Journal of AgriSearch, 6(2):92-95
An Open Access International Peer Reviewed Quarterly
ISSN : 2348-8808 (Print), 2348-8867 (Online)
https://doi.org/10.21921/jas.v6i02.15758

Preliminary Screening of Isolated Mycoherbicidal Fungi for the Management of Noxious Weed *Xanthium strumarium*

AJAY KUMAR SINGH* AND AKHILESH KUMAR PANDEY

ARTICLE INFO

Received on	:	27/04/2019
Accepted on	:	31/05/2019
Published online	:	05/06/2019

ABSTRACT

Natural phytotoxins of fungi are great source for the discovery of new herbicide and its offer a benign and eco-friendly alternative to manage weed. Thus, this study aimed to select potential fungi with potent herbicidal activity for control of weeds. In the present study, various phytopathogenic fungi were isolated from infected tissues of various weeds and evaluated against *Xanthium strumarium*, a problematic monocotyledonous weed of open lands, agriculture, horticulture and forests. Herbicidal potential of Cell Free Culture Filtrate (CFCF) of strains of *Phoma herbarum* (FGCCW#18, FGCCW#43) *Fusariummonilifrome* coded as FGCCW#35 and *Fusarium roseum* coded as FGCCW#55against *Xanthium strumarium* were evaluated by seedling and shoot cut bioassays. Maximum mortalities of shoots, seedlings and phytotoxic damage were obtained from 28 day sold cell free culture filtrate (CFCF) of FGCCW#18 at 100% concentration. Significant reduction in biological contents *i.e.* photosynthetic pigment and protein was observed in the host weed on treatment with the CFCF as determined by detached leaf bioassay. Phytotoxic damage such as severe wilting, chlorosis, necrosis and complete collapse of the entire parts of the weed were also noticed due to CFCF application.

KEYWORDS

Bioassay, Cell free culture filtrate, Mycoherbicide, *Xanthium strumarium*, Phytotoxicity.

INTRODUCTION

Xanthium strumarium L., is popularly known as common cocklebur an exotic plant responsible for several agricultural, environmental and health problems in India. The weed is considered as one of the world's worst weed (Holm, 1977). It is a serious weed in Australia, India, South Africa, and the Americas. It greatly reduces yield, and interferes with combine harvesting. It is a strong competitor for nutrients and water. It grows luxuriantly and seriously in infested paddy, sorghum, soybean, green gram, jawed, archer and other khaki annual crop fields in Andhra Pradesh, Maharashtra, Rajasthan and Madhya Pradesh (Deshpande, 1982). It contains several type toxins, carboxyatractyloside capable of killing cattle, goats, horses, sheep and poultry. Toxins present in cocklebur are sesquterpine, lactones, carboxyatractyloside causes hypoglycemia, vomiting, weakness, tremors, weak pulse, loss of appetite and convulsions. The allelochemicals produced from different parts of the weed also inhibit the seed germination and seedling growth of many crops viz. soybean, cotton, peanut, wheat, maize, pearl millet, chickpea, rapeseed, tobacco and lettuce(Cutler, 1983). It spreads fast specially in soybean and cotton fields and causes 70-80% loss in production. The weed is very variable in phenotype and adaptable. Some biotypes have developed resistance against herbicides including ALS inhibitors. Also create problems in forest margins, gardens, wastelands, roadsides area, and other open lands. Conventional methods of its control have failed due to one or other reasons. It grows on any type of soil and in a wide range of habitats. However, due to several undesirable side effect of synthetic herbicides, exploitation of microorganisms especially fungal strains have attracted the attention of a large number of workers worlds over (Saxena *et al.*, 2001). The use of mycoherbicides appears to have greatest potential for weed control in non cropped as well as use cropped areas, waterways, rangelands, pastures and other areas of public uses (Templeton, 1987).During the recent years the main focus in development of herbicides are on biodegradability. To overcome these problems herbicidal potential of secondary metabolites of FGCCW#18, FGCC#34, FGCCW#35 & FGCC#55 against *Xanthium strumarium* were evaluated and discussed in this paper.

MATERIALS AND METHODS

Recovery of strain: Four strains of fungi namely *Phoma herbarum* viz. FGCCW# 18, FGCCW# 34,*Fusarium monilifrome* FGCCW#35 and *Fusarium roseum* FGCCW# 55 were obtained from Fungal Germplasm Collection Centre (FGCC) of Mycological Research Laboratory, Department of Biological Science, R D University, Jabalpur, Madhya Pradesh. These were isolated earlier from seeds, seedlings and rhizospheric soil of the various weed.

Production of CFCF: 250ml Erlenmeyer's flasks containing 200ml Asthana and Hawker's broth were seeded with 5mm disc of inoculum separated from 7 days old cultures grown on PDA medium. Inoculated flasks were incubated at 27±1°C in aBio-Oxygen Demand (BOD) incubator (Remi, India) and the cell free culture filtrate (CFCF) was extracted after 7, 14, 21 and 28 days (Pandey *et al.*, 2004b).

Extraction of CFCF: The metabolized broth was passed through whatman no 1 filter paper under aseptic conditions through a pre-weighed whatman filter paper no.1 and was centrifuged at 400xg for 15-20 min (Saxena and Pandey, 2002). The pellet was thrown and the supernatant was again filtered *invacuo* by microfiltration using sterile microfilters, 0.45 µm pore size, Mininsart (Sartorius, Gottingen,

Mycology Research Laboratory, Department of Biological Sciences, Rani Durgawati University,Jabalpur, Madhya Pradesh, India
*Corresponding Author Email:drajay2009@gmail.com

Germany) making it cell free (Walker and Templeton, 1978). Thus Cell Free Culture Filtrate was obtained.

Bioassay:

Cut Shoot Bioassay: 20-25 days old *Xanthium strumarium*-seedlings shoot were taken and an inclined cut was made at the tip in sterilized water. They were then dipped in different dilutions *i.e.*25%, 50%, 75% and 100% of the CFCF in test vials. These were then incubated under daylight or artificial illumination (3.5 x 10^4 erg/cm^2/s). The vials were sealed with cotton buds and the effects of different days old fermented broths and different dilutions of the toxic metabolites were observed on the shoots of the test weed after 48 h at room temperature (27±1°C) (Vikrant *et al.*, 2006).

Seedling Bioassay: Seedlings of the *Xanthium strumarium* weed were raised in pots containing soil: sand: peat (1:1:1). 15-20 days old were treated with different days old CFCF (7, 14, 21, 28 days) and also with different dilutions (25%, 50%, 75% and 100%) of the phytotoxic metabolites and were then incubated. Observations were made regularly on a rating scale of 0-5 as described earlier (Abbas*et al.*,1992). Phytotoxicity of CFCF of various strains was determined by employing shoot cut and seedling bioassay tests as accordance to Thapar *et al,* (2002). Phytotoxicity was determined following the method of Abbas *et al.*,1992 on a rating scale of 0-5. Effect on biological content i.e. chlorophyll and protein contents were determined as per Pandey *et al.*, 2003.

RESULTSAND DISCUSSION

Data presented in Table 1 showed that CFCF of different strains of fungi of varied incubation periods had significant influence on cut shoots and seedling mortalities. Maximum shoot cut and seedling mortalities of the target weed were obtained with 28 days old fermented broth followed by 21 and 14 days old fermented medium in all the strains.

Table: 1: Study for effect of different Incubation period on Cell free culture filtrate of four different fungi against *Xanthium strumarium***

Strains	Shoot cut Bioassay Phytotoxicity				Seedling Bioassay Phytotoxicity			
	Incubation days							
	7 days DR*	14 days DR*	21 days DR*	28 days DR*	7 days DR*	14 days DR*	21 days DR*	28 days DR*
Control A	0	0	0	0	0	0	0	0
Control B	0	0	0	0	0	0	0	0
FGCCW#18	1	2	4	5	1	2	4	5
FGCCW#34	1	1	2	3	1	1	2	3
FGCCW#35	1	1	2	3	1	1	2	3
FGCCW#55	1	1	2	3	1	1	2	3

*Damage rating (DR): 0 =No symptoms, 1= slight chlorosis, 2= slight chlorosis and necrosis, 3=marked chlorosis and necrosis,4=marked chorosis and high necrosis, 5=acute chlorosis and necrosis leading to death of shoots.
**Results taken after 24 hrs during bioassay.
Cell free culture filtrate (CFCF) production condition:
Incubation temperature: 28°C ±1°C,Control A: Distilled Water, Control B: Growth medium broth
Data are means of three experiments each replicated thrice.

CFCF obtained from 7 days old utilized medium didn't cause significant mortalities. Out of these three test strains, CFCF of FGCC#18 exhibited maximum shoot cut, seedling mortalities and phytotoxic damage followed by FGCC#34, FGCCW#35 and FGCC#55. Phytotoxic damage rating represented in table 2 clearly indicates that in both the cut shoot and the seedling

Table 2: Study of different concentration of Cell free culture filtrate of four different fungi against *Xanthium strumarium***

Concentration	Shoot cut Bioassay Damage Rating				Seedling Bioassay Damage Rating			
	FGCCW#18	FGCCW#34	FGCCW#35	FGCCW#55	FGCCW#18	FGCCW#34	FGCCW#35	FGCCW#55
Control A	0	0	0	0	0	0	0	0
Control B	0	0	0	0	0	0	0	0
25%	1	0	0	0	1	0	0	0
50%	2	2	2	2	3	2	3	2
75%	3	3	3	3	4	3	3	3
100%	5	3	3	3	5	4	3	3

Damage rating: 0 =No symptoms, 1= slight chlorosis, 2= slight chlorosis and necrosis, 3=marked chlorosis and necrosis, 4=marked chlorosis and high necrosis, 5=acute chlorosis and necrosis leading to death of shoots.
CFCF production condition: Incubation time=21 days, Incubation temperature = 28°C ±1°C
**Results taken after 24 hrs during bioassay.
Control A: Distilled Water, Control B: Growth medium broth
Data are Means of three experiments each replicated thrice.

bioassays, toxicity gradually enhances with increase in concentration of CFCF. Maximum phytotoxic damage was recorded by 75% and 100% concentration of CFCF. 25% and 50% concentrations of fermented broth show minimal damage to shoots and seedlings of the test weed. 75% and 100% CFCF when sprayed on *Xanthium strumarium* seedling produced visible phytotoxicity i.e., browning at tip of leaves, which was more rapid, prominent and severe in later stages after 24 hours (Table 2).

Browning was followed by severe necrosis wilting and finally collapsed of seedlings completely. Data presented in Table 3 clearly reveals significant variation in the total chlorophyll, chl a, chl b contents when shoots of the target weeds were treated with cell free culture broth of*Phoma herbarum* (FGCCW# 18, FGCCW#34), *Fusarium monilifrome* (FGCCW#35) and *Fusarium roseum* (FGCCW#55) of different incubation periods. There was a gradual reduction of pigments with increased incubation periods. More or less all the seedlings and shoots died at higher concentrations of CFCF. Maximum reduction in chlorophyll and protein contents were recorded in case of shoots treated with CFCF of *Phoma herbarum* FGCCW# 18 obtained from 28 days old fermented broth (Table 3).

It was followed by CFCF obtained from fermented broth of FGCCW# 34, FGCCW#35 and FGCCW#55. Chl a was found to be more susceptible to the metabolites in comparison to chl b and total chlorophyll. Variation in phytotoxicity due to toxin has also been recorded by other researchers (Saxena and Pandey 2001; Joseph *et al*., 2002; Vikrant *et al*., 2006). Thapar *et al* (2002) also recorded significant reduction in biological contents of *Parthenium* treated with CFCF of *Curvularialunata.* Similar results regarding inoculum concentration versus growth stage for different bio-control agents and their respective hosts were earlier reported (Pandey *et al* 1997; Winder and Watson 1994). On the basis of above findings it can be concluded that the secondary metabolites produced by *Phoma herbarum* FGCCW#18 fungi have remarkable herbicidal potency need further evaluation for its large-scale application.

Table 3: Study for the effect of cell free culture filtrate of four different fungi on Chlorophyll and protein content of weed *Xanthium strumarium*

Items	Mean % Inhibition															
	Chl a				Chl b				Total Chl				Protein			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FGCCW#18	17.0	53.1	91.6	84.2	7.50	39.5	72.4	88.5	14.4	60.8	79.2	73.2	22	48.2	48.4	59
FGCCW#34	14.8	52.4	80.6	80.2	28.2	61.9	74.2	88.5	16.2	47.9	70.2	67.5	12	51.4	51.4	54
FGCCW#35	19.0	64.5	82.2	80.0	15.1	80.4	93.1	70.6	11	58.2	66.8	60.3	24	41.3	42.4	46
FGCCW#55	22.0	66.5	85.2	80.0	15.1	80.4	93.1	72.6	11	58.2	69.8	61.3	28	44.3	45.4	49

Incubation day A= 7 days; B= 14days; C= 21 days; D= 28 days.
Incubation temperature = 28^0C ±1^0C
Control A: Distilled Water
Medium B: Growth media broth
Data are Means of three experiments each replicated thrice

Variation in toxicity in relation to incubation period may be due to different phase of growth of the fungus. Metabolites required for fungal growth are normally synthesized during initial phase whereas most of the toxicants are formed during idiophase i.e. stationary phase of the fungus. Phytotoxin often act as the initiator factor for successful pathogenesis. Several phytotoxin are known to be the determinant factor in pathogenesis. Most of the phytotoxic metabolites acts by modifying the metabolism of the host plants, while some are toxic to the plant tissues once accumulated and poison the plant tissues (Amusa, 2006).

There was a gradual decrease in biological contents with increase in concentration of CFCF. Decrease in both the chlorophyll pigments (a and b and total chlorophyll were noted in phytotoxin treated detached leaves. Destruction of the two chlorophyll affects both the Photosystems of photosynthesis, since Photosystem I is associated with chl a and Photosystem II with chl b. Hill reaction which is a measure of the integrity of the photosystem II also gets adversely affected (Mishra, 1985). The toxin can create a change in the dynamic balance of synthesis and decomposition of chlorophyll pigments. The chlorophyll might have been destroyed by the enhancement of chlorophyllase activity or due to metabolic destruction. The reduction can either be due to an inhibition of chloroplast development or due to the destruction of pigments in mature chloroplasts. Similarly the biochemical basis for resistance is reflected in changes in the structural and functional proteins and products of their action in cells adjacent to the infected site.

CONCLUSION

On the basis of preliminary evaluation the secondary metabolites produced by *Phoma herbarum* FGCCW#18 fungi has shown herbicidal potency against *Xanthium strumarium* weed and it needs further evaluation for its large-scale application.

ACKNOWLEDGEMENTS

We are thankful to the Head, Dept of Biological Science for laboratory facilities. Financial assistance received from Council of Scientific and Industrial Research and Department of Biotechnology, New Delhi is also thankfully acknowledged.

REFERENCES

Abbas HK, Vesonder RF, Boyette CD and Peterson SW 1992.Phytotoxicity of fumonisin B, on weed and crop species.*Weed Technology* **6:** 549-552.

AmusaNA. 2006. Microbially produced phytotoxin and plant disease management. *African Journal. Biotech.* **5(5)**: 405-414.

Cutler HG. 1983. Carboxyatractyloside: a compound from *Xanthium strumarium* and *Atraetylis gummifera* with plant growth inhibiting properties. The probable & Inhibitor. *Journal Natural Products***46**: 609-613.

Deshpande K. 1982. Biocontrol of *Parthenium hysterophorus* L. and *Xanthium strumarium* L. through phytopathogens. pp. 48 In: Abstracts of paper & Annual conference of Indian society of weed science.

Holm LG, Pluncknett DL, Pancho, IV and Herberger, JP 1977.The world worst weeds.pp. 609.University Press of Hawaii, Honolulu.

Joseph S, Lal S and Pandey AK 2002. Preliminary evaluation of herbicidal potential of *Streptomyces* WC#150 against *Lantana camara*.*Annal Plant Protection Science.* **10:**134-136.

Mishra D. 1985. Ph. D. Thesis, Biosciences, Dept of Biological Sciences, R.D. University, Jabalpur.

Pandey AK, Singh AK, Quereshi Sadaf and Agrawal Deepa 2004b. Herbicidal activities of secondary metabolites of *Streptomyces* sp. against *Hyptis suaveolens. Journal Basic Applied Mycology***3**: 95-97.

Pandey AK, Singh Jaya, Shrivastava GM and Rajak RC 2003. In: *Plant Protection: A Biological Approach* (ed. Trivedi P.C.), Aavishkar Publishers, Distributors Jaipur, India: 305-39.

Saxena S and Pandey AK 2002. Evaluation of an indigenous isolate of *Alternaria alternata* LC#508 for use as a mycoherbicide for *Lantana camara*.*Crop Protection* **20(1)**: 73-75.

Saxena S and PandeyAK 2001.Microbial metabolites as eco-friendly agrochemicals for the next millennium.*Applied Microbiology Biotechnology* **55**: 395-03.

Saxena S, Pandey AK and Rajak RC 2001. Potential of idiolites from *Alternaria alternata* as biorational agrochemical.pp.22-28 In: Frontiers in fungal biotechnology and plant pathogen relationship(eds C. Manoharachary, G. Bagyanarayana, B. Bhadraiah, K. Satyaprasad, B. N. Reddy & A. Nagamani). Allied Publishers, Hyderabad.

Templeton GE. 1987. Mycoherbicides- achievements, developments and prospects. pp 489-497. In: Proc. Eights Austr. Weeds. Conf., Sydney, Australia 21-25 Sept 1987.

Thapar R, Singh AK, Pandey A and Pandey AK 2002. Bioactivity of CFCF of *Curvularia lunata* in *Parthenium hysterophorus* L. *Journal Basic Applied Mycology* **1(1)**: 126-29.

Vikrant P, Verma KK, Rajak RC and Pandey AK 2006. Characterization of a phytotoxin from *Phoma herbarum* for management of *Parthenium hysterophorus*. *Journal of Phytopathology* **154**: 1-8.

Walker HL and Templeton GE1978. In vitro production of phytotoxic metabolites by *Colletotrichum gloeosporioides* f sp *aeschynomene*. *Plant Science Letter* **13**: 91-97.

Winder RS and Watson K 1994.A potential microbial control for fireweed (*Epilobium angustefolium*).*Phytoprotection.* **75**: 19-33.

Citation:

Singh AK and Pandey AK. 2019. Preliminary Screening of Isolated Mycoherbicidal Fungi for the Management of Noxious Weed Xanthium *strumarium*. *Journal of AgriSearch* **6**(2):92-95