Potential biocontrol of Echinochloa crus-galli (Barnyard grass) by Curvularia lunata LD2 as a Mycoherbicide

Nandhini Chandrasekaran, Ganesh Punamalai, Yoganathan Kamaraj

Abstract: ***Echinochloa crus-galli (commonly known as barnyard grass) is a noxious weed causing loss of several economically important field crops in tropical regions . An intensive work on the screening of the bio control agents was accomplished through in vitro epidemic study to control weed populations. The foliar disease symptoms on infected weed plant caused by fungal pathogens represented as round to irregular maroon spots with dark borders and the epidemic was indent fields as leaf spot disease. The pathogen allied with the infection of barnyard grass was isolated from infectious propagules by inoculation of leaf bites on a potato dextrose agar (PDA). The caused agent of leaf spot was confirmed as barnyard grass by Koch's postulates. The mycoherbicide ability of Curvularia lunata to control barnyard grass has been examined through visual (standard are diagram) and statistical methods. The results revealed that the pathogen causes significantly severe infection on host weed and destructs the weed population by leaf spot diseases. The findings of the research suggested that the isolate barnyard grass is highly virulent and host specific and recommended for further studies as a promising bio- control agent against barnyard weed.***

KeyWords-Bioherbicides, Curvularia lunata, Echinochloa crus-galli, Mycoherbicides.

I. INTRODUCTION

Weeds are major contributing factor to crop yield loss on the prairies through competition yield for water and nutrient[1]. Presently, the most effective means of managing weeds are bioherbicides, which account for more than 60% of all pesticides used in crop production[2] . Weeds have spread unwantedly through the activities of human beings and domestic animals[3].So that way become a major problem to both crop as well as animals[4]. Biological weed control involves using living organisms, such as bacterial, Virus and fungi to reduce weed populations. *Echinochloa crus- galli* (Barnyardgrass) is a world's most harmful weed species[5]. *Echinochloa crus-galli* weed species compete with many economically important crops (Eg banana, cotton, corn, millet, potato, sorghum, and taro) and caused serious problem in rice production[6].

Excessive applications of agrochemicals are unhealthy for the crops as they directly and indirectly cause health problems in human, soil and water pollution. *Echinochloa crus-galli* is an annual pl[7]ant from cereal family, which is one of the most problematic weed. Microbial preparation of herb[8]icide is defined as bioherbicides that can control the weed. 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)[9,10].

Biological control suggests methods to set natural balance between the weeds and their surrounding environment and this process is done through introducing the insect and disease which attack the noxious plants[11,12]. Mycoherbicides are fungal pathogens that are applied for the sole purpose to control a population of weeds[13,14]. Microbial preparation of herbicide is defined as bioherbicides that can control the weed[15,16].

In 1984, a fungal strain belonging to *Helminthssporium sativum* was isolated from diseased barnyard grass in Portugal [17]. From then on, a lot of researches on biological barnyard grass control were conducted. Several fungi, belonging to *H. gramineum Colletotrichum graminicola*, *Alternaria alternate*, *A. tenuissima*, *A. triticina*, *A. brassicae*, *Exserohilum monoceras Epicoccosorus mematosporus*, *Bipolaris sorkiniana* , *Pyricularia grisea*, and *Ustilago trichophor* 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[18] .

Mycoherbicides are fungal pathogens that are applied for the sole purpose to control a population of weeds[19]. Biocontrol is an approach of using natural enemies to control or reduce the population of weed species. Using weed pathogens is a feasible way to control weed population[20]. 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[21]. 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[22]. Six 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[23]. 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[24,25,26,27,28,29].

Revised Manuscript Received on October 25, 2019.

Nandhini Chandrasekaran, Ganesh Punamalai[2]*, Research scholar, Department of Microbiology, Faculty of Science, Annamalai University.

Ganesh Punamalai, Assistant Professsor, Department of Microbiology, Faculty of Science, Annamalai University. Corresponding author E-Mail: drpg1974@gmail.com.

Yoganathan Kamaraj, Research Associate, Scigen Research and Innovation Pvt, Lt., Thanjavur, TamilNadu, India.

Thus, our research constitutes an important contribution in the biological control of *Echinochloa crus – galli* our results will be of great importance for India and several other countries where this terrestrial weed represent a major environmental, ecological and economic threat. Intensive work is still needed on the impact of the field environment and application technology on the efficacy of this pathogen as a mycoherbicide.

II. MATERIALS AND METHODS

A.Survey

Surveys were 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.

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

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.

B.Collection of infected leaves

Surveys were conducted to search naturally occurring fungal pathogens on *Echinocholacrus- 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.

C.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.

D.Identification of fungal pathogen

Morphology of various fungal isolates was studied by preparing lactophenol cotton blue mounts from moist chambers/plate cultures as well as from the infected host tissue sections. A morphological characteristic of fungal pathogens were studied at different stages for the identification of the pathogens based on features such as colonial morphology (Ainsworth *et al*., 1973; Lacap *et al*., 2003; Aneja, 2003).

E.Isolation frequency of pathogenic fungi from Echinochloa crus- galli

The isolation frequency of pathogenic fungi was calculated in different season i.e. summer, rainy and winter. The diseased specimens were collected and frequencies were recorded on the basis of their presence and were categorised as HF (high frequency, 70-100%), MF (moderate frequency, 1-30%) and A (Absent, when no disease was found in these seasons).

F.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 1×10^5 conidia ml-1 with a hand-hold sprayer at the 3-leaf stage and then kept in chambers for 48 hours.

G.Selection of best medium for growth and sporulation

To determine the best growth medium for the virulent fungal pathogen, four different media were selected. Growth and sporulation of the selected pathogens on these media were determined. All the media were sterilized at 15 psi & 121˚C for 15 minutes. Fifteen ml of a given medium was poured in to each sterile petri plates and allowed to solidify. The plates with solidified medium were kept in an inverted position for 24 hrs to remove the thin film of water from the surface. Mycelial discs of 8mm diameter of the test pathogen was cut from the periphery of seven days old culture (actively growing colonies) were placed in the center of each plate and were incubated at 25±1˚C for 6-9 days. Three replicates were taken for each medium. Fungal growth was determined by calculating the area of radial growth for each colony. The measurements of each colonies were taken along the perpendicular lines from the center of inoculated to the edge of colony and average diameter was determined for each Petridish. (Miller,1977; Abbas *et al.*, 1995).For measuring sporulation, the cultures were grown on various solid media, mycelial growth from petri plate was scraped out with distilled water and homogenized on a magnetic stirrer for 15-20 minutes and strained through what man's filter paper. Conidial concentration was determined by using haemocytometer (Tuite, 1969).

H.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 1×10^5 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.

I.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 Disease rating scale to assess the severity of symptoms on disease intensity (DI)

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	Maize	25	11	44%	35-45%	20-30%
4	Banana	15	9	60%	45-50%	15-25%
5	Sugarcane	20	12	60%	50-55%	30-35%
6	Black gram	17	15	88%	60-70%	70-85%

J.Disease intensity (DI)

Inoculum was applied onto the test plants of *Echinochloa cruss-galli* within 2 hoursof 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).

III. RESULTS AND DISCUSSION

A.Survey

In the year 2016-2018, extensive surveys of various pastures, gardens, lawn, croplands and non - 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 delta districts were found heavily affect by various types of leaf spots. During the extensive surveys conducted in the various delta districts Nagapattinam, Thanjavur, and Thiruvarur 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 (2017 - 2018)

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

B.Collection of infected leaves

Infected leaves with different types of symptoms were collected in sterile polythene bags and brought to the laboratory for the study of symptoms, isolation, identification and pathogenicity tests of the fungal pathogen/s involved.

Leafs spots and necrosis were the common symptoms observed. Although all the stages of leaves showed infection, the mature leaves were more heavily affected. During survey conducted to search for virulent fungal pathogens, *Echinochola crus – galli* population was found to be heavily infected by leaf spot diseases. The various parts of plant showing disease symptoms were collected from three sampling points per site and for each disease symptoms 3 -5 infected parts were collected. These infected parts were collected in sterile polythene bags and brought to the laboratory and stored at 4°C until further study.

C.Isolation of fungal pathogens

A total of six fungal sp namely *Colletotrichun sp., Fusarium sp., Curvularia sp , Myrothecium sp., Geotrichum sp., Pyricularia sp.,* were isolated from infected tissues on Potato dextrose agar (PDA) and Potato dextrose agar plus yeast extract (PDAY) 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, under sterile conditions in an inoculation chamber.

D.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.

E.Isolation frequency of pathogenic fungi from Echinochloa crus- galli

The perusal of data reveals that the frequency of *Curvularia lunata.* fungus was found to be high in all the three seasons where remaining pathogens have mild to moderate frequency. Highest frequency. Highest frequency of *Curvularia lunata*was recorded in rainy season and lowest in summer season.

F.Growth and sporulation on different media

To determine further the best growth medium, seven different media were used. It is evident from perusal of data presented in the fig that *Curvularia lunata* showed growth on all

the ten media tested, with various rate of growth and sporulation. However, fungus showed best growth on PDA, followed by CDA> PDAY media. Growth was good on MEA and very poor growth was noticed on NA media. Sporulation was best on PDA and CDA (36.99 X 10^5>34.18 X 10^5 >32.57 X 10^5 conidia/ml respectively). *Curvularia lunata* also sporulates well on MEA and SDA media (26.83x 10^5 >28.55X 10^5conidia/ml). Poor sporulation was observed on SDA and NA (24.11 X 10^5 >18.48 X 10^5 conidia/ml).

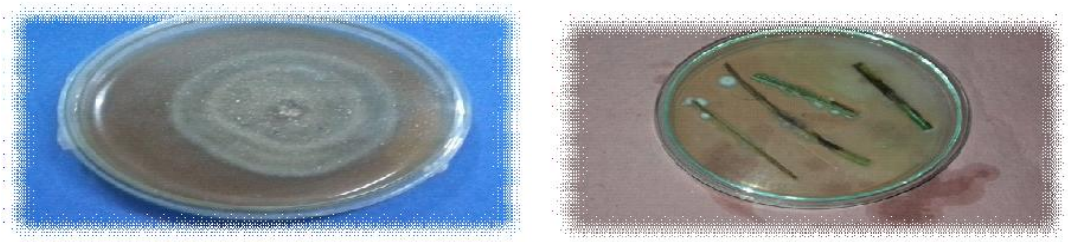

Fig.1 The isolation of causal agent of leaf spot from infected leaf propagules.
Diseased leaves colony (B) Culture of the isolate *Curvularia lunata*, on PDA

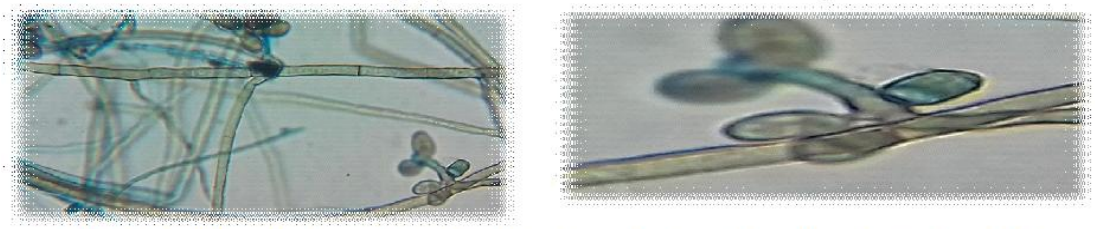

Fig. 2 Morphological characteristic of conidia and conidiophers of *Curvularia lunata* strain LD2

G.Pathogenicity tests

Table.3 Growth and spore count of different isolates ofCurvularia lunata after 7 days of incubation.

Growth Diameter (cm)		Average growth Diameter (cm)	Average spore count / ml (X 10^5)
Plate No.1	Plate No.2		
5.33	6.03	5.68±0.49	36.99±0.15
5.13	4.97	5.05±0.11	26.83±0.23
5.33	4.83	5.08±0.35	34.18±0.08
5.17	5.07	5.12±0.07	32.57±0.23
5.07	5.07	5.07±0	28.55±0.78
3.9	4.3	4.1±0.28	24.11±0.78
2.37	3.07	2.72±0.49	18.48±0.70

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 standarderror (Table 5). *Curvularia lunata* (host) pathosystemrevealed 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

In the treated pots, infection appeared on the leaves in the form of small pin point lesions, 3-4 days after inoculation, which eventually became enlarged, necrosis occurred and straw color appeared 10-15 days post inoculation (Fig.3). The difference in percent infection might be due to chance factor. Presence of inoculum in the environment might be a factor for more infection in inoculum applied pots as compared to control pots. There are several biological and environmental limitations which obstruct the efficacy of a biocontrol agent. In the present days, advances in adjuvants formulation and delivery system have been used to overcome some of these limitations and improve the efficacy of a biocontrol agent (Boyette *et al.*, 1996).

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

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

(A) Healthy un inoculated seedling (control), (B) Diseased seedling after two weeks of inoculation

IV. CONCLUSION

Echinochloa crus-galli (Barnyard grass) is a noxious weed due to competition for yields in various agricultural and vegetables crops such as paddy, maize, peanut, black gram and onion crops in south India. Although various pre- and post-emergence chemical herbicides are available to control this weed but keeping in view the pollution hazards created by chemicals, the need of the our research is to intensify the control of this weed either through biological agents or with an integrated approach of biological agents. From the present study, it may be concluded that several potential pathogenic fungi found in association with *Echinochloa crus-galli*which have a potential to control this terrestrial weed. Out of various isolates, *Curvularia lunata*can be highly aggressive towards *Echinochloa crus-galli*and has most of the characteristics that makes it suitable candidate as biocontrol agent of this weed, such as: capable of limiting population without eliminating the species; can be easily cultured on natural host good sporulation capacity; narrow host range, fast growth rate and hence can be mass produced in a short time and cloud be taken as a possible agent with biocontrol potential of this weed in India. Our study concluded that *Curvularia lunata* has good potential to control *Echinochloa crus-galli*in experimental pots. Phytotoxin produced by this phytopathogen may also play important role in biological control of *Echinochloa crus-galli*. Thus, our research constitutes an important contribution in the biological control of *Echinocloacrus-galli*, our results will be of great importance for India and several other countries where this terrestrial weed represent a major environmental, ecological and economic problem. Intensive work is still needed on the impact of the field environment and application technology on the efficacy of this phytopathogen as a mycoherbicide.

REFERENCES

1. Abbas, H.K., Tanaka, T. and Duke, S.O. 1995. “Pathogenicity of *Alternaria alternata* and *Fusarium moniliforme* and toxicity of AAL-toxin and fumonisin B-1 on tomato cultivars”. *Journal ofPhytopathology*. 143:329-334.
2. Ainsworth, G.C., Sparrow, F.K., and Sussman, A.S. 1973. “The fungi, an advanced treatise”. 621:39.
3. Aneja, K.R. 2003. “Experiments in Microbiology, Plant Pathology andBiotechnology”. *International Publishers*.4:437-438.
4. Bligtami K S, Sinha K K, Ansari A A. 1995. Wide spread occurrence of toxigenic *Alternaria* in cereals and oilseeds. *Indian Phytopathology*, 2, 150-153.
5. Charudattan R. 2005. Ecological, practical, and political inputs into selection of weed targets: what makes a good biological control target? *Biological Control*, 35, 183-196
6. Chaube, H. S., and Singh, U. S. Plant Disease Management: Principles and Practices. CRC Press, 1991.
7. Chen Y, Ni H W. 1999. Pathogenicity of indigenous fungi to *Echinochloa crusgalli* and rice. *Chinese Journal of Biological Control*, 15,73-76.
8. Chung Y R, Kim B S, Kim H T. 1990. Identification of *Exserohilum* species, a fungal pathogen causing leaf blight of barnyardgrass (*Echinochloa crus-galli*). *Korean Journal of Plant Pathology*, 6, 429-433.
9. Gianessi, L. P., and Puffer. C., 1991. “Herbicide use in the United States: National summary report”. Resources for the Future. 128
10. Gohbara M, Yamaguchi K, Petersen J B. 1992. Biological agents for the control of paddy weeds in Japan. *Integrated Management of Paddy and Aquatic Weed in Asia*, 45,184-194.
11. Holm, L.G., Pluknett, D.L., Pancho, J.V., Herberger, J.P., 1977. “The world’s worst weed: distribution and biology”. The university press or hawii.
12. *Huang S W, Yu L Q, Duan* G F, Luo K. 2005. Study on barnyardgrass (*Echinochloa crus-galli*) control by *Helminthosporium gramineum* and *Exserohilum monoceras*. *Acta Phytopathologica Sinica*, 35, 66-72
13. Jamil F F, Nicholson R L. 1987. Susceptibility of corn to isolates of *Colletotrichum graminicola* pathogenic to other grasses. *Plant Disease*, 71, 809-810.
14. Julien, M.H., 1992. “ Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds”.*Wallingford, Oxford*. 3:186.
15. Lekshmi Gangadhar and P. K. Praseetha, “A Novel Modeling of Quantum Dot Sensitized Solar cells”, *Journal of Computational and Theoretical Nanoscience*. ISSN: 1546-1955, Vol. 16, 389-392, 2019.
16. Lekshmi Gangadhar, Anusha Kannan and P. K. Praseetha ,”Quantum Dot-Sensitized Solar Cells Via Integrated Experimental And Modeling Study”, *Journal of Computational and Theoretical Nanoscience*, ISSN: 1546-1955, Vol. 16, 436-440, 2019
17. Lacap, D.C., Liew, E.C.Y. and Hyde, K.D. 2003. “An evaluation of the fungal morphotype‘ concept based on ribosomal DNA sequences”. *Fungal Diversity*. 12:53-66.
18. Li, Youngquan, Z., Sun, X., Zhuang, L., Xu, S., Chen and M., Li, 2003. “Research prograss on microbial herbicides”. *Crop prot*., 47:252.
19. Miller, J.H., 1977. “Kudzu eradication and management”. 137–149
20. Ray, P. and M. P. Hill. 2012. Impact of feeding by Neochetina weevils on pathogenicity of fungi associated with water hyacinth in South Africa. J. Aquat. Plant Manag. 50:79—84,
21. Reis E M. 1985. Manipulation of *Helminthosporium* spp. on sensscent tissues of graminaceous weeds and soybean under natural conditions. *FitopathologiaBrasileita*, 3, 643-648.
22. Templeton, G.E., 1991. “Use of *Colletotrichum* strains as mycoherbicides. In: *Colletotrichum*: Biology, pathology and control (Bailey JA and Jeger MJ Eds.)”. 358–380.
23. Tsukamoto H, Gohbara M, Tsuda M. 1997. Evaluation of fungal pathogens as biological control agents for the paddy weed, *Echinochloa* species by drop inoculation. *Annuals of the Phytopathological Society of Japan*, 63, 366-372.
24. Tuite, J. 1969. “Plant Pathological Methods: Fungi and Bacteria,” *Burgess Press*. 239.
25. Whipps J M, Lumsden R D. 2001. Commercial use of fungi as plant disease biological control agents: status and prospects. In: Butt T M, Jackson C, Magan N, eds., *Fungi as Biocontrol Agents.* CAB International, New York, USA. pp. 9-22
26. Yang M, Li J Q, Ni H W, Wang Q. 2007. Pathogenicity system of *Exserohilum monoceras* to barnyardgrass in greenhouse. *Acta Phytopathologica Sinica*, 37, 221- 224
27. Zhang, W.M. 1996. Biological control of *Echinochloa* species with pathogenic fungi. PhD Thesis.
28. Zhang W M, Watson A K. 1997. Efficacy of *Exserohilum monoceras* for the control of *Echinochloa* species in rice (*Oryza sativa*). *Weed Science*, 45, 144-150.