

## In Vitro Inhibition of *Botrytis Cinerea* - Causative Agent for Grey Mold by Crude Extracts of Basidiomycetes Fungi

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### ABSTRACT

*Botrytis cinerea* causes heavy economic losses to over 200 crop species world-wide and is one of the most difficult pathogen to control known to man. On-going *In vitro* antifungal screening has shown that acetone crude extracts obtained from fermented cultures of certain Basidiomycete fungal strains are able to inhibit the necrotrophic grey mold fungus *B. cinerea*. Crude extracts were prepared from sterile submerged liquid nutrient media where growth conditions were set to trigger production of the secondary metabolites. The antifungal testing of the crude extracts was done using agar diffusion assay technique.

**KEYWORDS:** *Botrytis cinerea*, grey mold, basidiomycetes, fermented cultures.

### INTRODUCTION

*Botrytis cinerea* the cause of grey mold is a well-known fungus with a wide host range that causes heavy economic losses of yield in more than 200 crop species including: onions, potato, strawberry, rose flowers, table grape and other ornamental plants (Guinebretiere et al., 2000). It is one of the most destructive plant pathogen species known to man (Elad et al., 2004) and is also regarded as one of the major threats to crop production (Hahn et al., 2008). Current commercial synthetic fungicides used for its control such as 'Mancozeb' have been shown to be carcinogenic (Marta et al, 2011). In addition, there have been documented evidences on traces of these fungicide residues persisting in vegetable crops and soil (Apladasarlis et al., 1994). Resistance of *B. cinerea* isolates from vegetable crops towards the major classes commercial anti-botrytis fungicides: anilinopyrimidines, phenylpyrroles, hydroxyanilides, benzimidazoles and dicarboximides have also been recently confirmed (Myresiotis et al., 2007). Natural antifungal compounds have been found to be comparatively much safer than synthetic ones in terms of toxicity in foodstuffs (Hanekamp and Kwakman, 2004). It is imperative that alternative fungicides from naturally occurring compounds that are easily biodegradable and of low mammalian toxicity be explored for safe control of crop fungal pathogens since low mammalian toxicity, minimal

environmental impact and novel modes of action are very important features of natural antifungal compounds.

Basidiomycetes fungi have been known to synthesize a vast array of secondary metabolites that possess beneficial biological activities (Dong-Ze et al., 2008). Some compounds obtained from them can be exploited through research for crop protection purposes (Loreto et. al., 2008). Current research on antifungal agents is based on the principle; that new generation fungicides should be practically non-toxic, except for the target organism (Komarek et al, 2009). Of relevance to this research, is the exploration of antagonistic strains belonging to the basidiomycete class of fungi, which are able to produce secondary metabolites that display antagonistic activity against *B. cinerea*. For this reason, armed with current methods in fungal biotechnology, there is high prospect of finding novel biologically active compounds that can be a potential fungicide for the control of grey mould disease.

### MATERIALS AND METHODS

Four hundred Basidiomycete strains which were identified based on their variations in morphological features were collected from Kakamega forest - Kenya and preserved in agar slants at the Integrated Biotechnology Research Laboratory (IBRL) - Egerton University.

All the glassware used in this work was standard quality and flasks as well as beakers were autoclaved before being used in the isolation of the test organism – *B. cinerea*.

The liquid nutrient media used in the fermentation process which was composed of molasses, glucose and yeast in a 10:4:4 mass ratio that was diluted in one liter of distilled water and placed in a 2.0L volumetric flask. The media and flasks were initially heat sterilized using an autoclave for 15 minutes at a temperature of 115 °C and pressure of 1.5 bars after which a single basidiomycete strain was introduced to grow inside as shown in figure 1. The flasks containing different strains were corked with sterile cotton wool. The fermentation flasks were regularly shaken and the glucose level in the cultures was monitored using glucose testing strips (Diabur-test® 5000 - Roche). The inoculation and monitoring of growth parameters were done under a lamina

flow hood backed with a hot flame produced by a Bunsen burner. The mycelium accumulation in each flask recorded at a day intervals.

At the end of the fermentation process, the growth was stopped. Crude extracts were then prepared using solvent extraction method using acetone which was recovered by concentrating using the Rotary evaporator apparatus (Buchi

R-205). *In vitro* antifungal testing was done by impregnating filter paper disc (Rundfilter, Ø6 mm, Schleicher & Schuell) with known amounts of the crude extracts (see figure 2). The inoculation and monitoring of growth parameters were done under a lamina flow hood backed with a hot flame produced by a Bunsen burner.



**Figure 1: Selected fermentation flasks containing the liquid nutrient media**

## RESULTS AND DISCUSSION

From the initial screening crude extracts using agar diffusion assay, 22 out of 400 strains produced appreciable antifungal activities against *B. cinerea* (see figure 2). The

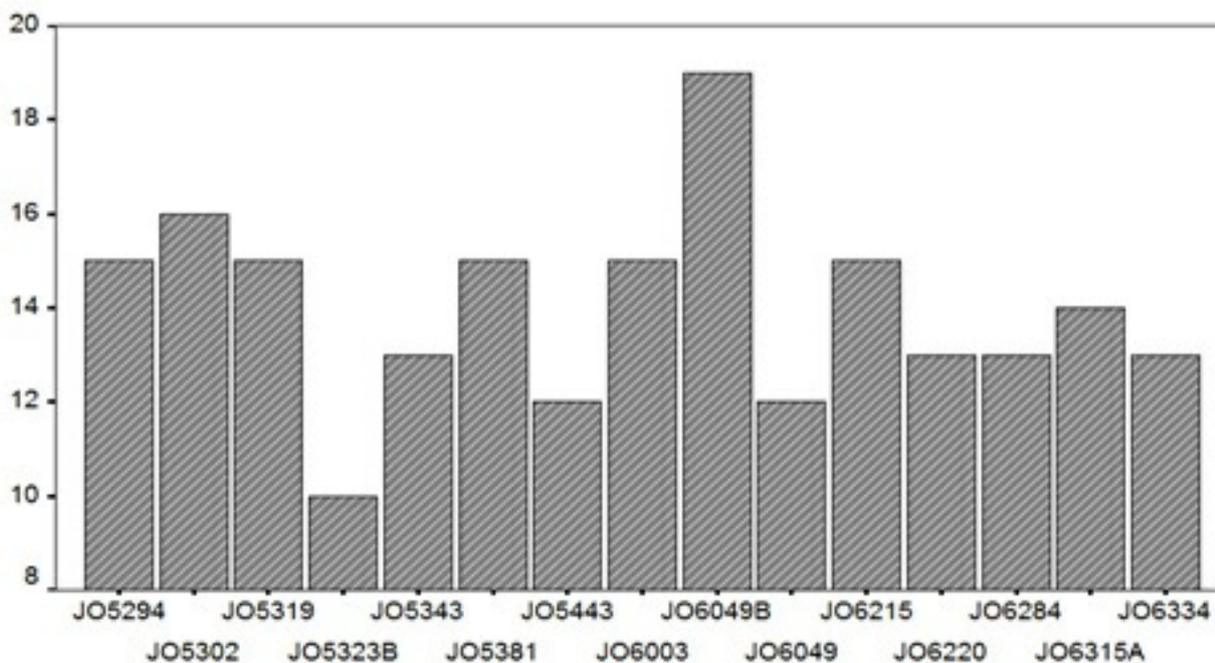
results significant since about 5% of the crude extracts screened showed significant activity against the *B. cinerea*, an accepted standard in microbial screening research (Rosa, 2003).



**Figure 2: Selected glass plates showing some of the bioactive basidiomycete strains**

Out of the 23 active strains, 15 of them showed reproducible antifungal activity accounting for a 65% reproducibility rate. It is possible that the differences in abiotic and biotic stimuli between laboratory culture and pristine conditions may

have played role in the production of the bioactive secondary metabolites as to explain the two-thirds reproducibility rate.



Fungal strains with reproducible activity  
**Figure 3: Graph of inhibition zone values for the active fungal strains**

The diameters of the inhibition zones (IZ) were measured in millimetres and analyzed using SPSS 11.5 and all the 22 strains collectively had mean of 14.2 mm, standard deviation of  $\pm 1.8$ , the greatest inhibition zone being 19 mm and the lowest being 10 mm (see figure 3).

## CONCLUSION

The results of this study at this stage suggest that naturally occurring antifungal compounds originating from certain strains of basidiomycete fungi do exist. Considering that *B. cinerea* is a serious threat to crop production, the anti-botrytis compounds contained in the active crude extracts shown can offer a potential alternatives to the current synthetic fungicides.

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## CONFLICT OF INTEREST

The authors of this article declare no conflict of interest.

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