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Potential control of *Echinochloa crus-galli* (Barnyard grass) by *Curvularia lunata* as a mycoherbicide

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ABSTRACT

Echinochloa crus- galli (Barnyard grass) is a world's most harmful weed species. *Echinochloa crus-galli* weed species compete with many economically important crops and causes serious problem in rice production. The virulent strain LD2 (leaf disease) of *Curvularia lunata* was screened out from 25 fungus strains isolated from the diseased leaves of barnyard grass (*Echinochloa crus-galli*). Greenhouse pot studies were conducted to the feasibility of the strain being as a mycoherbicide for barnyard grass control. The results of pathogenicity experiments showed that this strain was highly pathogenic to barnyard grass at the 2 to 3 leaf stages. Strain LD2 provided excellent barnyard grass control when it was applied at the concentration of 1×10^4, 1×10^6 conidia ml^1. This strain was very safe to rice and the most plant species except wheat, barley and corn. Findings of this study indicated that this strain could be a potential mycoherbicide for barnyard grass control in paddy fields in the future.

Keywords: Barnyard grass, Bioherbicides, *Curvularia lunata*, *Echinochloa crus-galli*, Mycoherbicide,

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INTRODUCTION

Weeds cause more economic losses on agricultural lands than all other pests combined. Currently, the most effective means of managing weeds are herbicides, which account for more than 60% of all pesticides used in crop production (Gianessi and Puffer 1991).Some widely used herbicides have been implicated in contamination of groundwater, soils, and food products, which may threaten public health and safety. Many of the weeds present in our surrounding environment, are the native different of regions and transferred to other areas by vectors seed, growth organ, fruit, wind, water and (insect). Unluckily, natural enemies of weeds are not transferred to new regions with them and therefore weeds are able to grow and expose. Excessive applications of agrochemicals are unhealthy for the crops as they directly and indirectly cause health problems in human, soil and water pollution. Barnyard grass (*Echinochloa crus-galli* (L.)) is one of the most troublesome weed species in rice crop production. Its infestation can inhibit the height of rice plants and reduce the number of panicles per plant, the number of grains per panicle, the weight of grains, and grain yield by 90% (Abeysekra 2003). In recent years, herbicide resistant biotypes of barnyard grass were reported (Fu *et al.* 2001; Maneechote 2003). It is essential to develop some new alternative tactics for the long term management of barnyard grass in the future. *Echinochloa crus-galli* is an annual plant from cereal family, which is one of the most problematic weed (Julien *et al.,* 1992). *Echinochloa crus- galli* (Barnyard grass) is a world's most harmful weed species. *Echinochloa crus-galli* weed species compete with many economically important crops (Eg banana, cotton, corn, millet, potato, sorghum) and caused serious problem in rice production (Holm *et al*., 1977). Microbial preparation of herbicide is defined as bioherbicides that can control the weed (Li *et al*., 2003).

In 1984, a fungal strain belonging to *Helminthssporium sativum* was isolated from diseased barnyard grass in Portugal (Reis 1985). From then on, a lot of researches on biological barnyard grass control were conducted. Several fungi, belonging to *H. gramineum* (Huang *et al*. 2005), *Colletotrichum graminicola* (Jamil and Nicholson 1987), *Alternaria alternate*, *A. tenuissima*, *A. triticina*, *A.*

brassicae (Bilgtami *et al.* 1995), *Exserohilum monoceras* (Chung *et al.* 1990; Gohbara *et al*. 1992; Zhang *et al*. 1996, 1997; Chen and Ni 1999; Huang *et al.* 2005; Yang *et al*. 2007), *Epicoccosorus mematosporus* (Gohbara *et al*. 1992; Zhang *et al*. 1996;), *Bipolaris sorkiniana* , *Pyricularia grisea*, and *Ustilago trichophora* (Tsukamoto *et al.* 1997) were reported as potential bio-control agents to manage barnyard grass.

The principal of biological weed control is to reduce and regulate weed populations below the economic injury levels, rather than to eradicate (Charudattan 2005). Mycoherbicides are fungal pathogens that are applied for the sole purpose to control a population of weeds (Templeton 1991). Biocontrol is an approach of using natural enemies to control or reduce the population of weed species (Charudattan and Dinoor 2000; Charudattan 2005).

Using weed pathogens is a feasible way to control weed population. There are lots of merits of this method such as convenient for practical, safe to human beings and animals or any other non-target organisms, and environmental friendly. These pathogens can be isolated from diseased weed plants or tissues. There is increasing interest in fungi as the control agents of arthropod pests, weeds and diseases (Whipps and Lumsden 2001).

Twenty five pathogenic strains were isolated from diseased leaves of barnyard grass strain leaf disease (LD2) collected from cauvery delta, Tamil nadu was highly virulent to this weed. The objective of this paper was to evaluate the potentiality of the strain LD2 as a biological control agent for barnyard grass in paddy fields.

MATERIALS AND METHODS

Survey

Survey was conducted in between 2017-2018 to see the percent occurrence and percent infestation of *Echinochloa crus-galli* weed in various agricultural important crops. However, the losses in yield were recorded simply on the basis of the information received from the farmers.

$$\%\ \text{occurrence of weed} = \frac{\text{No. of fields having } Echinochloa\ cruss-galli}{\text{Total no. of fields surveyed}} X\ 100$$

The percent infestation of a crop by the *Echinochloa crus-galli* was calculated by the quadrate method the most commonly used method, in ecological studies. The quadrate of 50 x 50 Cm^2 were used for the sampling purpose.

Collection of infected leaves

Surveys were conducted to search naturally occurring fungal pathogens on *Echinochola crus- galli* weed in cauvery delta districts of Nagapattinam, Thanjavur, Thiruvarur in the year 2016-2018. Infected plant were collected in sterile polythene bags and brought to the laboratory for the study of symptoms isolation, identification and pathogenicity test of the pathogens involved. Specimens were pressed, dried and kept as herbarium record, bearing details like name of the host, location, data of collection etc.

Isolation of fungal pathogens

The infected leaves were washed thoroughly in running tap water to remove the attached soil particles and cut in to 2 mm pieces by using a sterile scalpel. These were surface sterilized in 70% ethyl alcohol for 30, 60, 90, and 120 seconds followed by washing 6-7 times in sterile water. Two to three such surface sterilized pieces were aseptically transferred to potato dextrose agar (PDA) plates supplemented with 3.7 mg of streptomycin sulfate and 2.5 mg of chloramphenicol per liter of medium. The antibiotics were added to prevent bacterial contamination of cultures. Two methods were used for the isolation and identification of fungal pathogens from the infected leaves.

Barnyard grass plant preparation

Seeds of barnyard grass pre-germinated in Petri dishes at 28°C for 2 days were sown in 6 cm×6 cm plastic pots containing the mixture of vermiculite and peat (1:4, v:v) and kept in cultural chambers at 28°C in a 14hours photoperiods with light intensity 72 moL m^{-2} s^{-1}. Seedlings were thinned to 15 per pot before test. Unless indicated, seedlings were treated at the 3 leaf stage. Inoculum preparation and inoculation method the conidia of strain LD2 were harvested from potato dextrose agar plates after 2 week incubation at 28°C in dark. Conidial suspensions were prepared with sterilized water, containing 0.5‰ (v/v) Tween-20 as surfactant. Unless indicated, the test plants were inoculated by spraying the conidial suspension of 1×10^6 conidia ml^{-1} with a hand-hold sprayer at the 3-leaf stage and then kept in chambers for 48 hours.

Pathogenicity tests

Whole plant test when barnyard grass seedlings were treated with the conidial suspensions of strain LD2 were sprayed at the 1, 2, 3 and 4 leaf stage until runoff with a hand sprayer. Sterilized water containing 0.5% (v/v) Tween-20 was sprayed in untreated control for each seedling stage of barnyard grass. The fresh weight reductions were surveyed 10 DAT. The test plants were treated by spraying the conidial suspension of 1×10^6 conidia ml^{-1} with a hand-hold sprayer at the 3-leaf stage and then kept in dew chambers for 48 h. The fresh weights were measured and the symptoms were recorded 10 DAT for each species.

Measurement of disease intensity

The intensity of disease was measured in terms of disease incidence and disease severity (Chaube *et al.,* 1991). The disease severity was examined at 10 days of interval, and the leaf spot disease was evaluated using standard area diagram of infected leaves. The quantitative data on disease severity were calculated using the analysis of variance, as following Balyan *et al.,* (1986). For the estimation of leaf area diseased, the whole leaf surface area was considered as 100, and thereby the infected area was determined by eye estimation for percent of disease index (PDI). ie. Disease severity disease intensity and severity were rated by visual observation, and the infected leaves were scored using a 0.5 scale rating system. Using this rating system, a disease index (DI) was calculated per observation made at an interval of 10 days after treatment, for the assessment of disease severity individual leaf ratings were taken into an account until the death of weed.

Table 1 0-5 Disease rating scale used to assess the severity of symptoms on disease intensity (DI)

Disease rating scale	Disease description
0	No symptoms
1	1% - 10% of the leaf area covered by spots
2	11% - 25% of the leaf area covered by spots
3	26% - 50% of the leaf area covered by spots
4	51% - 75% of the leaf area covered by spots
5	≥75% of the leaf area covered by spots

Disease intensity (DI)

Inoculum was applied onto the test plants of *Echinochloa cruss-galli* within 2 hours of sunset to avoid drying and to allow for a natural dew period shortly afterwards. Plants were observed three days after treatment (DAT) for disease symptoms. The intensity of infection was determined visually, based on the initiation of disease and increase in disease area on the leaves, stems of test plants every day. The disease intensity of pathogen on test plants was determined using a score chart (-, no symptoms, a healthy plant; +, mild symptoms, a plant showing slight symptoms on $\leq$15% of the leaf area; ++, moderate symptoms, a plant showing definitely bigger patches of diseased areas on 16 to 59% of the leaf area; and +++, severe symptoms, enlarged lesions covering 60 to 80% of the leaf area) (Ray and Hill, 2012).

RESULTS AND DISCUSSION:

Survey

In the year 2017-2018, extensive surveys of various croplands were made with the objective to search for isolates and evaluate potential fungal pathogens on *Echinochola crus - galli*. During various surveys conducted in different regions of Nagapattinam, Thanjavur, and Thiruvarur in various cropland of cauvery delta districts were found heavily affect by various types of leaf spots between 2017 to 2018 during different seasons, infestation of *Echinochola crus – galli* was recorded in various crops such as paddy, black gram, peanut.

Table 2 Infestation of *Echinochloa crus- galli* in various agricultural crops as per my survey (2017 - 2018)

S.No	Crop	No. of fields visited	No. of fields having weed	%Occurrence of the weed	%infestation	% losses in yield
1	Paddy	30	25	83%	70-75%	85-90%
2	Cotton	20	13	65%	40-55%	32-60%
3	Sorghum	25	11	44%	35-45%	20-30%
4	Banana	15	09	60%	45-50%	15-25%
5	Maize	20	12	60%	50-55%	30-35%
6	Black gram	17	15	88%	60-70%	70-85%

Table 3 Diversity of Barnyard grass *(Echinochloa crus- galli)* in agricultural crops as per my survey

Table of Crop	English Name	Scientific Name	Weed Status
Food crop	Paddy /rice	*Oryza sativa* L.	Common
	maize/corn	*Zea mays* L.	Common
	Ragi/Finger millet	*Eleusina coracana* Gaertner	Common
Pulses	Black gram	*Vigna mungo* (L.) Hepper	Occasional
	Green gram	*Vigna radiata* (L.) Wilczek	Common
	Horsegram	*Macrotyloma uniflorum* (Lam.) Verdc.	Occasional
Vegetables	Brinjal/ Eggplant	*Solanum melongena* L.	Occasional
	Tomato	*Lycopersicon esculentum* Miller.	Common
Oil crop	Ground nut/pea nut	*Arachis hypogaea* L.	Common
	Sesamum/ Gingelly	*Sesamum indicum* L.	Common
Commercial crop	Sugarcane	*Saccharum officinarum* L.	Occasional
	Cotton	*Gossypium arboreum* L.	Common
	Tobacco	*Nicotiana tabacum*	Rare

Identical characteristics of *Curvularia lunata*

The identification features of the isolates, such as colony diameter, color, texture, sporulation, the shape and sizes of conidiophores and conidia, were carefully studied. The causal agent of leaf spot disease of *Echinochloa crus- galli* was isolated from wild plants and simultaneously from test plants inoculated with spore suspension. The pathogenicity of the isolate was confirmed by Koch's postulates, and the host specificity of the pathogen was tested using repeated spore treatments and reisolation of causal agent. At maturity stage, the profuse radial growth and sporulation of a fungal pathogen were recorded and the isolate was confirmed as *Curvularia lunata* by microscopic study of mycelium, conidia and conidiophores. Inoculated leaf lesions yielded white colored colonies of *Curvularia lunata* at initial stage on the surface of nutrient media. Subsurface mycelial growth was dense and dark on PDA. Sporulation was excellent at agar surfaces of CDA, and the moderate amounts of sporulation appeared on PDA. Significantly ($P < 0.05$) higher radial growth (mm) of the isolate was recorded on PDA (76.67 ± 1.76 mm) on the 12th day. *Curvularia lunata* often proliferates by means of a secondary conidiophore that arises immediately below the apical cell of the existing conidiophores.

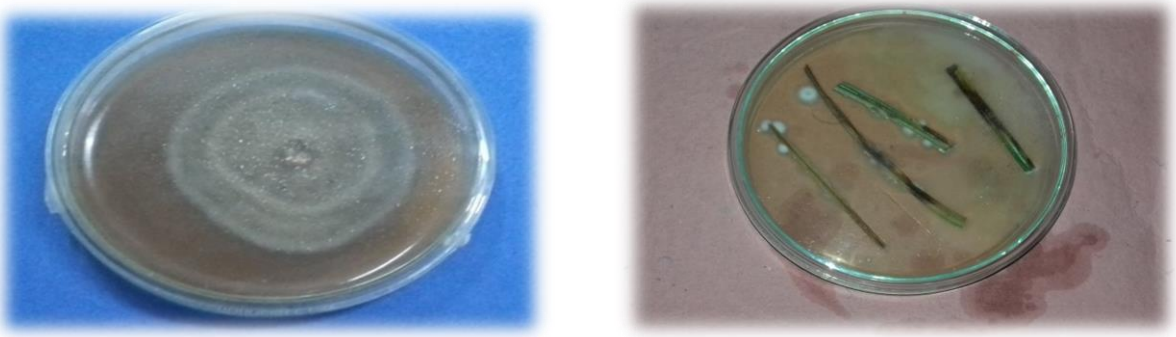

Fig. 1 The isolation of causal agent of leaf spot from infected leaf propagules.

(A) Diseased leaves colony (B) Culture of the isolate *Curvularia lunata*, on PDA

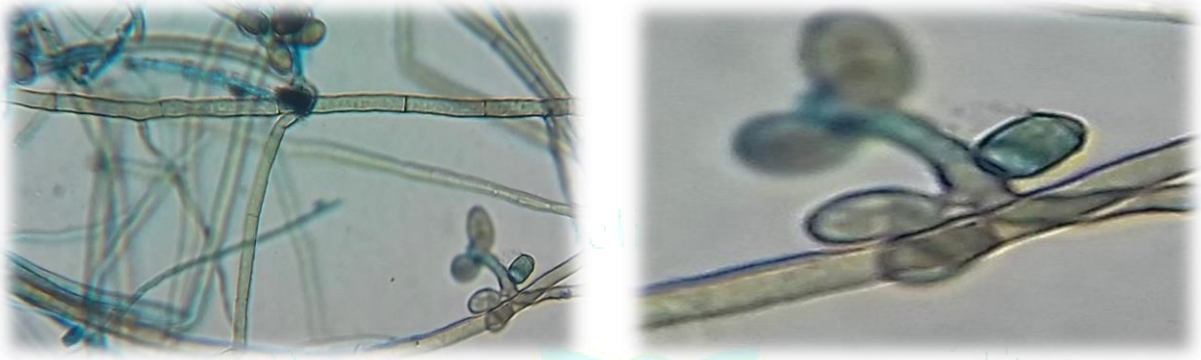

Fig. 2 Morphological characteristic of conidia and conidiophers of *Curvularia lunata* strain LD2

Pathogenicity tests

Pathogenicity test results showed that *C. lunata* strain LD2 had a strong pathogenic effect on barnyard grass (Fig. 3). In the leaf section test, lesions were observed on barnyard grass leaf sections and the leaf sections began to turn yellow 3 days after treatment (DAT). The whole leaf sections became wilt and conidial pustules appeared in 7th DAT. In the whole plants test, the leaves of barnyard grass began to turn yellow partially in 3th DAT and sporadic lesions were observed on 7th DAT. The lesions then expanded fast and almost all the leaves and stems were wilting 15 DAT. These minute lesions on leaves were the result of the penetration of the germ tube or infectious structures on host tissue. An infection rate of (85 ± 9.0 %) was observed at 20 days after treatment (DAT), and the epidemic increased timely with increase of incubation period. The significant (P < 0.05) virulence of pathogen in terms of PDI was determined as 30 ± 3.7%, 65 ± 7.3% and 85 ± 9.0 % on 7, 15 and 20 DAT, respectively. The values are represented mean of five replicates and standard error (Table 5). *Curvularia lunata* (host) pathosystem revealed the virulence of the pathogen as a promising mycoherbicide agent, which was highly potential to cause severe endemic on leaves, petioles, stem and other propagules of the target weed plant. Statistical analysis of the data on the inoculated plants revealed that percent infection was highly significant (P < 0.05) at various growth stages of the weed (Table 4). The early stage of the weed plant with 3-6 foliage favours the germination and penetration of the conidia of *Curvularia lunata* the infective propagules of the pathogen. The destructive damage of leaves and stems was examined on susceptible stage of the weed and caused 100% mortality of the weed within short period

Table 4 Disease intensity on test plant *Echinochloa crus- galli* inoculated with spore suspension (8X10^7 /ml) of *Curvularia lunata*

Days after treatment (DAT)	Disease intensity (DI)		DI Scale
	Control plants [a]	Test plants [b]	
3rd Day	- no symptoms	+ mild symptoms on 10% of the leaf area	1
7th Day	- no symptoms	+ mild symptoms on 20% of the leaf area	2
11th Day	- no symptoms	++ moderate symptoms on 35% of the leaf area	3
15th Day	- no symptoms	++ moderate symptoms on 59% of the leaf area	4
18th Day	- no symptoms	+++ severe symptoms, enlarged lesions covering 80% of the leaf area	5
20th Day	- no symptoms	Affected leaves became chlorosis and dried up causing severe defoliation and withering of stems.	5

'a' inoculated with cool sterilized distilled water, *'b'* inoculated with spore suspension of isolate

(Fig.3) Effect (infected plants) of *Curvularia lunata,* on *Echinochloa crus – galli*

Table 5 Effect of foliar application of *Curvularia lunata* (percent infection) to *Echinochloa curs- galli*, 20 days after inoculation

Percent infection on leaves		
No. of Days	After Inoculation (%)	Control (%)
7 days	30 ± 3.7%	3.4 ±4.5
15 days	65 ± 7.3%	5.2 ±6
20 days	85 ± 9.0 %	6 ±6.5

The results of the 'whole plant tests' showed that the strain LD2 was highly pathogenic on barnyard grass seedlings in controlled conditions. De Luna *et al* (2002) mentioned different rice cultivars had different reactions with pathogens. However, our result showed that the strain LD2 was safe to different types of rice. *C. lunata* is one of important pathogens of corn and causes leaf-spot diseases. The strain LD2 infected wheat, barley and corn, but the infection was slight and did not re-infect the crop plants, and furthermore, the production of rice is separated spatiotemporally from wheat, barley, and corn in some regions. So this separation could be utilized for the safe use of this pathogen. Strain LD2 was very convenient to incubate in laboratory. It grew fast on PDA media and enough conidia were produced 10 d after inoculation under 28°C. This characteristic is propitious to the mass production of conidia.

The present data suggest that *Curvularia lunata* was approved as highly aggressive towards control of Barnyard grass and has certain characteristics suggested by various workers. Barnyard grass is one of most noxious limiting factors in rice production in the world. *Curvularia lunata* LD2 was highly pathogenic on this weed and very safe to rice. The results of this study indicated that this strain could be a potential mycoherbicide to control barnyard grass in the future.

CONCLUSION:

Microbial pesticides (bioherbicides/ mycoherbicides) for the management of weeds are host specific, inexpensive and eco-friendly approaches. There has been a great number of naturally occurring fungal strains investigated for possible use as mycoherbicides, but only a small proportion has been developed to commercial products. The findings in this paper enlightened the mycoherbicide properties of *Curvularia lunata* and concluded that it is an effective bio control agent to the target weed. The results also revealed that the Barnyard grass weed was controlled by the fungal pathogen *Curvularia lunata* at field as well as in greenhouse conditions. The fungal pathogen *Curvularia lunata* is highly virulent as well as host specific. The quantitative data on disease severity revealed the bio control potential of *Curvularia lunata* as a successful mycoherbicide. The more extensive work is required to study pathogenicity, adaptability, dispersal and survival efficiency of the pathogen for the development of a commercial mycoherbicide. Type of strain and its virulence, infection structures produced by germ tubes, the number of appressoria per conidia, penetration of plant parts, toxin productions, inoculum adjuvants such as surfactants, interaction with other phylloplane microbes etc can affect the successes of a particular fungal isolate. The research in the direction of the development of mycoherbicide based on several field applications should be needed, and also the exploration of different virulent strains of *Curvularia lunata* is obligatory to develop a successful mycoherbicide.

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