STUDIES ON *LECANICILLIUM MUSCARIUM* AS A MYCOPARASITE OF THE SOYBEAN RUST FUNGUS, *PHAKOPSORA PACHYRHIZI*

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DISSERTATION SUMMARY

Soybean rust (SBR) caused by *Phakopsora pachyrhizi* Syd. & P. Syd is the most important foliar disease of soybean. The disease starts on the lower canopy and from there it colonizes the whole plant. When conditions are favorable for disease development, SBR can cause serious damage and is able to cause up to 100% of yield loss. Spraying with fungicides is currently considered to be the only effective method for SBR control. However, there are undesirable consequences such as fungicide resistant strains of *P. pachyrhizi* and increased costs of production. Therefore, alternative control strategies, aiming for integrated disease management practices, are needed to control rust on soybean.

An isolate of *Lecanicillium spp*, strain Nesta-08, was observed colonizing and feeding on *Hemileia vastatrix* Berk. and Broome, the rust of coffee at the Assagay Coffee Farm, Cato Ridge, KwaZulu-Natal, South Africa. The fungus was isolated on Sabouraud dextrose yeast extract agar (SDYA) and the pure culture obtained was subcultured onto potato dextrose agar (PDA). Morphological studies were done using light and scanning electronic microscopy (SEM). Polymerase chain reaction (PCR) was performed to amplify the internal transcribed spacer (ITS), and specifically the portion of the mitochondrial encoded NADH dehydrogenase subunits 1 (MT-ND1) and 3 (MT-ND3) genes. Based on morphology and molecular studies, the isolate Nesta-08 was identified as a strain of *Lecanicillium muscarium* Zare et Gams and it was deposited into the National Collection of Fungi (accession number PPRI 13715).

Optimization of growing conditions is an essential aspect that must be taken into consideration to produce an economically viable biocontrol agent. Laboratory experiments were conducted to assess the effects of different growing conditions (temperature, artificial growing media, natural substrates and UV radiation) on colony growth and conidia production. Strain N-08 of *L. muscarium* was cultured on plates and incubated in the dark at five different temperatures: 18, 21, 24, 25 and 28°C for 30 days. The best growth was observed at 24°C. It was also grown on four different media, namely potato dextrose agar (PDA), malt extract agar (MEA), V-8 juice agar (V8A), and Sabouraud dextrose agar (SDA). The best growth was seen on V8A. Six different agro-industrial solid substrates were tested for suitability for sporulation by *L. muscarium* strain Nesta-08. These were wheat bran (*Triticum aestivum* L.), rice (*Oryza sativa* L.), rolled oats (*Avena sativa* L.), pearl

millet [*Pennisetum glaucum* (L.) R.Br.], pearled barley (*Hordeum vulgare* L.), and sorghum (*Sorghum bicolor* L.). The best production of conidia occurred on pearl millet followed by wheat bran and pearled barley. The lowest conidial production was observed on rolled oats. Under UV light mycelial growth was unaffected.

The effect of *L. muscarium* strain Nesta-08 on SBR was investigated using a bioassay on detached leaves, and in a greenhouse experiment. In a bioassay study conducted on soybean leaves infected with *P. pachyrhizi*, 1ml of a conidial suspension (10^6 conidia ml^{-1}) was sprayed on the abaxial surface of detached infected leaves using a hand sprayer. The isolate was observed growing and colonizing the SBR fungus. Under ESEM (Environmental Scanning Electron Microscope), the mycelium of *L. muscarium* was observed coiling around *P. pachyrhizi* urediospores. Penetration holes were also observed, where the infection hyphae had pulled away during sample preparation. *L. muscarium* Nesta-08 was further tested against *P. pachyrhizi* under greenhouse conditions. Potted soybean plants were artificially infected with *P. pachyrhizi* at Stage R_1. Once SBR pustules emerged, the plants were sprayed by conidia of *L. muscarium* at various conidial doses (10^8, 10^6 and 10^4 conidia ml^{-1}). All treatments significantly reduced SBR severity (P=0.0001) by 83.92%, 79.86% and 70.15%, respectively.

L. muscarium strain Nesta-08 was used in a field experiment at three doses (10^8, 10^6 and 10^4 conidia ml^{-1}), using the fungicide Score® as a comparison. All tested treatments significantly reduced SBR severity (P=0.0001). There were no significance differences in the disease ratings of plots treated with three doses of *L. muscarium* and Score® (0.0001). However, none of the treatments caused a significant increase in either plant dry weight or seed weight. Larger field trials are needed to test the effect of *L. muscarium* strain Nesta-08 on *P. pachyrhizi* for yield increases in soybean.

Keywords: Biocontrol, *Lecanicillium muscarium*, soybean rust, *Phakopsora pachyrhizi*.