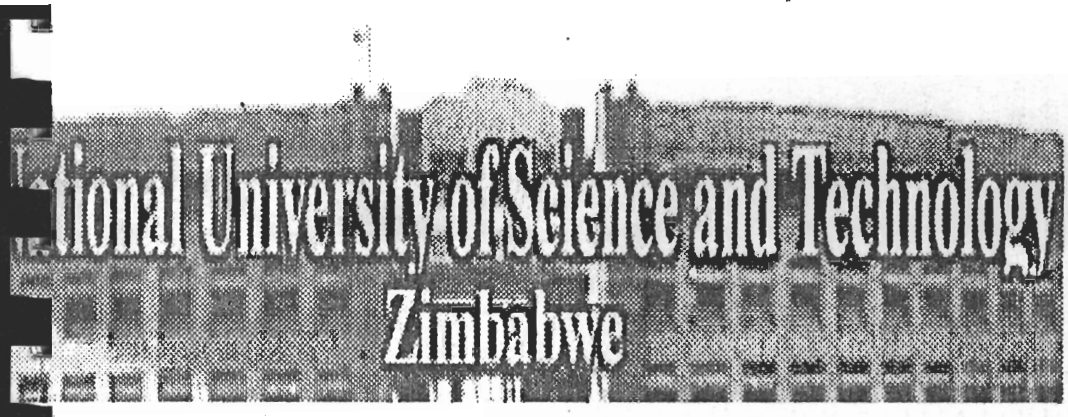

FACULTY OF APPLIED SCIENCE

DEPARTMENT OF APPLIED BIOLOGY AND BIOCHEMISTRY

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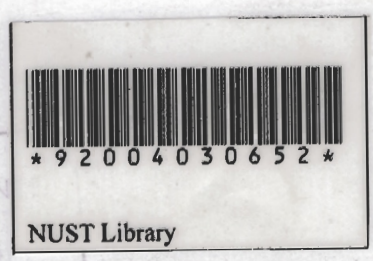
PROJECT TITLE

BIOCHEMICAL CHARACTERISATION OF *CLAVICEPS* ISOLATES THAT COLONIZE *HYPARRHENIA RUFA*(THATCHING GRASS) IN ZIMBABWE.

NONTOBEKO MLILO

N990 104M
2003



ABSTRACT

Claviceps africana is a well known pathogen of *Sorghum* in Zimbabwe. Recently, *Claviceps* isolates of unknown identity (species) were found colonizing *Hyparrhenia rufa* a thatching grass. The current study aimed at characterizing these isolates from *H. rufa*. Biochemical and growth characteristics of four *Claviceps* isolates (R1, R2, R3 and R4) colonizing *Hyparrhenia rufa* were compared with two representative isolates of *Claviceps africana* (Nov-1, B1/1). Analyses involved growth of the isolates on eight carbon sources which include glucose, fructose, carboxymethyl cellulose, crystalline cellulose, sorghum grains, Msasa cell walls, pectin and polygalacturonic acid. The culture medium (Medium F) included one percent (1 %) of each carbon source. Maximal growth was observed on sucrose and fructose carbon sources for all the isolates. The *Claviceps africana* isolates grew on all carbon sources except on polygalacturonic acid *H. rufa* isolates did not grow on crystalline cellulose and Msasa cell walls in addition to polygalacturonic acid but grew on the other carbon sources. The production of cell wall degrading enzymes using pectin and carboxymethyl cellulose as carbon sources was also investigated. Four enzymes which include polygalacturonase, pectin lyase and endo β 1,4-glucanase were assayed from the culture filtrate. Polygalacturonase (PG), exo-β 1,4-glucanase and endo β-1,4 glucanase were assayed using the dinitrosalysilic acid method to estimate the increase in reducing sugars. Pectin lyase was detected using the thiobarbituric acid method with pectin as the substrate. Endo β 1,4 glucanase and pectin lyase activities were detected in all the six isolates while PG and exo-β-1,4 glucanase were not detected. The detection of pectin lyase in the culture filtrate suggests that the pathogen may use pectin lyase to gain access to host tissues by degrading the pectin that is part of the middle lamella where pectin is a major constituent. Failure of *H. rufa* isolates to grow on Msasa cell walls and crystalline cellulose coupled with morphological colony differences of *H. rufa* and *Claviceps africana* isolates could suggest that *H. rufa* isolates are not the same as *Claviceps africana*, hence *H.rufa* may not be an alternative host of *Claviceps africana*. The close similarity in the biochemical characteristics of the two groups of isolates could also support that *H. rufa* isolates use a similar mechanism in penetrating its hosts as common members of the genus *Claviceps*.