# MANAGEMENT OF FUSARIUM HEAD BLIGHT OF WHEAT USING ANTAGONISTIC MICROORGANISMS

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### ABSTRACT

Laboratory and green house studies were conducted to evaluate the efficacy of *Epicoccum*, *Alternaria*, *Trichoderma*, and *Bacillus* spp. in control of *Fusarium* head blight caused by *F*. *graminearum*. Fungicides folicur<sup>®</sup> and copper oxychloride were used as standard checks. In laboratory, antagonism was measured as reduction in pathogen colony diameter while green house effectiveness was determined as reduction in head blight severity and mycotoxin deoxynivalenol (DON).

The antagonists and fungicides significantly reduced the growth of *F. graminearum* colonies in culture. Fungicides folicur and copper oxychloride completely inhibited growth of pathogen colonies while *Trichoderma* sp. showed 64% reduction in colony growth. The least effective was *Epicoccum* sp. However, the antagonists showed limited reduction in head blight severity in green house trials. Among the antagonists, *Trichoderma* sp. showed higher disease severity reduction (18%) while fungicide folicur was most effective with a reduction of 28%. All the antagonists had little or no significant effect on grain yield. However, co-inoculation of *F. graminearunm* with *Alternaria* and *Epicoccum* spp. reduced deoxynivalenol content in the grain but co-inoculation with *Trichoderma* and *Bacillus* spp. increased DON in the grain.

The results indicated that some of the antagonist might be useful in the management of *Fusarium* head blight and the associated mycotoxins. However, more studies are required to determine the effectiveness of the antagonists under field conditions and to screen more microorganisms for potential usefulness in management of *Fusarium* head blight and trichothecene mycotoxins.

Key words: Antagonists, Deoxynivalenol, Fusarium head blight, Fungicides, Wheat.

#### **INTRODUCTION**

*Fusarium* head blight (FHB) is a serious disease of small grain cereals. In addition to grain yield reduction, FHB can result in the reduction of grain quality, either by affecting grain processing qualities or by producing a range of toxic metabolites that have adverse effects on humans and livestock (Goyarts *et al.*, 2007). Mycotoxin deoxynivalenol (DON) can accumulate to unacceptable levels in harvested grain (Browne, 2007). Levels of DON above 2 ppm may render grain and their by-products unfit for commercialization and consumption. Efforts to minimize the impact of FHB and DON have been centred on the use of management strategies such as crop rotation, host plant resistance, tillage, and fungicides application (Kriel *et al.*, 2006; Browne, 2007). An integrated approach to management of FHB that includes chemical, cultural and host plant resistance seems the most logical way to reduce losses (Pereyra and Dill-Macky,

2004). Conflicting evidence exists regarding the effect of fungicides on the development of FHB and accumulation of trichothecene mycotoxins in grain (Halley *et al.*, 2005). All labelled systemic fungicides appear to increase yield, but those that contain a 'triazole', instead of a 'strobilurin' active ingredient are more effective in reducing DON levels in infected grain (Müllenborn, *et al.*, 2007). However, use of fungicides on wheat ears has the disadvantage of accumulation of residues in the resulting grain. The use of biological control would lead to reduction, if not elimination of the possible chemical residues in grain, environmental pollution and potential hazards to humans. Therefore, this study was carried out with the objective of evaluating the efficacy of fungal and bacterial antagonists in management of FHB caused by *F. graminearum*.

## MATERIALS AND METHODS

Laboratory and green house studies were conducted in 2006 to evaluate the efficacy of *Epicoccum* sp, *Alternaria* sp., *Trichoderma* sp., and *Bacillus* sp. to control *Fusarium* head blight of wheat caused by *F. graminearum*. Laboratory experiments involved growing the microorganisms together with the pathogen in paired cultures, where the pathogen agar disc was inoculated at the middle of plate and the antagonist at 4 equidistant points located 2 cm from plate edge. Fungicides folicur<sup>®</sup> (1000 ppm) and copper oxychloride (1000 ppm) were used as standard checks. Degree of antagonism was determined by measuring the pathogen colony diameters and percentage inhibition calculated as follows:

Percent inhibition = <u>Colony diameter of pathogen alone (control) – Colony diameter of pathogen + antagonist × 100</u> Colony diameter of pathogen alone

In the green house, ears of a highly susceptible wheat variety 'Mbuni' were inoculated at 50% flowering with antagonistic microorganisms together with *F. graminearum* or with *F. graminearum* together with fungicide folicur<sup>®</sup> (1.5 g/L) and copper oxychloride (6g/L). Head blight severity (proportion of ear bleached) was assessed five days after the last inoculation and after every 5 days thereafter until ripening. At maturity ears were harvested and threshed for grain weight determination and DON analysis by ELISA method.

# RESULTS

All the antagonists and fungicides tested significantly ( $P \le 0.05$ ) reduced colony diameters of *F*. *graminearum* (Table 1). Folicur<sup>®</sup> and copper oxychloride completely inhibited the growth of *F*. *graminearum in vitro*. The highest colony diameter reduction (64%) was observed in the treatment with *Trichoderma* sp. while the least reduction (45%) was observed in paired cultures with *Epicoccum* sp.

Head blight severity was significantly different ( $P \le 0.05$ ) among the antagonists (Table 1). Folicur<sup>®</sup> reduced the disease severity by up to 47% while copper oxychloride reduced disease severity by up to 36%. Among the antagonists, *Trichoderma* sp. was the most effective with a significant reduction of FHB by up to 25%. *Epicoccum and Alternaria* spp. had minimal effect on FHB while *Bacillus* sp. had no significant effect on head blight severity. Minimal amounts of disease were observed on plants inoculated with antagonist alone and the control. *Fusarium graminearum* was re-isolated at very high levels from kernels harvested from ears inoculated with the pathogen alone but the re-isolation rate differed for the kernels from ears inoculated with different antagonists.

The antagonists had little or no significant effect on grain weight (Table 1). However, folicur<sup>®</sup> and copper oxychloride significantly (P $\leq$ 0.05) increased grain weight by between 47 and 94%, respectively, compared to ears inoculated with *F. graminearum* alone. Folicur<sup>®</sup>, copper oxychloride and *Alternaria* sp reduced DON content in the grain by between 76 and 93% but *Trichoderma* and *Bacillus* spp increased DON compared to treatments with *F. graminearum* alone (Table 4). Head blight severity, kernel weight and DON content in grain were significantly (P $\leq$ 0.05) correlated..

| Table 1. Effect of antagonistic microorganisms on colony size, head blight severity, kernel    |
|--|
| weight and pathogen re-isolation for F. graminearum in culture and when inoculated on to wheat |
| ears   |

|                          | % Colony  | % FHB    | %Kernel wgt | DON     | Pathogen     |
|--------------------------|-----------|----------|-------------|---------|--------------|
|                          | reduction | severity | reduction   | content | re-isolation |
| Fusarium + Alternaria    | 51.5      | 76.0     | 44.7        | 125     | 48.0         |
| Fusarium+ Epiccocum      | 45.5      | 72.3     | 43.5        | 1,250   | 50.0         |
| Fusarium+ Trichoderma    | 64.1      | 59.7     | 41.0        | 3,100   | 58.5         |
| Fusarium+ Bacillus       | 52.0      | 80.5     | 44.7        | 2,450   | 73.5         |
| Fusarium+ Folicur        | 100.0     | 39.9     | 19.5        | 100     | 41.5         |
| <i>Fusarium</i> + copper | 100.0     | 52.9     | 26.1        | 337.5   | 41.5         |
| Fusarium alone           | 0.00      | 77.9     | 47.7        | 1,450   | 83.0         |
| Control                  | _         | 17.9     | 0.00        | 204     | 26.5         |

### DISCUSSION

All the antagonists inhibited the growth of F. graminearum in culture, indicating a possible release of extracellular volatile metabolites that diffused through the media. However, folicur® and copper oxychloride were the most effective, completely inhibiting the growth of the pathogen. In the two greenhouse trials, the fungicides reduced the disease severity by between 28-58%, although no complete control was observed, therefore confirming earlier findings by Müllenborn, et al. (2007). The low disease levels observed on plants inoculated with antagonist alone and the control could have been caused by pathogen conidia spread by wind from the inoculated ears. Among the antagonists, Trichoderma sp. reduced disease severity. Other microorganisms reported to have inhibitory effect on plant pathogens include Bacillus sp. (Parello et al., 2002), Epicoccum purpurascens (Brown et al., 1987), Trichoderma and Bacillus spp (Schumacher et al., 2007; Seddon 2007). Application of folicur® and copper oxychloride led to an increase in yield confirming earlier findings by Mesterhazy et al., 2003). The effect of fungicides on Fusarium spp. is dependent on timing and frequency of applications (Parry et al., 1995) and treatments at anthesis seems to be the best time for reduction of Fusarium infection (Mesterhazy et al., 2003). Trichoderma sp. reduced both disease severity and slightly increased the grain yield. This shows that biological control has considerable promise in reducing FHB as reported by Seddon, (2007). The antagonistic effect could be due to production of antifungal metabolites (Seddon, 2007) or production of a biosurfactant, which modifies the plant surface reducing surface wetness. Testing of a broader spectrum of other possible antagonists under field conditions and determination of their mechanisms of action would be necessary.

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