tolerance or resistance to these pathogenic agents, but are largely unavailable to cocoa farmers.

Cultivation methods aimed at reducing initial inoculation through sanitary harvesting and modifying the microclimate in plantations to create conditions that discourage the development of the disease cannot alone guarantee the total protection of the cocoa orchards (Ndoumbe-Nkeng *et al.*, 2004).

Biological control through the use of micro-organisms that help manage black pod disease produced promising results in earlier work in Cameroon (Tondje *et al.*, 2007; Deberdt *et al.*, 2008).

However, the level of efficacy of biological control agents has remained below that of chemical molecules (Deberdt *et al.*, 2008). Several factors may be behind this, notably environmental factors, biotic factors and the inadequacy of the formulation of the spores of *T. asperellum*, which is not conducive to the best expression of antagonistic activity. The main aim of this study is to develop a formulation of conidia of *Trichoderma asperellum* that could be used in the biological control of cocoa black pod disease.

## MATERIALS AND METHODS

### Formulation of conidia of *Trichoderma asperellum* and evaluation of the stability of the formulations

The formulation developed consisted of dispersing conidia of *T. asperellum* in a vegetable oil (groundnut oil or palm oil) to which are added emulsifying-dispersing agents and a structural agent. In total, six dispensable formulations in the form of emulsions in water (EC) were produced and tested. The evaluation of the stability of the dispersion of the formulations in water was done using the CIPAC MT 36 test. 95 ml of water of medium hardness (CIPAC D water at 342 ppm in $CaCO_3$) was introduced into graduated glass tubes and placed in a bath thermostatised at 30°C±1°C for 24 hours. 5 ml of each formulation was added to each tube containing the CIPAC D water. Two test tubes were used for each formulation tested, and their contents were homogenised. Observations were made after 30 minutes, 1 hour, 2 hours and 24 hours. They consisted of assessing the dispersion, degree of coloration of the walls of the tubes immediately after homogenisation, the homogeneity of the dispersion and of measuring the conidia deposits (volume of the cap).

### Evaluation of the effect of the different co-formulants on the growth of *Trichoderma asperellum*

The wells of a multiwall plate were sequentially filled with 150 µl of the sterile liquid culture medium PDB (Potato Dextrose Broth, 24 g/l). 50 µl of each sample of formulation at $1.10^6$ conidia/ml are then added to the wells that have first been filled with the liquid culture medium. The different treatments being compared were the two formulations that presented the best dispersion capabilities, their controls without conidia, a treatment with conidia only

and a *blanco* treatment consisting solely of the culture medium, to which 50 µl of sterile distilled water had been added. Each treatment was repeated twelve times and the optical density of the cultures in the multiwall plate was read using a plate reader every 24 hours at 630 nm. This experiment was repeated twice.

### Test of the efficacy of the formulations on detached pods

The test consisted of pulverising cocoa pods from a sensitive clone (SNK10) with formulae at $1.10^7$ conidia/ml. For each pod, 10 ml of formulation was used. The "negative control" treatments consisted of pods treated with the different formulations without conidia and cocoa fruit treated with water. The "positive control" treatment consisted of pods treated with a chemical fungicide, RIDOMIL GOLD PLUS 66 WP (6% metalaxyl + 60% copper oxide). A treatment based on pure conidia of *T. asperellum* had also been carried out.
The treated pods were placed in moistened trays to create conditions favourable to the development of the disease. The inoculation of the pods with a suspension of zoospores of the strain NKOM III of *P. megakarya* at $1.10^5$ zoospores/ml was done 24 hours after treatment.
The trays were incubated at 25±2 ºC and the diameter of the necrosis on the fruit was measured each day, for five days. These measurements were taken in both directions of the pod and the mean value was calculated. Ten pods were used for each treatment.

### Treatment and statistical analysis of data

The measurements of the diameters of the necrosis on the detached pods were recorded using Excel 2007 software and subjected to analysis using the appropriate General Linear Model with the SAS software (Statistical Analysis System; version 9.1). The Student-Newman-Keuls multiple comparison test of means at the 5% level of significance was used for the classification of the different groups.

## RESULTS AND DISCUSSION

### Characterisation and stability of the formulae developed

The formulations developed (Table 1) differ in terms of their colours, degree of homogeneity, pH, density and viscosity. All the formulations for which the Tensiofix NTM had been used as an emulsifying-dispersing agent have a homogeneous appearance. Conversely, those that combine the Tensiofix DB08 and the Tensiofix IW60 remain heterogeneous.
The emulsification test (Figure 1) revealed a white-cream coloration of the walls of the tubes containing formulae 1 and 2 after homogenisation. The presence of foam was noted in the tubes containing formulae 3, 4 and 6. The thickness of the foam varied from 3 to 5 ml.
The observation made after half an hour revealed a separation of phases in the tubes containing formulae 4 and 6, with a reduction in foam. This phase separation is apparently due to a coalescence of droplets. In the tubes containing formula 3, creaming was noted on the surface. No sedimentation was observed in any of the tubes after 30 minutes.
The observations taken after one hour and two hours revealed traces of sedimentation in the tubes containing formulae 1 and 2. Conversely, in the tubes containing the other formulae, a higher degree of sedimentation was observed.
After 24 hours, the sedimentation was approximately 1% in formula 1 and 0.5% in formula 2. As for the other formulae, the sedimentation was approximately 5%, or all the formulation that had been diluted.

Formulations 1 and 2 were subsequently selected for the biological and antagonism tests on the plant material.

**Table 1.** Composition (in g) and characteristics of the formulations tested

| Ingredients | Number of the formulation | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Conidia | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Groundnut oil | 75.50 | 0 | 75.50 | 0 | 75.50 | 75.50 |
| Palm oil | 0 | 75.50 | 0 | 75.50 | 0 | 0 |
| Tensiofix NTM | 15.00 | 15.00 | 15.00 | 15.00 | 0 | 0 |
| Tensiofix DB08 | 0 | 0 | 0 | 0 | 9.50 | 9.50 |
| Tensiofix IW60 | 0 | 0 | 0 | 0 | 5.50 | 5.50 |
| Tensiofix OC653 | 0 | 0 | 0 | 0 | 1.00 | 1.00 |
| Tween 20 | 0 | 0 | 3.00 | 3.00 | 0 | 0 |
| Tensiofix 869 | 3.00 | 3.00 | 0 | 0 | 2.50 | 0 |
| Water | 0 | 0 | 0 | 0 | 10.00 | 10.00 |
| **Characteristics** | | | | | | |
| Colour | Dark brown | Yellowish | Green | Light brown | Light brown | Greyish |
| Homogeneity | | Homogenous | | | Heterogeneous | |
| pH | 6.1 | 5.2 | 5.3 | 5.2 | 6 | 5.4 |
| Density (g/cm³) | 0.75 | 0.66 | 0.83 | 0.82 | 0.79 | 0.83 |
| Viscosity (cps) | 666 | 3849 | 189 | 2250 | 1620 | 9250 |

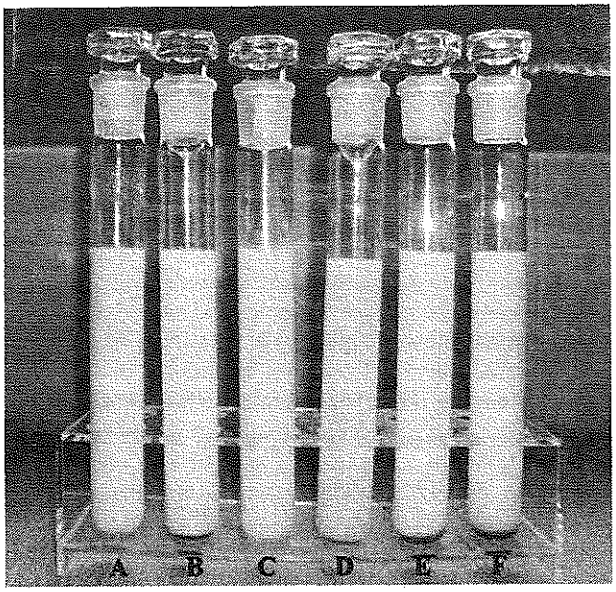


**Figure 1.** Emulsification tests on the formulations developed. (A, B, C, D, E and F represent the dispersion results of the samples of formulations 1, 3, 2, 4, 5 and 6 in CIPAC D water).

nd antagonism tests on

ions tested

| | 6 |
|---|---|
| ) | 0.10 |
| ) | 75.50 |
| ) | 0 |
| ) | 0 |
| ) | 9.50 |
| ) | 5.50 |
| ) | 1.00 |
| ) | 0 |
| ) | 0 |
| ) | 10.00 |
| t | Greyish |
| | |
| rogeneous | |
| | 5.4 |
| ) | 0.83 |
| ) | 9250 |

e 1. Emulsification tests on
rmulations developed.
C, D, E and F represent the
sion results of the sam-
f formulations 1, 3, 2,
nd 6 in CIPAC D water).



## Effect of the different co-formulants on the viability of the conidia of *Trichoderma asperellum*

The inoculation of the multiwall plates containing the liquid culture medium PDB (Potato Dextrose Broth) with formulations 1 and 2 allowed us to highlight the lack of effect of the different co-formulants on the viability of the conidia of *T. asperellum*. Indeed, the growth of *T. asperellum* in the wells resulted in an increase in optical density over time (Figure 2).
This figure shows that the three curves describe a sigmoid with an initial phase that is a growth start-up phase for the dry conidia or a 48-hour lag phase for the formulated conidia, followed by an accelerated growth phase and finally a last phase during which growth becomes almost zero.

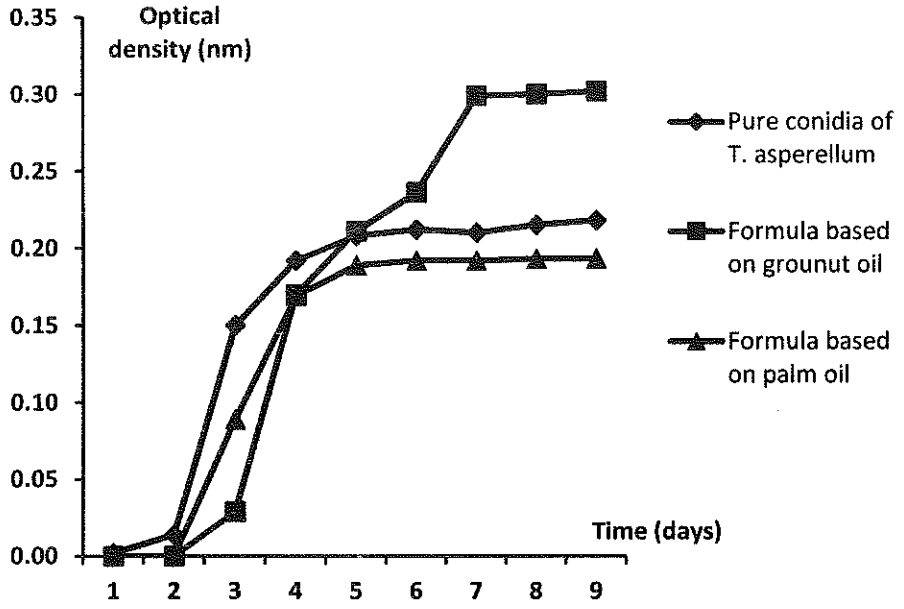


**Figure 2.** Optical densities of the formulae as a function of time

## Effect of the different formulations on the development of cocoa black pod disease

Analysis of the variance in daily growth of the necrosis on the cocoa pods (Table 2) shows that the conidia of *T. asperellum* have an effect on the development of the disease.

**Table 2.** Analysis of the variance in growth speeds of necrosis on the cocoa fruit

| Source of variation | d.o.f | S.S. | Mean square | *F* | *Pr > F* | Progression of the necrosis (mm/d) |
|---|---|---|---|---|---|---|
| Treatment | 6 | 8840.906 | 1473.484 | 11592.1 | <0.001 | |
| Error | 63 | 8.008 | | | | |
| Total | 69 | 8848.914 | 0.127 | | | |
| Water control | | | | | | 31.61 a |
| Control formulation 2 | | | | | | 25.20 b |
| Control formulation 1 | | | | | | 23.92 c |
| Pure conidia | | | | | | 7.25 d |
| Formulation 2 | | | | | | 6.89 e |
| Formulation 1 | | | | | | 6.29 f |
| Fungicide | | | | | | 0.00 g |

Treatments: i) Formulation 1 (groundnut oil, Tensiofix NTM, Tensiofix 869, conidia); ii) Formulation 2 (palm oil, Tensiofix NTM, Tensiofix 869, conidia); iii) Control formulation 1; iv) Control formulation 2; v) Simple water control; vi) Pure conidia; vii) Chemical fungicide.

This effect results in a reduction in the speed of growth of the necrosis compared with that of pods treated with simple water. The formulations allow the efficacy of the strain of *T. asperellum* PR11 to be improved. The speed of development of the necrosis was 6.29 mm/d for the treatment with formula 1 and 6.89 mm/d for the treatment with formula 2, whereas it was 7.25 mm/d for the treatment with non-formulated conidia. The co-formulants had an effect on the development of the necrosis. Compared with the control treated with simple water, a significant difference was noted between the speeds of development of the disease. Chemical treatment was the best under the conditions of this study.

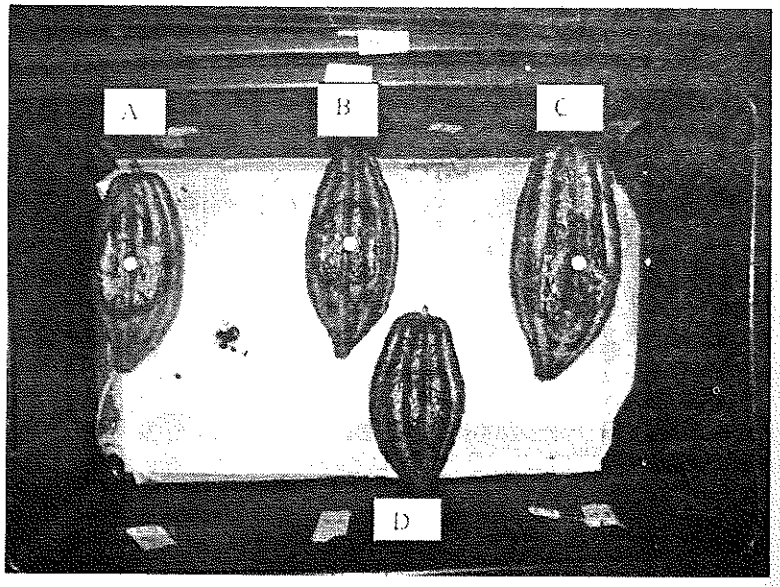


**Figure 3.** Development of necrosis on cocoa fruit three days after inoculation of *P. megakarya*. (A: Pod treated with simple water; B: Pod treated with the co-formulants of formulation 1; C: Pod treated with the co-formulants of formulation 2; D: Pod treated with formulation 1)

fruit

| F | Progression of the necrosis (mm/d) |
|---|---|
| 01 | |
| | 31.61 a |
| | 25.20 b |
| | 23.92 c |
| | 7.25 d |
| | 6.89 e |
| | 6.29 f |
| | 0.00 g |

ii) Formulation 2 (palm oil, Ten-
2; v) Simple water control;

osis compared with that of
y of the strain of *T. asperel-*
sis was 6.29 mm/d for the
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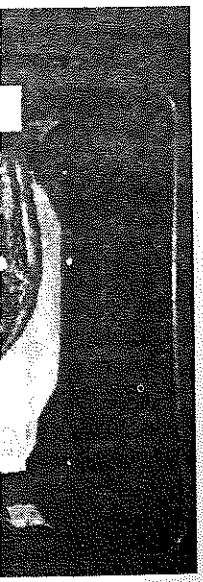

ation of *P. megakarya*. (A: Pod
nulation 1; C: Pod treated with



## DISCUSSION

Oily dispersions are not generally used for the formulation of biofungicides. The most common formulations are inverse emulsions, granules or tablets for dispersion in water, soluble powders or granules and capsule suspensions (Lewis and Lumsden, 2001; Batta, 2004; 2007; Sunil *et al.*, 2009). However, these various formulae do not show excellent properties upon dilution compared with oily dispersions, which are more stable. In this study, the results of the emulsification tests showed that none of the formulae was appropriate for the formulation of conidia of *T. asperellum*. Several parameters may be behind the instability of the formulae. The first source of instability could be attributed to the incompatibility of the emulsifying-dispersing agents and the vegetable oils tested (groundnut oil and palm oil). The second source of instability of the formulations upon dilution in water could relate to the nature of the active substance. The conidia cannot be dissolved, as is the case with the active substance in chemical formulations. These conidia must remain suspended in the emulsion. But this was not always the case with the formulations tested. Thus, for example, the rapid sedimentation observed with certain formulae could be due to the fact that the structural agents used do not allow the conidia to remain in suspension. The characteristics of the formulae developed in this study showed that the viscosities ranged from 185 to 9250 cps. These viscosities are high compared with those obtained with the inverse emulsions developed by Batta (2004; 2007), which range from 15 to 27 cps. This difference is due to the composition of the two types of formulation.

The evaluation of the effect of the co-formulants on conidia growth revealed a 48-hour lag time for the formulated conidia compared with the dry conidia. This lag time is apparently due to the fact that the oil forms an envelope around the conidium and slows down the diffusion of water within the fungal cell. Conversely, when the dry conidia are in contact with water, germination is faster. The optical density variation curves all describe a sigmoid. The first phase corresponds to the lag time, the germination of the conidium and the start of growth; the second phase represents the active growth of the fungus and the last phase shows a slowdown in growth, probably following the depletion of nutrients in the culture medium. The growth of the fungus in the formulation based on groundnut oil remains greater than that of the dry conidia and of the conidia formulated with palm oil. *Trichoderma asperellum* apparently finds nutrients in the groundnut oil that enable it to better pursue its development.

The pulverisation of *T. asperellum* on the cocoa pods allowed the size of the necroses due to *P. megakarya* to be reduced. This reduction in necrosis translates the antagonistic potential of the PR11 strain of *T. asperellum*. Similar results were obtained by Tondje *et al.*, (2006) and Mpika *et al.*, (2009). These authors demonstrated that the application of propagules of *Trichoderma* spp. and *Geniculosporium* spp. on pieces of cortex or cocoa pods allowed the number and size of lesions caused by *P. megakarya* or *P. palmivora* to be reduced. The inhibitory activity of the growth of *P. megakarya* by *T. asperellum* apparently results from the ability of this biological control agent to germinate and release onto the pod enzymes capable of intervening directly in the breakdown of the cell wall of the pathogen. The research carried out by Tondje *et al.*, (2007) in the study of the mechanisms of action used by *T. asperellum* vis-à-vis *P. megakarya* enabled two types of enzyme to be identified, laminarinases and caboxymethylcellulases, which intervene in this wall breakdown function. The reduction in necrosis on the fruit is apparently also the result of the ability of *T. asperellum* to establish itself as an endophyte fungus of the pod. This survival in the tissues of the plant could then induce activation of defence mechanisms that would make the pod less sensitive to infection by *P. megakarya*. Activation of the plant's defence mechanisms was demonstrated by Bigirimana *et al.*, (1997),

Howell *et al.*, (2000), Sid Ahmed *et al.*, 2000 and Harman *et al.*, (2004) in haricot beans, cotton, peppers and corn pre-inoculated with strains of *T. virens* and *T. harzianum*, subjected to attacks by *Rhizoctonia* sp., *Colletrotrichum* sp. and *Phytophthora* sp.
The oil formulations enabled the efficacy of *T. asperellum* to be improved. These results are similar to those obtained by Rogério *et al.* (2009), who showed during their research into the biological control of *P. palmivora* that the formulated conidia of *T. martiale* had a greater tendency to reduce the incidence of the disease compared with non-formulated conidia. This improvement in efficacy is apparently due to the fact that the co-formulants used allow a better distribution of the conidia in solution in water and therefore a better cover of the pod during treatment. Oil-based media could also allow the ability to germinate of the conidium to be maintained, since oil dries out less quickly than water, and is a source of nutrients for the conidium. This difference in efficacy between formulated conidia and dry conidia could be further amplified in naturally infected fields. Indeed, the work carried out by Ndoumbe-Nkeng *et al.*, (2009) into the epidemiology of black pod disease in Cameroon showed that rain is the main factor triggering the disease on cocoa farms. Thus, the intensity of the rain not only encourages leaching of the conidia onto the fruit, but also a greater dispersion of the disease in the plot. With the oil formulations, the adhesiveness of the conidia on the pods is greater and the risks of leaching fewer.

## CONCLUSION

The aim of this study was to formulate conidia of *T. asperellum*, a biological control agent against cocoa black pod disease in Cameroon. The grading of the various co-formulants and the evaluation of the stability of the formulae upon dilution resulted in the selection of two oily dispersions. The first combines the conidia of *T. asperellum* with groundnut oil and Tensiofix 869 and NTM, while the second oily dispersion is a mixture of conidia with palm oil and Tensiofix 869 and NTM. The test of the viability of the conidia of *T. asperellum* showed that the different co-formulants involved in the composition of the two selected formulae do not inhibit the growth of *T. asperellum*. A reading of the optical density of the formulated samples showed that groundnut oil improves the growth of *T. asperellum*. The test of the efficacy of the different formulae that was carried out on the detached pods showed an improvement in the antagonistic ability of *T. asperellum* vis-à-vis *P. megakarya*. This improvement in antagonistic ability resulted in a reduction in the speed of development of necroses on the cocoa pods treated with the various formulae, compared with treatment with non-formulated conidia. The different results obtained in this study represent a significant advance in the development of a strategy of biological control of cocoa black pod disease.

## REFERENCES


BATTA Y.A.(2004). Postharvest biological control of apple grey mold by *Trichoderma harzianum* Rifai formulated in an invert emulsion. *Crop Protection*, **23**: 19-26.

BATTA Y.A.(2007). Control of postharvest diseases of fruit with an invert emulsion formulation of *Trichoderma harzianum* Rifai. *Postharvest Biol. Technol.*, **43**: 143-150.

BIGIRIMANA J, DE MEYER G, POPPE J, *ET AL.* (1997).Induction of systemic resistance on bean (*Phaseolus vulgaris*) by *Trichoderma harzianum*. *Med. Fac. Landbouww. Univ. Gent*, **62**: 1001-1007.

DEBERDT P, MFEGUE CV, TONDJE PR. (2008). Impact of environmental factors, chemical fungicide and biological control on cacao pod production dynamics and black pod disease (*Phytophthora megakarya*) in Cameroon. *Biol. Control*, **44**: 149-159.

HARMAN GE, HOWELL CR, VITERBO A.(2004).*Trichoderma* species-opportunistic avirulent plant symbionts. *Nat. Rev. Microbiol.*,**2**: 43-56.

HOWELL CR, HANSON LE, STIPANOVIC RD.(2000).Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. *Phytopathol.*, **90**: 248-252.

KUBICEK CP, AKÉ S.(2009). Antagonist potential of *Trichoderma* indigenous isolates for biological control of *Phytophthora palmivora* the causative agent of black pod disease on cocoa (*Theobroma cacao* L.) in Côte d'Ivoire. *Afr. J. Biotechnol.*, **20**: 5280-5293.

LEWIS JA, LUMSDEN RD.(2001). Biocontrol of damping-off of greenhouse-grown crops caused by *Rhizoctonia solani* with a formulation of *Trichoderma* spp. *Crop Protection*, **20**: 49-56.

MPIKA J, KEBE IB, ISSALI AE, *ET AL.*(2004). Impact of removing diseased pods on cocoa black pod caused by *Phytophthora megakarya* and on cocoa production in Cameroon. *Crop Protection*, **23**: 415-424.

NDOUMBE-NKENG M, EFOMBAGN MIB, NYASSE S.(2009). Relationship between cocoa *Phytophthora* pod rot disease and climatic variables in Cameroon. *Can J Plant Pathol.*, **31**: 309-320.

NYASSE S, EFOMBAGN MIB, KEBE BI.(2007).Integrated management of *Phytophthora* diseases on cocoa (*Theobroma cacao* L.): impact of plant breeding on pod rot incidence. *Crop Protection*, **26**: 40-45.

ROGÉRIO EH, ALAN WVP, SOBERANIS W. (2009). Biocontrol potential of *Trichoderma martiale* against the black-pod disease (*Phytophthora palmivora*) of cacao. *Biol. Control*, **50**: 143-149.

SID AHMED A, SANCHEZ PC, CANDELA EM. (2000). Evaluation of induction systemic resistance in pepper plants (*Capsicum annuum*) to *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidiol accumulation. *Eur. J. Plant Pathol.*, **106**: 824-824.

SONWA DJ, OUSMANE C, WEISE FS. (2008). Management of cocoa: Constraints during acquisition and application of pesticides in the humid forest zones of southern Cameroon. *Crop Protection*, **27**: 1159-1164.

TONDJE PR, HEBBAR KP, WEISE S.(2006).Bioassay of *Geniculosporium* species for *Phytophthora megakarya* biological control on cacao pod husk pieces. *Afr. J. Biotechnol.*, **8**: 648-652.

TONDJE PR, ROBERTS DP, BON MC, *ET AL.* (2007). Isolation and identification of mycoparasitic isolates of *Trichoderma asperellum* with potential for suppression of black pod disease of cacao in Cameroon. *Biol. Control*, **43**: 202-212.