Pathogenicity of *Fusarium oxysporum* and *Curvularia lunata* as a mycoherbicide for the control of *Echinochloa crusgalli* (Barnyard grass)

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ABSTRACT

Echinochloa crusgalli (Barnyard grass) is one of the most invasive paddy (*Oryza sativa* L.) weed. Pure culture isolates of several fungi including *Fusarium oxysporum* (SBT # 17) and *Curvularia lunata* (SBT # 30) obtained from field sites were tested as candidates for the microbial control of barnyard grass. A survey was conducted in the paddy fields of eight southern districts of Andhra Pradesh to document the fungal pathogens of barnyard grass. Pure cultures were isolated from the naturally-infected barnyard grass leaves collected from different paddy - growing areas. A total of 51 fungi were isolated and two isolates were pathogenic on *E.crusgalli* under laboratory condition (> 70%). Furthermore *F.oxysporum* (SBT # 17) *and C. lunata* (SBT # 30) exhibited more pathogenicity under green house conditions on E. *crusgalli* and holds a great promise as biocontrol agents of barnyard grass.

Key words : Biological control, *Fusarium oxysporum, Curvularia lunata, Echinochloa crusgalli* .

INTRODUCTION

Echinochloa crusgalli (Barnyard grass) is one of the most invasive paddy weeds of Rice (*Oryza sativa* L) (Holm, L 1977). This species severely reduces the yield and the quality of rice (Smith, 1983). Biological control using living organisms can control or at least reduce the population of undesirable weed species. This classical approach with exotic plant pathogens for controlling the weeds was developed during 1970's (Alan, 1991). An alternative approach to bioherbicide development is based on the idea that an endemic pathogen might control its weed hosts through a massive dosage of inoculum at susceptible stages of growth (Daniel *et al*., 1973; Charudattan, 1991). Much research on the development of new mycoherbicides has been conducted during the two past decades worldwide. A mycoherbicide should be highly virulent and destructive and does not necessarily mean the killing of plant as suppression or debilitation may be achieved by the same desired level of control (Templeton and Heiny, 1990; Watson and Wymore, 1990).

Recently four different indigenous fungal species have been isolated from naturally infected *E. crusgalli* in Andhra Pradesh. To select the best candidate for further development as biocontrol agents for *E. crusgalli* species in rice, this study was designed to determine the pathogencity of these fungi on *E. crusgalli and* Pathogencity of selected isolates against *E.crusgalli* under green house conditions.

MATERIALS AND METHODS

Isolation and Identification of Fungi

A survey was conducted to document various fungal pathogens of *Echinochloa crusgalli* in eight southern districts of Andhra Pradesh *viz.*, Adilabad, Warangal, Guntur, Nalgonda, Medak, West Godavari, Krishna and Kurnool. In each district three locations were surveyed and leaf and stem tissues which developed diseased symptoms like spots, yellowing, rots and browning were collected from the paddy fields and air dried in a plastic bags, cut to appropriate size, and stored at 4^0C in paper envelops. Leaf pieces with lesions were surface sterilized with 0.5%sodium hypochlorite solution and incubated on fresh potato dextrose agar plates. Fungi that grew from the lesions were isolated and maintained on full - strength potato dextrose agar (PDA) slants at 4^0C. Based on the percentage of pathogenicity the selected isolates were submitted to the Department of Botany Osmania University, Hyderabad, for further confirmation and identification.

Inoculum Production

Fungal stock culture (3mm fungal discs) was aseptically transferred onto PDA plates and incubated for 2 weeks at 20^0C under continuous white fluorescent light. Spores were harvested by flooding the plates with sterile distilled water and lightly scraping the surface. The resulting spore suspension was filtered through cheese cloth and adjusted

to the appropriate density using a haemocytometer.The suspension of freshly harvested spores was added with 0.1% Tween - 80.

The list of fungal pathogens isolated from infected portions of *E.crusgalli* are *Fusarium* spp (SBT # 1, 10,11,15,17), *Alternaria* spp (SBT # 13,14,21), *Curvularia spp* (SBT # 2,30) *and Exserohilum* spp (SBT # 7,8,22).

Test for the Pathogencity of the Isolates

Pathogenicity of the isolates was assayed by the leaf bioassay. Healthy leaves of *E.crusgalli* were collected and covered immediately with a moistened sterile filter paper and transferred to the laboratory in polyethylene bags under moistened conditions. The leaves were washed in a running tap water rinsed thrice with sterile distilled water and placed in petri dishes lined with moist filter paper. The *E. crusgalli* leaves assigned to the treatments were sprayed with spores suspension (10^6 conidia / ml), while the control leaves were dipped in a sterile distilled water. Leaves were maintained in petri plates (5/plate) at 26 - 28° C under natural photoperiod. The leaf bioassay was repeated for three times and the leaves were daily observed for the development of symptoms, its nature and the time taken for its development, which were subsequently recorded. In addition, the extent of damage was evaluated at five rating levels: 1- Healthy; 2 - up to 25% shoot area chlorosis (less virulent); 3-26-50%chlorosis (moderately virulent); 4 - 70 - 90% chlorosis (virulent) and 5 - 76-100 % chlorosis (highly virulent) and death**.** The disease index was calculated using the formula

$$\text{Disease index (DI)} = \frac{\text{Sum of the score for each leaf}}{\text{No.of leaves scored} \times \text{Maximum score}} \times 100$$

Green House Tests

Production of plant material

Barnyard grass and Rice seeds collected from paddy fields were used in this experiment. Fresh seeds were soaked in sterile water for 4-5hrs and germinated on moistened filter paper in petri dishes maintained at 25°C for 3 days. Twenty fully germinated seeds were planted in each 14.5-cm x 11.5cm diameter tall plastic pots containing paddy growing soil. Plants were provided greenhouse conditions giving 30/25± 5°C day/night temperature with a 12-h photoperiod under natural sun-light. Seedlings were regularly watered. Unless otherwise indicated, 2-3 leaf stage plants were used in all experiments.

Inoculum production

The inoculum of each fungi used for all experiments were harvested from cultures grown on potato dextrose agar (PDA). Cultures were placed in an incubator at 28°C. A 12-h photoperiod was provided by two 20 W black fluorescent lamps positioned 20 cm above the PDA plates. Unless otherwise indicated, conidia were harvested from 7-14 day-old cultures by rinsing the cultures with sterile distilled water containing 0.1% (v/v) Tween-80. Freshly harvested conidia was filtered by using pre-sterilized muslin cloth and used as inoculum for all experiments. Conidial concentrations were determined and adjusted prior to use with the aid of a haemocytometer. Barnyard grass and Rice seedlings were sprayed with the inoculum amended with 0.1% (v/v) Tween 80, $1x10^6$, $1x10^5$ conidia / ml using a hand sprayer. All the plants were placed in a mist chamber (25°C) for 24 h, and then returned to the greenhouse. Likewise, the control treatments were done by spraying with sterile distilled water.

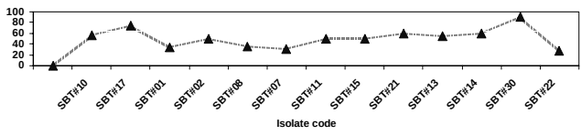
Disease severity and differences among treatments were assessed by rate of infection. Mortality of weed seedlings per pot was measured 10 days after inoculation. Diseased leaves were collected and the fungi isolated from symptomatic lesions to fulfill Koch's postulates.

RESULTS AND DISCUSSION

E. crusgalli leaves developed disease symptoms when inoculated with *Fusarium* spp, *Alternaria spp, Curvularia spp and Exserohilum spp.* Leaves were supported by water but upon removal from water they were limp and flaccid. Microscopic examination confirmed that fungal hyphae invaded the plant cell within 72 hrs, causing collapse and disruption of the infected tissues. Disease severity increased with inoculum concentration and the highest level of disease occurred at 10^6 conidia/ml. Based on the time taken for the development of symptoms, the pathogens were classified into four categories *viz*., less *virulent, moderately virulent, virulent and highly virulent* (Figure 1). The highly virulent pathogens developed symptoms within 3 days of artificial inoculation, while virulent (70 - 90%) pathogens developed symptoms within 6 days ,the moderately virulent(26-50%) needed more than 7 to express the symptoms and the less virulent (up to 25%) produced less symptoms within 20 days. *Fusarium* spp (SBT # 17) and *Curvularia* spp (SBT#30) were the only fungus in this study that could be grouped under the virulent category and the isolates were characterized as *F. oxysporum* and *C. lunata*, based on the development of symptoms within 5 - 6 days of artificial inoculation. Some other isolates of *Fusarium spp, Alternaria* spp and *Curvularia* spp are examples of moderate virulent pathogens, while *Exserohilum spp* constituted the less virulent category. A short dew duration requirement, quick and virulent infection of target weeds, good adaptation to the environment in which it

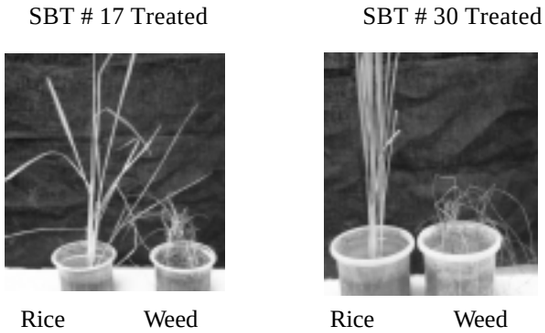
will be used, a high level of safety to crops and easy mass - production are the main criteria which a potential biological control agents must meet (Charudattan 1989, 1991).

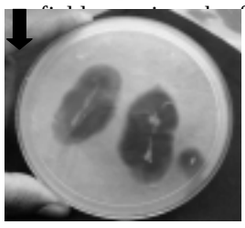
Figure 1. Pathogenicity of fungi isolated from infected *Echinochloa crusgalli* in paddy field (SBT # 1, 10, 11, 15 & 17 denotes *Fusarium* spp, SBT # 13, 14 & 21 denotes *Alternaria* spp, SBT # 2 & 30 denotes *Curvularia* spp, SBT # 7, 8 & 22 denotes *Exserohilum* spp)



In green house experiments, *F. oxysporum* isolate (SBT#17) showed infections i.e., yellowing of leaves after 48hr which slowly became dry after 7- 10 days. The isolate was able to kill 50% of the plants after 7 days of inoculation and 90% after 3 weeks with 10^6 conidia/pot, where as the requirement of more than 12 h of dew period for severe infection is a significant limiting factor for many fungal bioherbicides (Ghorbanl *et al.,* 2000; Kadir *et al.,* 2000; Zhang *et al.,* 2002). The percentages of innoculum density also play a major role in the infection process. Innoculum conc. of 10^6 conidia/ml subjected to 70 - 80% RH exhibited quick mortality rate when compared to 10^5 conidia / ml. Very low conc. of inoculum did not cause any infection. The plants got completely dried after 10 days of inoculation. No infections were observed in rice plants inoculated with the pathogenic isolates SBT # 30 and SBT # 17. Based on these results SBT # 30 isolate was considered as a best biocontrol agent against *E.crusgalli*

Figure 2. Pathogenicity of *Fusarium oxysporum* (SBT # 17) and Curvularia *lunata* (SBT # 30) against *Echinochloa crusgalli* under green house condition.





Reisolation from infected leaf

C. lunata (SBT # 30) exhibited black colour necrotic lesions within 24-48hr followed by necrosis and wilt within 3 days. The plants got completely withered after 5-6 days (Fig. 2). The isolate killed nearly 50% of the plants within 5 days of inoculation and 95% after 2 weeks of inoculation at 10^6 conidia/pot. Inoculation with less than 10^5 conidia/ pot failed to kill the host seedlings. Infection was not observed in rice plants inoculated with SBT # 30.

The infected leaves were collected and surface sterilized using 0.5%Naocl followed by 5 - 6 times washing with sterile distilled water. The leaves were later subjected to drying under sterile conditions and transferred onto PDA plates and incubated at 28^0C in inverted position for 7-8 days. After the complete spore formation they were subjected to microscopic observation to confirm the Koch's postulates.

Among the eight districts surveyed, paddy fields in West Godavari district were less frequently infested with *E. crusgalli*. Out of the 51 fungi recorded in the survey, 13 were pathogenic (Figure 1). Of these, *Fusarium* spp, *Alternaria* spp, *Curvularia* spp *and Exserohilum* spp *were* observed consistently in all the areas surveyed. The first stage in the development of mycoherbicide for paddy fi[illegible]ollowing characteristics: 1) Ease of [illegible] A wide range of target weeds and 3) [illegible] rice plants. *C.lunata* (SBT # 30) and [illegible] 17) satisfies these requirements.

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