MOLECULAR CHARACTERISATION OF Verticillium dahliae Kleb ISOLATES AND COMPARISON OF THEIR VIRULENCE ON SELECTED COTTON VARIETIES IN ZIMBABWE

By

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The undersigned certify that they have read and recommend to the Department of Crop Science for the acceptance of the thesis titled: **Molecular characterisation of Verticillium dahliae Kleb isolates and comparison of their virulence on selected cotton varieties in Zimbabwe.**

Submitted by **Nyamande Mapope** in partial fulfilment of the requirements of the degree of **Master of Science in Crop Protection**

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ABSTRACT

A study to determine the virulence of five *Verticillium dahliae* isolates on five varieties of cotton (SZ 9314, G 501, FQ902, BC 853 and 563-97-12) was carried out under both field (at five sites Cotton Research Institute (CRI), Henderson, Rafingora, Chisumbanje and Chinhoyi) and greenhouse conditions. The molecular differences of the five isolates were also compared using the Polymerase Chain Reaction (PCR) at Tobacco Research Board's Molecular Biology Laboratory. In the field experiments to determine virulence, the five varieties named above were planted at each site in a Completely Randomised Block Design with four replications. Monthly infections and fortnight scoring were done from January to the end of May. Infection percentages were calculated at the end of the season. They were arc sine transformed and then subjected to analysis of variance using Gestat 3.2. Fortnightly scores were used to calculate the area under the disease progress curves (AUDPC) using Sigma Plot 8. Significant differences ($p<0.05$) in infection percentages (arc sine transformed) were obtained at CRI, Chisumbanje and Chinhoyi but not at Rafingora and Henderson. There were no significant varietal differences for AUDPC at four sites (CRI, Chisumbanje, Rafingora and Henderson) except Chinhoyi, which had significant AUDPC differences between the varieties. From the five sites, soil samples were taken to isolate *Verticillium dahliae*. The isolates were tested for their virulence on five varieties used in the field. The trial was laid in a split plot design with *Verticillium dahliae* isolates as main plots and varieties as subplots. A single pot with one plant constituted a plot. Inoculum was prepared from the isolates and plants were stem pricked at the base at six weeks after planting. Severity scores were done a week after infection for the next four weeks. All the isolates were virulent, with isolates from CRI and Chinhoyi producing significantly higher AUDPC on all varieties than other isolates form other sites. Significant AUDPC were observed with G501 and BC853 having the lowest AUDPC while SZ9314 and F902 had the highest AUDPCs and variety 563-97-12 had intermediate AUDPC. PCR was run to compare five *Verticillium dahliae* isolates against the standard fungus from imported from South Africa at the Tobacco Research Board. Two primers were used (primer 19/22 and 42/70). Primer 22/70 managed to produce more bands (eleven bands) than primer 19/22 which produced five bands. Bands were scored and a cluster analysis was done to compare the differences of the banding patterns. Results show that there were differences at molecular level between the isolates. Differences were evident between the Rafingora isolate and the other isolates. Relationship between virulence and molecular differences was no established. However our results are preliminary and further research still need to be carried out on the isolates.

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