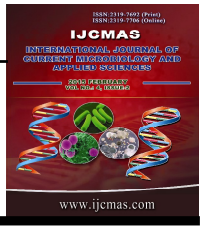
Int.J.Curr.Microbiol.App.Sci (2015) 4(2): 623-631

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 2 (2015) pp. 623-631**
http://www.ijcmas.com



Original Research Article

Bioherbicidal Activity of *Curvularia lunata* on Common Cocklebur (*Xanthium strumarium* L.)

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Keywords

Curvularia lunata, Biological control, Common Cocklebur, Weed, *Xanthium strumarium*

A B S T R A C T

Common cocklebur (*Xanthium strumarium* L.) is a biotype which has different morphology and higher seedling production ability. Greenhouse studies were conducted to investigate the bioherbicidal activity of *Curvularia lunata* on common cocklebur. Xanthium strumarium L. is an exotic plant responsible for several agricultural, environmental and health problems in Sudan. Due to non-acceptability of conventional methods of control, the possibilities of its management through an indigenous strain of Curvularia lunata had been explored. . The present study experimentally emphasizes on the development of Curvularia lunata as a mycoherbicide in the management of the weed, Xanthium strumarium L., so *C. lunata* showed a good potential as a biocontrol agent for *X. strumarium.* Virulence and toxin production of *C. lunata* was of special interest. Spores of *C. lunata* easily germinated on *X. strumarium* leaves and invaded them through the stomata causing discrete leaf spots, which later coalesce causing leaf blight and ultimate death of leaves. The disease affected the productivity of *X. strumarium* significantly. It increased the number of dead leaves for single plants and competing plants. However, the production of new leaves was reduced for single plants and competing plants.

Introduction

Cocklebur (*Xanthium strumarium* L.) is an economically important weed of soybean [*Glycine max* (L.) Merr.], cotton (*Gossypium hirsutum* L.) and peanut (*Arachis hypogaea* L.) (11) ,(20); (21), (9) showed that heavy infestation of cocklebur resulted in yield reduction of 50 to 75% in soybean. In addition to the substantial yield reduction, this weed is becoming a concern because several biotypes are resistant to some conventional herbicides.

(4) showed 12 different biotypes of cocklebur, in which the biotypes from Bolivar county, Mississippi, are resistant to imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinoline carboxylic acid) and the biotypes from Duck Hill county, Mississippi, are resistant to MSMA (monosodium methylarsonate). A common cocklebur biotype resistant to imidazolinone herbicides was found by (8). (13) identified a biotype

resistant to the organic arsenical herbicides.

Common cocklebur has two seeds per bur and usually produces only one seedling (5) Higher seedling production ability increases the weediness of this particular biotype (5); (7); (9); (10); (22).

As some biotypes of cocklebur have become resistant to the conventional herbicides, (8); (13), (14), the need for an alternative to the chemical weed control methods like biological control has become obvious. *Alternaria helianthi* (Hansf.) Tubaki and Nishih. has been well documented as a potential bio-control agent for cockleburs (2); (3); (19). This is a pathogen of sunflower (*Helianthus annus* L.) and can infect other plants in compositae family (6).

Microbial weed control represents an innovative means to manage troublesome weeds and utilize the naturally occurring biological herbicides produced by soil microorganisms. These compounds kill or hinder the growth of weeds so that beneficial plant species can gain a competitive advantage. The vast diversity of microorganisms in our environment is largely untapped, and the potential discovery and characterization of these microbial compounds represents an opportunity to complement chemical herbicides, or reduce the potential for erosion or soil degradation due to tillage for weed control. Invasive weeds continue to threaten the productivity of agricultural lands and natural areas; however, for many weeds adequate, cost-effective control measures presently are not available (16).

Discovery of biological controls for invasive plants represents an alternative way to slow the spread of these weeds using natural enemies (16). Further advances in microbial genetics will continue to improve our understanding of the wealth of genetic diversity and potential in the soil and to better use plant-microbe interactions. The development of biocontrol agents would lessen the need for chemical herbicides and Provide greater options for weed management. Microbes have a place in integrated, Ecologically based weed management and their potential are only just being realized.

In the present study, the objective was to apply *Curvularia lunata* on the weed (*Xanthium strumarium*) and its potential as biocontrol agents

Materials and Methods

Glasshouse experiments

This study was done at Faculty of Agriculture, Sudan University of Science and Technology, Khartoum, Sudan

The temperature of the Glasshouse ranged from 19 to 21°C during winter, 29 to 32°C during the summer and 25 to 29°C during the rainy season. The relative humidity ranged from 45-75% with normal day light cycle.

Xanthium strumarium seeds were surface sterilized and sown in pots Containing sterilized soil,and were left to grow for 4 weeks. Prior to inoculation, the plants were predisposed to 95 percent humidity for 24 hour. Thereafter, they were inoculated with spore suspension of *Curvularia lunata* diluted to approximately $1x10^6$ spores/ml. The spore suspensions were sprayed using a pressurized sprayer. After inoculation the plants were exposed in the same conditions for 24 hours suitable control plants were maintained by spraying of sterile distilled water.

Fungal inoculum preparation

Spore suspensions of *Curvularia lunata* was prepared by pouring 15 ml of sterile distilled water into the culture plates. The surface of the colony was scraped with a glass rod to free the conidia. The resulting spore suspension was passed through 4 layers of cheese cloth to separate spores from mycelia. The concentration of spores in each suspension was estimated with a Haemocytometer.

Three slides were prepared from each spore suspension and four counts for the number of spores per ml of the suspension were made per slide. The inoculum concentration was expressed as the number of spores per ml, counted in three slides (spore counts throughout this study were made with above procedure).

The resulting spore suspension was then atomized on to the leaves of *Xanthium strumarium*. The plants were then left to dry for 30 min. and then covered with plastic bags to maintain high humidity and maintained in the glasshouse. After 48 hours the plastic bags were removed. The plants were left for 7 days so as to express the symptoms.

Infection and pathological histology

To determine the mode of penetration of the pathogens, plants were inoculated with a drop of a spore suspension, covered with plastic bags and kept in the glasshouse. Portions of *Xanthium strumarium* leaves were selected 48 hours after inoculation. They were placed on microscope slide and 2 drops of 15% potassium hydroxide solution were added.

The slides were gently heated when the leaf portions were sufficiently softened, they were flooded with water. Two leaf portions were dried with a blotting paper and stained with lacto phenol cover slips were applied and the slides were examined under the microscope.

Spore preparation

Spores were collected by placing 15 ml sterile distilled water on the cultures in Petri plates. The surface of the cultures was then scraped with a glass rod to release the conidia. The mycelia fragments were separated by passing the spore suspension through 2 layers of cheese cloth. The spore suspensions were calibrated to obtain 1 x 10^6 spores per ml dilution using a haemocytometer.

The effect of culture age and temperature on the virulence of *C. lunata* was investigated. The experiment was conducted to determine the length of time the fungus retained, virulence to *Xanthium strumarium* when incubated at 25°C.

Preparation of test plants

Plants used in this experiment were having 3 leaves.

Evaluation of test pathogen as biocontrol agents

This experiment was designed to study the effect of *Curvularia lunata* on the productivity of *Xanthium strumarium*. Two levels of treatment were tested on single plant to test the effect on the plant under density-independent conditions and 3 plants to test the effect on the plant under competition (density-dependent). The productivity of control plants was also tested as single plant and under competition and replicated 3 times.

The treatments were arranged in a completely random design (each plant has 3 leaves). A spore suspension of $1x10^6$ spore per ml from freshly isolated cultures was sprayed on the plants.

The plants were covered with polythene bags for 48 hours then removed. Control plants were sprayed with distilled water and incubated as above.

Biocontrol potential of *Curvularia lunata*

Dead leaves

The number dead leaves per plant infected with *C. lunata* were recorded at weekly intervals for seven weeks, for single plant and competing plants and statistically analyzed.

Number of new leaves

The number of new leaves per plant was recorded at weekly intervals for the single plant and competing plants and statistically analyzed

Total number of leaves per plant

This variable represented a count of all leaves both emergent and sub-emergent. There were three leaves per plant at the beginning of the test.

Roots length (cm)

The length of roots was measured for the single plant and competing plants, then the data was statistically analyzed.

Plant height (cm)

The plant height (cm) was measured for all plants (single and competing plants) then the data analyzed statistically.

Disease parameters

1-Disease incidence

Disease incidence was recorded weekly as percentage value for seven weeks.

Disease incidence (1)

(Frequency) = Number of infected plant units x 100
Total number (healthy and infected) of units assessed

2-Disease severity

Disease severity was estimated in terms of % age of necrosis of leaf area by following the method of (23).

Disease severity area(s) =
Area of plant tissue affected by disease x 100
Total area

The rating scale of (15) modified by (17) was employed to determine the severity of disease. Accordingly 12 class values corresponding to different levels of disease severity have been used for rating.

However the rating scale used in the present study consisted of 11 class values representing the percentage of disease severity as:

1 = 0; 2 = 1-5%; 3 = 6-10%; 4 = 11-15%; 5 = 16-30%; 6 = 13-50%; 7 = 51-65%; 8 = 66-75%; 9 = 75-85%; 10 = 86-95%; 11 = 96-100% necrosis of total leaf area (plant death).

Statistical method

Statistical analysis was accomplished in SAS 9.0 version, and the Duncan's multiple range test (DMRT) was adopted to compare means. Least significant difference values at ($P \leq 0.05$) were used to separate treatment means when ANOVA indicated significant

F value. Standard error and coefficient of variation were calculated for each treatment.

Results and Discussions

Biocontrol potential of *Curvularia lunata*: dead leaves:

The number of dead leaves per plant infected with *C. lunata* was recorded weekly intervals and presented in Table (1,)no dead leaves were recorded on the plants at the end of the first week after inoculation.

There was no significant differences between treatments were recorded on the second and third weeks. By the 4th week there was a significant increase in the number of dead leaves per plant for single and competing plants. The disease accounted for 71.69% for single plants and 77.99% for competing plants. The number of dead leaves for single diseased plants increased steadily up to the 7th week where an average of 1.34 leaves died per week.

These data confirm the effectiveness of *C. lunata* as a bioherbicidal pathogen for *Xanthium strumarium*. This agrees with the findings of (1)where *A.helianthi* caused severe disease infestation and growth reduction of multiple-seeded cockleburs.

Number of new leaves

The results were presented in Table (2). No significant effect of the disease was observed on the number of emergent leaves on the second, third, fourth and five weeks after inoculation with the pathogen. From the 5th week up to the end of the test, the increase of new leaves per plant was constant one leaf/week. The highest number of new leaves per plant was recorded for competing plants on control of 9.67 that means the competition had no effect on the emergent of leaves but the decrease of the number of new leaves due to the effect of disease.

Total number of leaves

This variable represented account of all leaves both emergent and sub-emergent Table (3). There were three leaves per plant at the beginning of the test. The data indicated that the total number of leaves per plant increased in all the treatments. There was no enough evidence to reject the hypothesis that the disease has no effect on the total number of leaves both dead and living. These results are in line with the results of. (12).

Plant height

The results were presented in Table (4). There was a significant effect of the disease on the plant height. At the end of the test the plant height of the single plants averaged 43 cm for the diseased and 45.33 for the control plants. For competing plants the average plant height of diseased 40.67 and 42.67 for control.

Disease incidence of *Xanthium strumarium* inoculated with *C. lunata* at different spore age

Figure (1) the disease incidence showed steadily increase attaining maximum incidence in the spore age 20 day for single plants and competing plants. The disease incidence ranging from 56.03 to 73.5 for the single plants where spore age was 20 days and from 62.30 to 78.93 for competing plants. Disease incidence for spore age 40 days, ranging from 42.87 to 53.67 for single plants and 38.33 to 63.40 for competing plants. Disease incidence in the spore age 60 was

0.00 that means the pathogen loose the viability with increase of age. These results are in line with the results of (12).

Disease severity of *Xanthium strumarium* inoculated with *C. lunata*

The results were presented in Table (5). There was a significant difference on the disease severity was recorded in spore age 20 days and 40 days the highest disease severity recorded was 77.67 where spore age 20 days.

Table.1 Effect of *Curvularia lunata* on *Xanthium strumarium* (number of dead leaves per plant)

Weeks	Single plants		Competing plants	
	Control	Diseased	Control	Diseased
1	0.00^{k}	0.00^{k}	0.00^{k}	0.00^{k}
2	0.67^{jk}	2.67^{hi}	2.00^{i}	1.67^{ij}
3	2.33^{hi}	3.67^{gh}	3.33^{h}	3.33^{h}
4	3.67^{gh}	5.00^{ef}	4.67^{fg}	5.00^{ef}
5	5.33^{ef}	6.33^{de}	5.67^{ef}	6.33^{de}
6	6.33^{de}	8.33^{b}	7.00^{cd}	7.67^{bc}
7	8.33^{b}	9.67^{a}	8.67^{ab}	8.33^{b}
C.V%	16.16%			
$Lsd_{0.05}$	1.191			
SE±	0.4199			

Any two mean values bearing different superscripts with columns and rows are significantly different (P≤0.05).

Table.2 Effect of *Curvularia lunata* on *Xanthium strumarium* (number of new leaves per plant)

Weeks	Single plants		Competing plants	
	Control	Diseased	Control	Diseased
1	2.00^{l}	2.33^{l}	2.00^{l}	2.33^{l}
2	2.33^{l}	4.00^{hij}	3.67^{ijk}	3.00^{jkl}
3	2.67^{kl}	4.67^{ghi}	4.00^{hij}	4.33^{hi}
4	4.67^{ghi}	5.00^{gh}	5.67^{fg}	5.67^{fg}
5	5.67^{fg}	5.67^{fg}	6.67^{ef}	6.67^{ef}
6	7.00^{de}	6.67^{ef}	8.33^{bc}	8.00^{bcd}
7	8.33^{bc}	7.67^{cde}	9.67^{a}	9.00^{ab}
C.V%	11.60%			
$Lsd_{0.05}$	1.001^{**}			
SE±	0.3531			

Any two mean values bearing different superscripts with columns and rows are significantly different (P≤0.05)

Table.3 Effect of *Curvularia lunata* on *Xanthium strumarium* (Total number of leaves per plant)

Weeks	Single plants		Competing plants	
	Diseased	Control	Diseased	Control
1	3.00^{q}	3.33^{p}	3.33^{p}	4.67^{o}
2	5.67^{n}	6.67^{m}	6.67^{m}	7.67^{l}
3	8.33^{kl}	8.67^{k}	8.67^{k}	9.33^{j}
4	10.00^{j}	10.67^{ij}	11.33^{i}	12.67^{hi}
5	13.33^{h}	12.67^{hi}	14.00^{g}	15.67^{f}
6	17.67^{e}	15.33^{f}	19.67^{c}	18.67^{d}
7	22.00^{ab}	19.33^{cd}	23.67^{a}	21.33^{b}
C.V%	17.34%			
$Lsd_{0.05}$	1.548^{**}			
SE±	0.5459			

Any two mean values bearing different superscripts with columns and rows are significantly different (P≤0.05)

Table.4 Effect of *Curvularia lunata* on plant height (cm) of *Xanthium strumartium*

	Single plants	**Competing plants**
Diseased	43.00^{b}	40.67^{c}
Control	45.33^{a}	42.67^{b}
C.V%	1.86%	
$Lsd_{0.05}$	1.597^{*}	
SE	0.4615	

Any two mean values bearing different superscripts with columns and rows are significantly different (P≤0.05)

Table.5 Disease severity of *Xanthium strumartium* weed sprayed with *Curvularia lunata* at different spore age

Spore age (days)	Severity (%)
Control	0.00^{c}
20	77.67^{a}
40	62.80^{b}
60	0.00^{c}
C.V%	4.79%
$Lsd_{0.05}$	5.083
SE	1.295

Any two mean values bearing different superscripts with a column are significantly different

(P≤0.05)

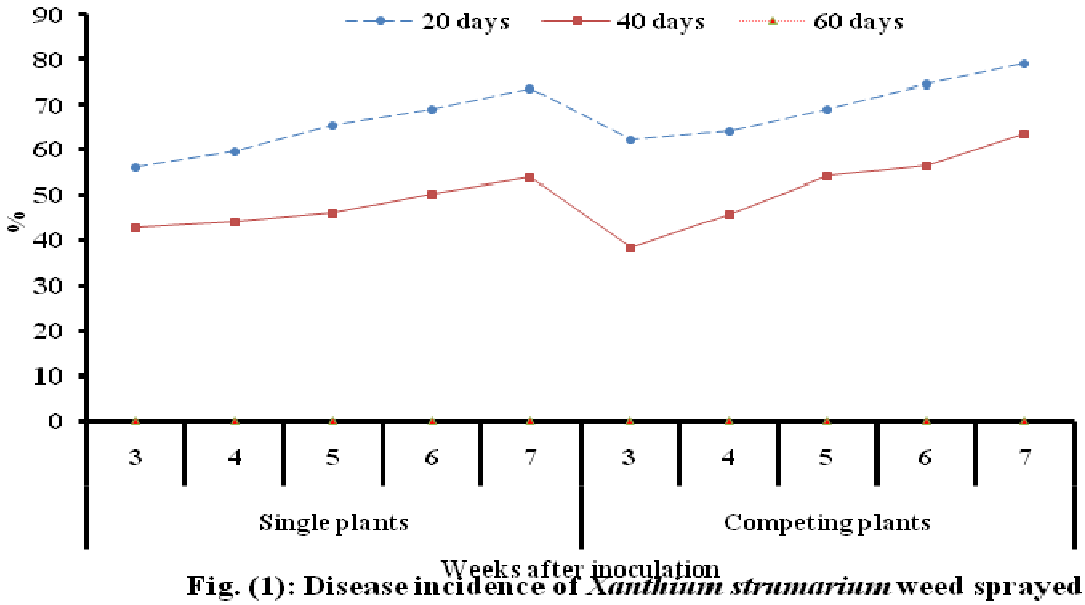

Fig. (1): Disease incidence of *Xanthium strumarium* weed sprayed with *Curvularia lunata* at different spore age

The lengthy period for inoculum build up could be overcome by spraying the host species with mass cultured *C. Lunata* spores. Freshly isolated spores should be used, because both pathogens tend to lose virulence rapidly. The inability to produce spores in liquid culture was a constraint which could be overcome by producing spores on solid media. There have been many investigations of potential products for weed management. Some have been successful at suppressing weeds in the field and a select few are marketed products that now reduce weed infestations. Further studies are needed to continue to search for additional tools to control weeds. Increasing our understanding of plant-microbe interactions will assist in this effort. Biocontrol agents need to be specific, competitive and well –matched with the weed of interest.

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