J.Biol.Control, 5 (2), 116-118, 1991

Evaluation of *Epicoccum nigrum* for the Biological Control of Waterhyacinth

K.R. ANEJA and B. SRINIVAS
Department of Botany, Kurukshetra University
Kurukshetra - 132 119

ABSTRACT

Surveys for natural enemies of waterhyacinth were conducted during 1988-91 at different places in Haryana State. Leaf spots characterised by compact zonations, starting from tip of the leaf and spreading backwards were observed. The pathogen was identified as *Epicoccum nigrum* L. Koch's postulates were fulfilled. Waterhyacinth plants, when inoculated responded differently to infection depending upon the morphotypic state of development of the plant. Leaf infections were 20 to 40% and 25 to 50% in covered and uncovered pits respectively. Maximum growth of the fungus occurred on waterhyacinth dextrose agar.

KEY WORDS : *Eichhornia crassipes,* biocontrol, *Epicoccum nigrum*

Waterhyacinth, *Eichhornia crassipes* (Mart.) Solms is the most predominant, persistant and troublesome aquatic weed which ranks second among the eighteen important sub-tropical and tropical weeds (Evans, 1987). It infests over 500,000 ha of water areas (Rao, 1983) and has attained the status of Number 1 aquatic weed in India. Biological control of this weed is under investigation all over the world including India, as it is considered to be most efficient method, long lasting, and is less costly with minimum detrimental environmental impacts (Gopal, 1987). In order to control waterhyacinth by biological means, surveys were initiated to search for natural fungal pathogens of this weed.

MATERIALS AND METHODS

Surveys were conducted to find out naturally occurring fungal pathogens of waterhyacinth throughout Haryana during 1988-1991. Diseased leaves showing leaf spot symptoms were collected and plated on waterhyacinth dextrose agar (WHDA) and potato dextrose agar plates supplemented with streptomycin sulphate, with the help of sterilized forceps under aseptic conditions. The constituents of waterhyacinth dextrose agar (WHDA) medium were as follows

Waterhyacinth leaves	200.0 g
Dextrose	15.0 g
Agar	20.0 g
Distilled water	1.0 l

Pure culture of the pathogen was maintained on PDA slants.

Pathogenicity of the isolate was determined both in detached leaves and whole plants. Fresh and healthy waterhyacinth leaves were washed with distilled water, surface-sterilized with rectified spirit and wounded by pricking with a sterilized needle and inoculated by placing mycelial discs from 7-day old fungal cultures. Inoculated leaves were kept in moist chambers and incubated at 25 ± 1°C for 3 days. A total of 8 leaves, 4 wounded and 4 non-wounded, were used for inoculations.

Waterhyacinth plants were grown in 16 cemented pits, (50 x 50 x 50 cm). The mycelial mat from 20 Petri dishes was ground with 500 ml sterile water and sprayed on eight pits. Each pit consisted of 2 plants each with 4 to 6 leaves. Four pits were covered with polyethylene sheets and the remaining 4 pits were left uncovered. An equal number of controls was kept. Observations were made after one month.

RESULTS AND DISCUSSION

During 1988-91 surveys, a leaf spot disease of waterhyacinth showing 50% infection

Table 1. Disease incidence and severity on small, medium and large waterhyacinth leaves, one month post inoculation with *Epicoccum nigrum* in experimental pits

Nature of the leaves	Condition of the pits											
	Uncovered						Covered					
	Without inculum (control)			With inoculum			Without inoculum (Control)			With inoculum		
	H	D	%	H	D	%	H	D	%	H	D	%
Small	9	-	-	20	7	25	9	-	-	4	1	20
Medium	7	-	-	8	5	38	13	2	13	7	3	30
Large	3	-	-	5	5	50	17	3	15	4	3	42

H = No. of healthy leaves ; D = No. of infected leaves one month post inoculation

$$\% = \frac{\text{No. of infected leaves one month post inoculation}}{\text{Total no. of leaves present}} \times 100$$

was observed. Leaf spots had compact zonations, starting from tip of the leaf and spreading backwards. Petiole infection was also seen. Isolations from diseased waterhyacinth leaves on WHDA and PDA yielded *Epicoccum nigrum* Link. The identity was confirmed from the International Mycological Institute, Kew, Surrey, England under reference No. 333324. It has been reported from waterhyacinth by Aneja *et al.* (1990). Symptoms were observed on both wounded and non-wounded leaves, in detached leaves and whole plants. The pathogen was reisolated from inoculated leaves. Waterhyacinth leaves responded differently in different environment conditions. The infection in covered pits ranged between 20 to 40 per cent while in uncovered pits it ranged between 25 to 50% (Table 1). The wounded leaves showed more infection.

Table 2. Infection* of leaves by *Epicoccum nigrum* one month post inoculation (* Mean of three readings taken between 1989-1991)

Plant size	Percentage infection of leaves			
	Covered pit		Uncovered pit	
	Inoculated	Uninoculated	Inoculated	Uninoculated
Small	20	0	25*	0
Medium	30	13	38*	0
Large	42	15	50*	0

* Significant values at 0.05% level

Another observation made in the present study is that small plants (leaf 1 < 15 sq. cm) showed lower degree of infection, than large plants (Leaf 1 < 40 sq. cm) in the field as well as in experimental pits showing that small leaves are resistant to *E. nigrum*. Per cent infection on waterhyacinth leaves caused by E. nigrum was more in uncovered pits than in the covered pits, one month post inoculation. The values of per cent infection in both covered and uncovered pits were found to be statistically significant (Tables 2 and 3).

WHDA was found to be the best medium for the growth of this pathogen. However, there was not much difference in growth response on PDA, PSA and CDA (Table 4). This study is in conformity with the observation of Gopal and Jamil (1986) who have suggested that waterhyacinth leaf extract can be used as a better substrate for culturing certain species of fungi and bacteria.

E. nigrum is a weak pathogen and has been reported on several hosts from India, such as *Sorghum vulgare* and *Zea mays* (Bilgrami *et al.*, 1979, 1981; Mukerji and Bhasin, 1986; Sarbhoy *et al.*, 1986). Extensive host-range tests of the isolated strain of *E. nigrum* need to be conducted before considering it as a biocontrol agent for waterhyacinth.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, International Mycological Institute, England

Table 3. t - ratios showing the percentage of infection of waterhyacinth leaves one month post inoculation caused by *Epicoccum nigrum* between inoculated and uninoculated leaves in relation to different leaf sizes in covered and uncovered pits

	Covered pit					Uncovered pit				
	Mean	S.D.	SE_D	df	t	Mean	S.D.	SE_D	df	t
Inoculated (WIU)	30.6	9.68	7.86	4	2.706	37.66	8.8	7.12	4	5.289*
Uninoculated (WIU)	9.33					0.00				
Inoculated (SIU)	20.3	0.4	0.32	3	63.43*	24.66	0.56	0.45	3	54.06*
Uninoculated (SIU)	0.0					0.33				
Inoculated (MIU)	30.3	0.57	0.46	3	38.47*	37.30	0.57	0.46	3	79.65*
Unioculated (MIU)	12.6					0.66				
Inoculated (LIU)	41.6	0.81	0.65	3	40.92*	51.66	1.34	1.08	3	46.60*
Uninoculated (LIU)	15.0					1.33				

* Significant at 0.05 W = Whole plant S = Small sized leaves
M = Medium sized leaves L = Large sized leaves I = Injured U = Uninjured

Table 4. Growth response of *Epicoccum nigrum* to different media (26°C) after 8 days

Medium	Diameter of colony (mm)
Czrapek-Dox Agar	23
Potato Dextrose Agar	25
Potato Sucrose Agar	24
Waterhyacinth Dextrose Agar	30

for confirming the identification of the isolate; and the Department of Environment, Govt. of India for sanctioning a major research project to the senior author Dr. K.R. Aneja, to control waterhyacinth by the use of mycoherbicides.

REFERENCES

ANEJA, K.R., SRINIVAS, B. and SINGH, K. 1990. Three new pathogenic fungi of waterhyacinth from India. *Trop. Pest Mgmt.*, 36, 76.

BILGRAMI, K.S., JAMALUDDIN and RIZWI, M.A. 1979. Fungi of India. Part-I. List and references. Today and Tomorrow's Printers and Publishers, New Delhi, pp. 467.

BILGRAMI, K.S., JAMALUDDIN and RIZWI, M.A. 1981. Fungi of India, Part-II. Host index and addenda. Today and Tomorrow's Printers and Publishers, New Delhi, pp. 128.

EVANS, H.C. 1987. Fungal pathogens of some subtropical and tropical weeds and the possibilities for biological control. *Biocon. News Inform.*, C.A.B. International, 8, 7-30.

GOPAL, B. 1987. Waterhyacinth. Elesvier Science Publishers, B.V. pp 471.

GOPAL, D.R. and JAMIL, K. 1986. Potential of waterhyacinth extract as a new growth medium for culturing fungi and bacteria, *IBC*, 3, 1-10.

MUKERJI, K.G. and BHASIN, J. 1986. Plant diseases of India. A Source book. Tata McGraw Hill Publishing Company Limited, New Delhi, pp. 468.

RAO, V.S. 1983. Principles of weed science. Oxford and IBH Publishing Co., New Delhi, pp. 540.

SARBHOY, A.K., AGARWAL, D.K. and VARSHNEY, J.L. 1986. Fungi of India (1977-1981). Associated Publishing Company, New Delhi, pp. 274.