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Full Length Research Paper

Mycoherbicidal potential of *Alternaria alternata* for management of *Chenopodium album* under field condition

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A field experiment was conducted to evaluate the mycoherbicidal potential of *Alternaria alternata* to control *Chenopodium album* L., a problematic weed of wheat. Two wheat cultivars viz. Inqalab 91 and Punjab 96 were co-cultivated with *C. album* in 1:1 crop-weed ratio. Mycoherbicidal formulation was prepared in 20% canola oil emulsion with 10^7 conidia mL^{-1} of *A. alternata*. The mycoherbicidal formulation was sprayed at 2 - 3 and then at 4 - 5 leaf stage of the weed. Application of mycoherbicide significantly reduced the biomass of the target weed species up to 90%. Mycoherbicidal application significantly enhanced number of fertile tillers and 100 grains weight in both wheat cultivars. As a result of mycoherbicidal application, root and shoot biomass was significantly increased in cv. Inqalab 91 but not in cv. Punjab 96. The present study concludes that application of *A. alternata* in 20% canola oil can reduced growth of *C. album* resulting in enhanced growth and yield of wheat.

Key words: *Alternaria alternata*, canola oil emulsion, *Chenopodium album,* mycoherbicide.

INTRODUCTION

Chenopodium album L. is an annual weed of cultivated ground, especially on rich soils and old manure heaps. It is considered to be a very serious weed in the world. It is one of the most frequently occurring weeds in wheat fields in Pakistan (Siddiqui and Bajwa, 2001). It is a highly competitive weed and can cause drastic yield reduction under heavy infestation. The yield reduction by weeds in wheat may be up to 65% (Siddiqui, 2005).

Several management strategies including manual, mechanical, cultural and chemical methods are in practice to control *C. album*. However, these methods have certain limitations. Manual and mechanical practices are becoming costly with increasing cost of manual labour. Cultural practices are not always helpful in controlling the weed due to its high adaptability potential in different conditions. Chemical herbicides although are effective but health and environmental problems are associated with their use (Soares and Porto, 2009). These factors have opened new avenues for weed management like the use of plant pathogens as biological control agents (Ellison and Barreto, 2004; Cipriani et al., 2009; Siddiqui et al., 2010).

Recently, Siddiqui et al. (2009a) reported a new fungal pathogen *Alternaria alternata* responsible for causing leaf blight in *C. album*. The pathogen causes up to 70% mortality of the host plant. Previously, Siddiqui (2005) found that a formulation of *A. alternata* in 20% canola oil emulsion can cause severe disease in *C. album* under green house conditions. The present study was conducted to evaluate the mycoherbicidal potential of *A. alternata* against *C. album* under field conditions.

MATERIALS AND METHODS

Field experiment

Experiment was conducted in Botanical Garden, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan. Soil of the experimental field was sandy loam in texture having organic matter 0.69%, pH 7.8, nitrogen 0.035%, available phosphorus 6.3 mg kg^{-1} and available potassium 100 mg kg^{-1} of soil. The micronutrient boron (B), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) were 1.06, 22.8, 10.8, 1.9 and 1.3 mg kg^{-1} of soil, respectively. A

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basal dose of N, P_2O_5 and K_2O at 35, 50 and 50 kg ha^{-1} in the form of urea, diammonium phosphate and sulphate of potash was appied three days before sowing. N at 35 kg ha^{-1} as urea was also top dressed at initiation of flowering. These chemical fertilizers were applied as recommended by the Agriculture Department of Punjab, Pakistan.

Experiment was conducted in a split-split plot design. Two wheat cultivars namely Inqalab 91 and Punjab 96 were kept in main plots, weed species in subplots and mycoherbicidal application in sub-sub plots. Each sub-sub plot measured 2.5 x 1.4 m^2. Seeds of commercial wheat varieties Inqalab 91 and Punjab 96 were sown with inter and intra row spacing of 22 cm. One month after germination of wheat, *C. album* plants at 2 - 3 leaf stage were transplanted in the respective plots in 1:1 ratio with wheat plants. Plots without weeds served as control. Each treatment was replicated thrice. Plots were irrigated with underground water of good quality whenever required.

Preparation of mycoherbicidal formulation

A. alternata was isolated from leaf spots of *C. album.* Inoculum of *A. alternata* was produced on potato dextrose agar (PDA) medium. The PDA medium was sterilized by autoclaving at 121°C for 30 min. Twenty milliliter aliquots of sterilized medium were poured in sterilized Petri plates of 9 cm diameter and allowed to solidify. A 5 mm diameter disc from 8 days old stock cultures of *A. alternata* was transferred in the centre of each Petri plate and incubated at 25 ± 1°C in a growth incubator (Company: REVCO, Model: BOD30). Conidia were collected from 8 days old cultures by scraping the agar surface with a rubber spatula. The conidial suspension was then passed through a single layer of cheesecloth to separate the conidia from mycelial debris. Mycoherbicidal formulation was prepared in 20% canola oil having 10^7 *A. alternata* conidia mL^{-1} (Siddiqui et al., 2010).

Application of mycoherbicide

First mycoherbicidal application was employed at 5 - 10 leaf stage and second application was done at 10 - 15 leaf stage of *C. album.* In case of control, weed was sprayed with the formulation having no *A. alternata* conidia.

Host specificity

Seeds of wheat, maize and sunflower were sown in field plots of 1.5 × 1.5 m with inter-plant spacing of 20, 22 and 23 cm, respectively. Each treatment was replicated thrice. Plants were sprayed with mycoherbicidal formulation having 10^7 conidia mL^{-1} of *A. alternata*, both at vegetative and reproductive stages, and disease symptoms were monitored.

Harvesting schedule and data analysis

Wheat plants were harvested after 120 and 150 days of sowing. At each harvest, plants were carefully uprooted, thoroughly washed under tap water and roots were separated from shoots. Number of total tiller, fertile tillers, and shoot and root dry weights were recorded. At final harvest, grains were separated from ears and weight of 100 grains was recorded. All the data were analyzed statistically by applying t-test using computer software COSTAT.

RESULTS AND DISCUSSION

Effect of mycoherbicide on biomass of *C. album*

First mycoherbicidal application was employed at 5 - 10 leaf stage of *C. album.* The weed at this stage did not respond to applied formulations due to low prevailing temperature. Temperature is considered to be the most important component of the environment that limits the utilization and effectiveness of biological control agents as mycoherbicides (Smith et al., 2006; Siddiqui et al., 2009b). Application of second spray of mycoherbicide significantly reduced the fresh biomass of *C. album* as compared to control treatment (without mycoherbicde application). Effect of mycoherbicide application on *C. album* biomass was similar in field plots of both wheat varieties. There were 90 and 88% reduction in biomass of *C. album* due to mycoherbicide application in field plots of wheat cv. Inqalab 91 and Punjab 96, respectively (Figure 1). These results are in line with the findings of earlier workers who reported severe losses in weed biomass due to mycoherbicide applications (Sauerborn et al., 2007; Zahran et al., 2008).

Effect of mycoherbicide on number of tillers per plant of wheat

The effects of mycoherbicide application on number of tillers in two wheat varieties are shown in Figures 2a-b and 3a-b. In both wheat cultivars, mycoherbicide application significantly enhanced the total number as well as number of fertile tillers. Effect was significant at both harvest stages viz. 120 and 150 days after sowing (DAS). At 150 days growth stage, there were 46 and 29% increase in number of fertile tillers in cv. Inqalab 91 and Punjab 96, respectively, due to the application of mycoherbicide.

Effect of mycoherbicide on shoot and root dry biomass of wheat

In Inqalab 91, mycoherbicidal application enhanced shoot dry biomass both at 120 and 150 days growth stages. The effect was significant at latter growth stage where 47% increase in shoot dry biomass was recorded due to mycoherbicide application (Figure 2c). In case of cv. Punjab 96, the effect of mycoherbicide application on shoot dry weight of wheat was nonsignificant (Figure 3c).

Data regarding the effect of mycoherbicide application on root dry weight of two wheat cultivars are shown in Figures 2d and 3d. In Inqalab 91, mycoherbicide application significantly increased root biomass at 120 as well as at 150 days growth stage. The effect was more pronounced at 150 than at 120 days growth stage. There

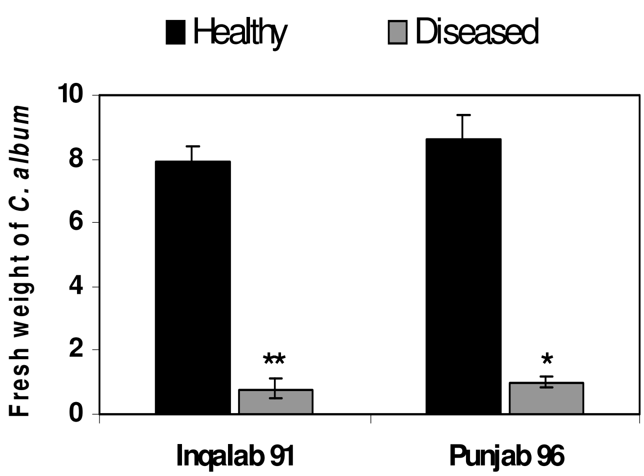

Figure 1. Effect of *A. alternata* blight on fresh weight of *C. album* co-cultivated with wheat varieties Inqalab 91 and Punjab 96. Vertical bars show standard errors of means of three replicates. *, **Significant difference between healthy and diseased *C album* plants at p ≤ 0.05 and 0.01, respectively.

were 40 and 175% increase in root biomass, respectively, due to mycoherbicide application. In contrast, in cv. Punjab 96, the effect of mycoherbicide application on root dry biomass was insignificant.

Effect of mycoherbicide on weight of 100 grains

The data regarding the effect of mycoherbicide application on 100 grain weight of cv. Inqalab 91 and cv. Punjab 96 are shown in Figure 4. Application of mycoherbide significantly enhanced 100 grain weight. There was 34 and 66% increase in 100 grain weight in wheat cv. Inqalab 91 and cv. Punjab 96, respectively, due to mycoherbicde application. The greater grain weight in mycoherbicidal than in non-mycoherbicidal treatments could be attributed to the reduced *C. album* biomass due to mycoherbicide application. Weeds compete with crops for water, light and nutrients (Tollennaar et al., 1997; Gibson, 2000; Wright et al., 2001). In case of diseased weed plants, the weed-crop competition is reduced; consequently, more nutrients and other resources are available to crop plants, resulting in enhanced grain yield.

Host specificity

No disease symptoms were observed on plants of any of the three crops viz. wheat, maize and sunflower. Due to the high specificity of *A. alternata* isolate used in the present study, it is preferable as a more specific mycoherbicide formulation agent for the management of *C. album*. Host specificity is of great importance when

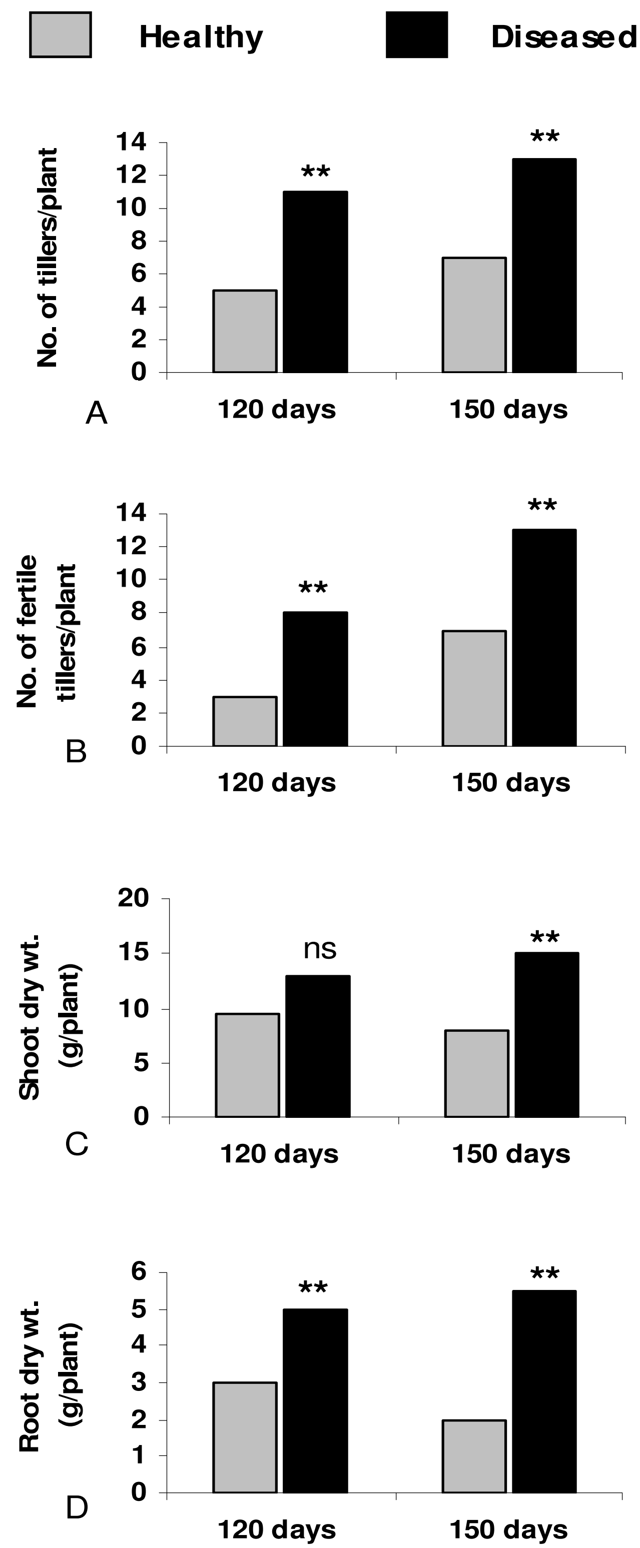

Figure 2. Effect of co-cultivation of healthy and diseased (suffering from *A. alternata* blight) *C. album* plants on vegetative growth of wheat cv. Inqalab 91. *, **Significant difference between healthy and diseased *C. album* plants at p ≤ 0.05 and 0.01, respectively; ns, non-significant.

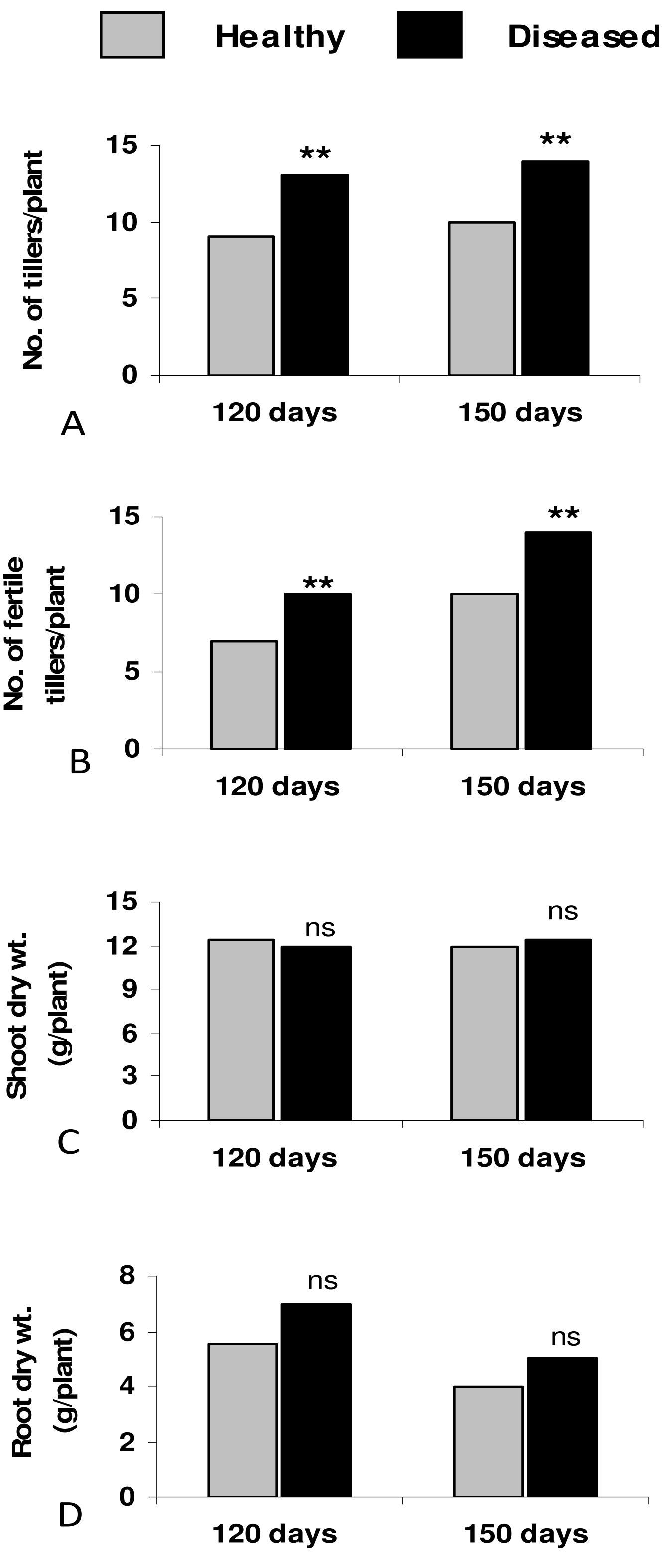

Figure 3. Effect of co-cultivation of healthy and diseased (suffering from *A. alternata* blight) *C. album* plants on vegetative growth of wheat cv. Punjab 96. *, **Significant difference between healthy and diseased *C. album* plants at p ≤ 0.05 and 0.01, respectively; ns, non-significant.

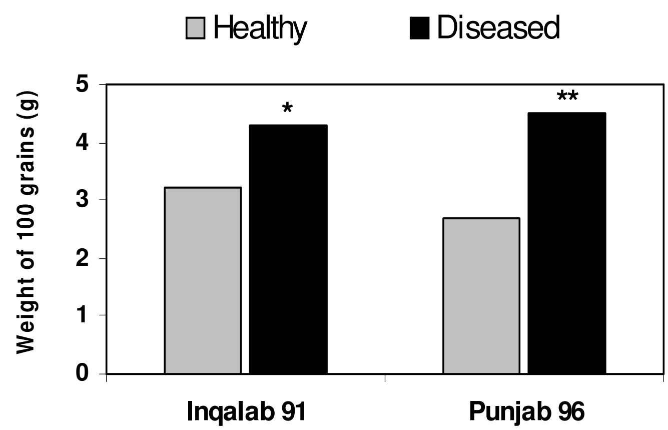

Figure 4. Effect of co-cultivation of healthy and diseased (suffering from *A. alternata* blight) *C. album* plants on 100 grains weight of wheat varieties Inqalab 91 and Punjab 96. *, **Significant difference between healthy and diseased *C. album* plants at p ≤ 0.05 and 0.01, respectively.

considering a pathogen for biological weed control (Nemat-Alla et al., 2008; Sands and Pilgeram, 2009). A narrow host range provides higher environmental safety of a bioherbicide, but can also limit effectiveness if more than one weed species has to be controlled (Winder, 1999; Elzein et al., 2006). In conclusion, *A. alternata* conidial formulation in 20% canola oil significantly reduced the biomass of *C. album*; consequently, the growth and yield of the two wheat varieties were enhanced significantly.

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