World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

PHYTOTOXICITY ASSESSMENT OF SECONDARY METABOLITE OF FUNGI ISOLATED FROM HYPTIS SUAVEOLENS. L

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Article Received on 03/06/2017 **Article Revised on 18/06/2017** **Article Accepted on 03/07/2017**

ABSTRACT

During survey undertaken at R.D.V.V. campus, 4 fungal isolates were recovered from diseased parts of Hyptis suaveolens. Out of all the isolated strains, Nigrospora sp.FGCC#74 was found to be the most dominant pathogen with maximum herbicidal potential against Hyptis. Nigrospora is known to incite severe wilt, chlorosis and necrosis in Hyptis suaveolens. More or less all the strains recovered are known to synthesize phytotoxic metabolites. Primary and secondary screening test conducted to select potential strains revealed that Nigrospora sp. produces secondary metabolite with very high herbicidal activity against Hyptis suaveolens. Further, the toxic metabolite residing in Cell Free Culture Filtrate was extracted with carbon tetra chloride, chloroform, ethyl acetate, and butanol .The residue obtained after solvent evaporation were evaluated separately for there phytotoxicity against the Hyptis leaves by detached leaf bioassay .Residue obtained from butanol fraction exhibited maximum toxicity i.e. by showing chlorosis and necrosis when compared with others. This is the first report confirming the potential of this particular toxin from Nigrospora sp. as a biorational, which can be applied as phytotoxin against Hyptis suaveolens.

KEYWORDS: During survey undertaken at R.D.V.V. campus.

INTRODUCTION

Hyptis suaveolens is a ruderal weed (Walter, 1963, Keller and Armbuster, 1989; Aluri, 1990) and is capable of heavy infestations displacing native flora and is said to be potent invader of Vindhya high lands. (Sharma et al., 2007). Hyptis is of common occurence along the rail tracts, road side (Verma and Mishra 1992), foot hill of open forest, forest clearing (Mudgal et al.,1997) and can heavily infest waste lands particularly arid and rocky substrate. Allelopathic properties of Hyptis and its unpalatability to live stock is due to presence of essential oil like Terpene 4 ol (Peerzada,1997) so because of avoidance as fodder, other species are heavily used as fodder by live stocks resulting in loss of other species (Raizada, 2006). Conventional methods of weed management have failed due to several reasons. Biorational strategy of weed management is an effective and cheaper ecofriendly strategy involving the use of microorganisms including fungi. Fungi have long been recognized as plant pathogen and many of them produce a variety of bioactive extracellular toxic compounds. Herbicidal properties of such toxic metabolite of microorganism have been exploited in weed management (Pandey, 1999, 2000; Pandey et al., 2001, 2002, 2003, 2004; Saxena et. al. 2001). Certain of such product have been patented and few such as phosphinothricin (glufosinate), bialaphos, hydantocidin, have been commercialized (Saxena and Pandey, 2001; Pandey et al.,2003, 2004). Therefore present study was carried out to screen out potential fungal strain for its herbicidal potential against Hyptis suaveolens.

MATERIALS AND METHODS

Recovery of fungal strains

Tissue from diseased portion (of leaves and stem) of the weed were cut down into about 1mm pieces with the help of sterilized blade and foreceps and under aseptic condition transferred to petridishes containing presterilized PDA medium. The petridishes were later incubated at 28±1ºC in BOD (Yorco, India) incubator and examined regularly.

Production of CFCF

150 ml Erlenmeyer flasks containing 50 ml of Richard's broth (KNO_3-10gm,KH_2PO_4-5 gm,$MgSO_4$ 7 H_2O-2.8gm, Sucrose-35 gm, $FeCl_3$-100µg(trace), Distilled water-1000ml) were seeded with 5 mm disc that were separated from 7 days old actively growing culture on PDA medium at 28±1ºC in BOD (Yorco,India) inoculated

flask were incubated at 28±1ºC in BOD incubator for 7, 14,21,28 days.

Extraction of CFCF
Extaction of CFCF was done as per (Vikrant et al. 2006).

Shoot cut bioassay
Shoot cut bioassay was done as per (Sharma and Sharma, 1969 and Chaing et al., 1989).

Detached leaf bioassay
Detached leaf bioassay was done as per (Sharma et al., 2004).

Effect of CFCF on Biological content
Effect of CFCF of Nigrospora sp. on biological content of Hyptis suaveolens were determined.

A) Chlorophyll content
Determination of chlorophyll a, b and total chlorophyll was done by the method of Arnon, 1949.

B) Protein contents
To determine total protein method suggested by Lowry et al., (1951) was followed.

Thermal nature of phytotoxic moiety
Determination of Thermal nature of phytotoxic moiety was done as per Siddaramaiah et al., (1979).

Solvent extraction of CFCF
A volume of 25ml of CFCF was taken in a separating funnel. Various organic solvents were used for extraction. A volume of 15ml of carbon tetra chloride was added to 25ml of filtrate, shaken well and kept until the two phases got separated. The lower carbon tetrachloride layer was separated from beaker and was vacuum dried. The remaining filtrate was extracted similarly in succession with chloroform, ethylacetate and n-butanol. All the organic fractions were evaporated to dryness in a vaccum dessicator (Rotary evaporator, Buchi) at 45ºC (Templeton, 1979).

Phytotoxic activity of various fractions
All fractions separated by solvent extraction were subjected to in vaccuo dessication at 45ºC to remove any trace of solvents and to obtain the final residue. Residues were named as obtained, viz. fraction A (carbon tetra chloride); fraction B (chloroform); fraction C (ethylacetate); fraction D (n-butanol). Test residue were tested for their phytotoxic activity using detached leaf bioassay.(Nakjima et al.,1991)

RESULT AND DISCUSSION

Data depicted in Fig.1 indicates the interrelation ship between growth (biomass), final pH and toxin production by the four primarily screened fungi against Hyptis suaveolens .There was a gradual increase in final pH and mycelial dry weight (biomass) with increasing incubation days. Maximum biomass was obtained after 28 days of fermentation.

Fig.2 shows phytotoxic damage rating, CFCF of Nigrospora sp.(FGCC#74) causes maximum phytotoxic damage after 48 hpt followed by Fusarium oxysporum(FGCC#72), Alternaria alternata (FGCC#71)and Acremonium sp. (FGCC#73). Symptoms include, appearance of slight curling after 24 hrs of treatment. At advanced stage rapid wilting of leaves, epinasty veinal chlorosis and necrosis of leaves was observed resulting in death of entire shoot. Similar observation regarding interrelation ship between biomass, pH and phytotoxin production has been observed by several other workers (Pandey et al., 2000; Saxena et al.2000; Saxena and Rajak, 2001; Chandla, 1999).

As evident from fig. 3, 21 days old fermented broth of Nigrospora sp. (FGCC#74) imparted maximum phytotoxic damage to Hyptis leaves as assessed by detached leaf bioassay, the phytotoxicity of secondary metabolite was maximum after 72 hpt followed by 48 hpt and 24 hpt.

Fig. 4 represents that with the increase in dilution there is decrease in phytotoxic damage rating. On treating Hyptis leaves with different concentrations of 21 days old CFCF of Nigrospora sp. maximum phytotoxic damage was exhibited by 100%concentration followed by 75%, 50%, 25% and Thakur (2006) and Sanodiya (2006) have reported similar results.

Similarly in fig.5 effect of different concentration of the phytotoxin of 21 days old CFCF of Nigrospora sp. On chlorophyll content and protein content is shown. Thus reducing total chlorophyll to 86.06% while, chl a and chl b were less effected. There was 75.62% reduction in protein content with 100%, 75%, 50% and 25%. Photobleaching of chloroplast pigments in given tissue was observed after 48 hpt.

The effect of FBI toxin on Jimson weed is identical to herbicide action (Abbas et al., 1992) .Toxin causes the photodynamic porphyrin intermediate, protoporphyrin IX to accumulate in the plasma membrane lipid prooxidation .These result were in accordance with those observed by other worker (Pandey et al.,2003; Pandey et al., 2006; Joseph 2000; Abbas et al., 1992).

Fig.6 shows percentage reduction in biological contents of leaves of Hyptis on treatment with different days old CFCF of Nigrospora sp.(FGCC#74). Maximum damage was observed in 21 days old CFCF as is evidenced by reduction of total chlorophyll to 97.2% followed by chl a and chl b. Maximum reduction in protein content reported was 75.62% after 72 hpt.

It evident from fig.6 that the phytotoxicity of Nigrospora sp. was stable at 50^0C, 100^0C and 121^0C (15 psi). Thus,

it could be concluded that the phytotoxic moiety was thermo-tolerant. Thus it was inferred that the toxic moiety (ies) was thermostable and non proteinaceous compound(s). Similar observations have been earlier reported by Kurien et al.,(1977), while working with Cristulariella pyramidalis respectively.The toxic compound extracted by each of the organic solvents varied up to certain extent with the duration of treatment. Maximum toxicity at 72 hrs post treatment was observed by detached leaf bioassay treated with Butanol fraction.

Similar observations have also been made by Siddaramaiah et al., (1979) while working with Phaephleospora indica. Saxena (2000) with Alternaria alternata FGCC # 508, Thapar et al. (2002) and Shukla R.and Pandey (2006) with Sclerotium rolfsii. Kurian et al. (1977) recorded thermostable and non proteineous nature of toxin produced by Cristularia pyramidalis.

Fig.8 shows phytotoxic damage rating when Hyptis leaves were treated with different solvent extracted fraction of CFCF. Maximum phytotoxic damage was obsereved in case of butanol followed by chloroform, ethylacetate and carbon tetrachloride which started at 24 hpt.

In contrast to this result, Pandey et al., 2001 have reported maximum phytotoxic damage to Lantana at 48 hpt by the active metabolite extracted from CFCF of Phoma herbarum FGCC#3 with Benzene. Less phytotoxic damage was reported with Ethyl acetate and Butanol fractions of CFCF. Similarly, Vikrant et al., 2006 extracted and characterized a novel herbicidal compound 3-nitrophthalic acid against Parthenium from CFCF pf Phoma herbarum with ethyl acetate as the organic solvent.

Fig 9 shows reduction in biological contents of leaves treated with partially purified, CFCF of Nigrospora sp. FGCC#74. N-Butanol extracted fraction caused remarkable reduction i.e. 90% in chlorophyll a followed by total chlorophyll and chlorophyll b. Protein content was reduced considerably to 89.31% with this fraction. Reduction in biological contents by n-butanol extracted fraction was followed by Ethyl acetate, chloroform, carbon tetrachloride extracted fraction. Similar results have been shown by other workers. Several secondary metabolites have been extracted by scientists worldwide. Fumonisin, Ophiobolin A were extracted in chloroform; Alternariol in ether/benzene; tentoxin could be extracted by ether, Chloroform and benzene (Orsengo, 1957; Bassett et al., 1967; Saad et al., 1970).

Metabolite A produced from Nigrospora oryzae has been found to show weak antibiotic properties and mild toxicity to brine shrimp and chick embryos but not to be toxic to mice or ratsat the level tested (Wilson et al., 1986). A phytotoxic t3 compound (5,6-di hydro-5-hydroxy-6-propenyl-2H-pyr-2-one a host specific toxin showing phytotoxic effect to various plants including turf grasses was assessed for its phytoxicity by the leaf wounding assay and the whole plant test and the cellular leakage test (Choi, et al., 2006). Nigrospora A and B, two new phytotoxin and antibacterial metabolite were isolated from culture filtrate of Nigrospora oryzae. A culture broth of Nigrospora sacchari showed strong herbicidal activity in treatment of intact green house grown plants. The major compound which exhibited the most significant effect of the fungal metabolite in the assay procedure was identified as (+)-phomalactone, 6 (1-propenyl)-5, 6-dihydro-5-hydroxy-2H, pyran-2-one others were 5-[1-(1-hydroxybut-2-enyl)]-furan-2-one and 5-[1-(1-hydroxybut-2-enyl)]- dihydrofuran-2-one (Fukushima et al., 1998).

Figure 1: Interrelationship between final pH and biomass production by some fungi isolated from *Hyptis suaveolens* .

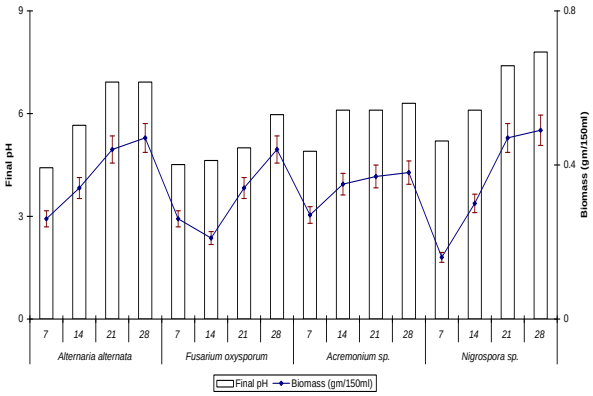


Figure 3: Assessment of phytotoxic damage rating of Hyptis treated with different days old CFCF of Nigrospora sp. FGCC#74 by detached leaf Bioassay

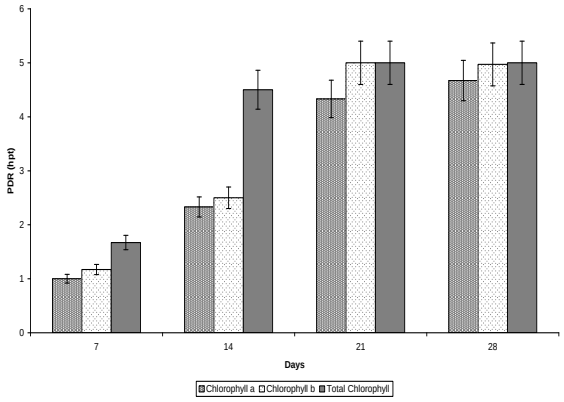


Figure 4: Herbicidal potential of Cell Free Culture Filtrate of *Nigrospora* sp. against *Hyptis* suaveolens

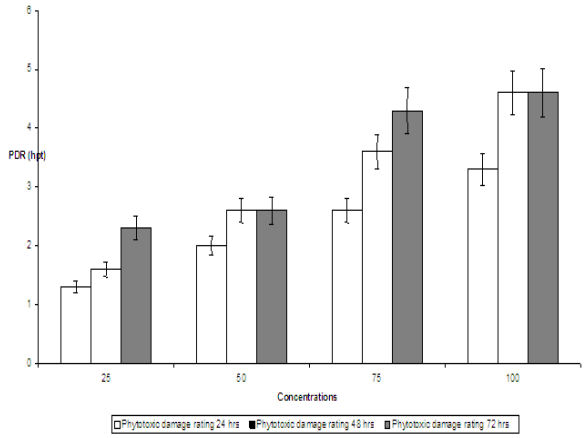


Figure 6: Percentage reduction in biological contents of *Hyptis suaveolens* leaves treated with different days old CFCF of *Nigrospora* sp. (FGCC#74)

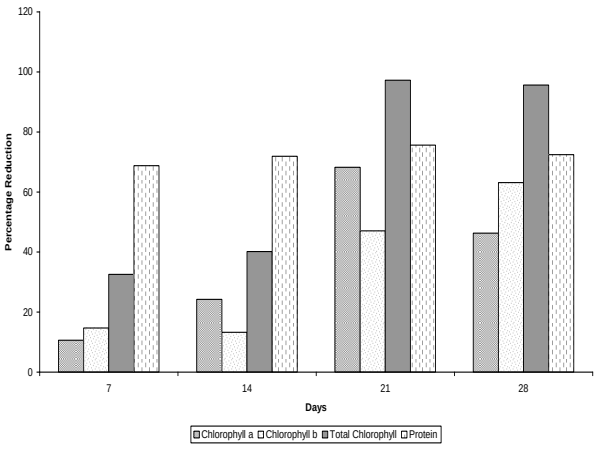


Figure 7: Thermal stability of phytotoxin from Nigrospora sp.(FGCC#74) against Hyptis suaveolens by shoot cut bioassay.

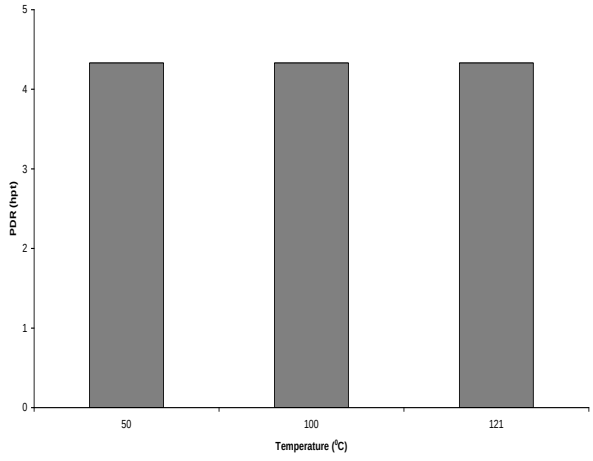


Figure 8: Phytotoxic damage rating of solvent extracted fraction of CFCF of nigrospora sp. (FGCC#74) against Hyptis suaveolens (DLB).

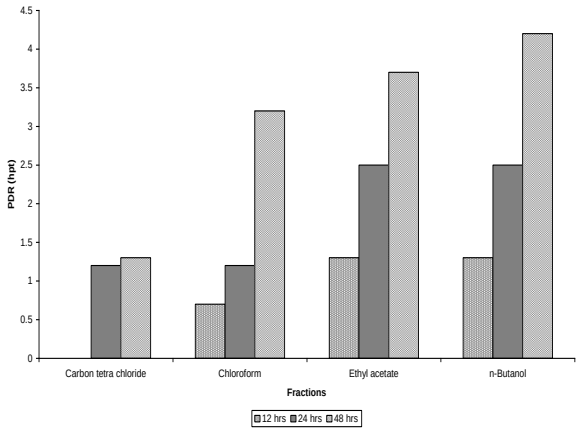
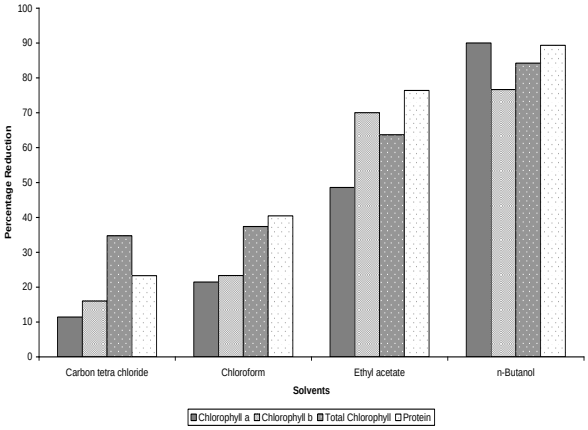


Figure 9: Percentage reduction in biological contents of Hyptis suaveolens leaves treated with partially purified CFCF of Nigrospora sp. (FGCC#74)



ACKNOWLEDGEMENT

We are grateful to the Head, Dept of Biological Sciences, R.D. University, Jabalpur for laboratory facilities. Financial assistance received from Madhya Pradesh Biotechnology Council, Bhopal and DoEn, New Delhi are also thankfully acknowledged.

REFERENCES

1. Abbas, H. K., Vesonder, R. F., Boyette, C.D., Hoagland, R.E. and Krick, T. Production of fumonisins by Fusarium moniliforme culture isolated from Jimson weed in Missisipi. J. Phytopathol, 1992; 136: 199-203.
2. Aluri, JSR. The explosive Pollination mechanism and mating system of the weedy Hyptis suaveolens (L).Plant Species Biology, 1990; 5: 235-241.
3. Arnon, D.I. Chlorophyll adsorption spectrum and quantitative. Biochem. Biophysics Acts, 1949; 20: 449.
4. Bassett, C., Sherwood, R.T., Kepler, J. A. and Hamilton, P.B. Production and biological activity of fommanosins. A toxic sesquiterpene metabolite from Fomes annonus. Phytopathol, 1967; 57: 1046-1052.

5. Choi, G.J., Kimand, J.C., Shon, M.J. and Kim, H.T., Cho, K.Y. Phytotoxin production of Nigrospora sphaerica pathogenic on Turfgrasses. The Plant Pathology Journal, 2006; 16(3): 137-141.
6. Das C.R. and Anima pal, Rhizopin: An antibiotic produced by Rhizopus nigerians Indian phytopath, 1974; 27(1): 33-36.
7. Fukushima,T., Tanaka, M., Gohbara, M. and Fujimori, T. Phytotoxicity of three lactones from Nigrospora sacchari. Phytochemistry. Oxford: Elsevier Science Ltd, 1998; 48(4): 625-630.
8. Gyan P.Sharma,Purnima Raizada and A.S. Raghubanshi, New Report of invasion in the Vindhya highlands:Hyptis suaveolens Poit, Asia Pacific Forest Invasive speciese Network News letter (Accepeted In Press), 2007.
9. Joseph, Shanatamima. Studies on the herbicidal potential of some indigenous strains of Streptomyces against Parthenium and lantana, Dissertation thesis, R.D. University), 2000.
10. Keller, S and S Armbuster.Of Hyptis capitata by Eumenid wasp in panama.Biotropica, 1989; 21: 190-192.
11. Kurian, P., Steleig D.A., Baniecki Toxin production by Cristullariella pyramidalis. Mycologia, 1977; 69: 1203-1206.
12. Lowry, O.H., Rosen lerough, N.J., Fan, A.L. and Randal, R.J., Protein measurement with the folin – phenol reagent. J. Bio. Chem, 1951; 193: 265-275.
13. Mudgal,V, Khanna KK and Hazra PK. Flora of Madhya Pradesh II Botanical Survey of India, 1997; 403-404.
14. Nakjima, M., Itoi, K., Takamatsu, Y., Sato, S., Furukawa, Y., Honwa, T., Furuya, K., Kadotini, J., Kozasa, M., and Haneishi, T. Cornextistin: A new fungal metabolites with herbicidal activity. J Antibiot, 1991; 44(10): 1065-1072.
15. Orsenigo, M. Extraction and purification of Ophiobolin, a toxic product from Helminthosporium oryzae. Phytopath Z., 1957; 29: 189-196.
16. Pandey A.K.,Quereshi Sadaf, Singh A.K.,Yadav K.K. Herbicidal Potential of Phoma sp. FGCCW#54: A Preliminary evaluation. J. Basic Appl. Mycol, 2006; 5(I and II): 60-61.
17. Pandey, A.K. Herbicidal potential of microorganism: Present status and future prospects. In: Microbial Biotechnology for sustainable development and productivity. Prof. S.K. Hasija Festschrift vol. (R.C. Rajak ed.) Scientific publishers, Jodhpur, 1999, 86-105.
18. Pandey, A.K., Lal S. and Joseph S., Herbicidal activity of partially purified metabolites of Streptomyces sp. WC # 150 on Parthenium. PICN, 2000; 32: 11-23.
19. Pandey, A.K., Lal S. and Joseph S., Herbicidal activity of partially purified metabolites of Streptomyces sp. WC # 150 on Parthenium. PICN. 2000; 32: 11-23.
20. Pandey, A.K., Rajak R.C., Hasija, S.K. Biotechnological development of ecofriendly mycoherbicides In. Innovative Approaches in Microbiology (D.K. Maheshwari and R.C. Dubey) Published by Bishen Singh Mehendra Pal Singh, Dehra Dun, 2001.
21. Peerzada, N. Chemical Composition of the Essential Oil of Hyptis suaveolens Molecule, 1997; 2: 165-168.
22. Riazada, Purnima, Ecological and vegetative characteristics of a potent invader, Hyptis suaveolens Poit. from India. Pdf, 2006.
23. Saad, S.M., Hallion, J.M. and Hagedorn, D.T. Production, purification, and bioassay of tentoxin. Phytopathol, 1970; 60: 415-418.
24. Saxena, Sanjai and Pandey, A.K. Preliminary evaluation of fungal metabolites as natural herbicides for the management of Lantana camara J. Indian phytopathol, 2000; 53(a): 490-493.
25. Sharma M.C. and Sharma B.C. Toxic metabolite production by Colletotrichum gloeosporioides causing citrus dieback. Indian Phytopathol, 1969; 23: 67-74.
26. Sharma. P., Sharma, S. R. and Sindhu A detached leaf technique for evaluation of resistance in cabbage and cauliflower against three major Pathogens. Indian Phytopathol, 2004; 57(3): 315-318.
27. Shukla, R. and Pandey, A.K. Maximization of production oxalic acid form Sclerotium rolfsii, a mycoherbicidal agent against Parthenium. Ann. Pl. Protect. Sci, 2006; 14(1): 202-205.
28. Siddaramaiah, A.L., Hedge, R.K., Kulkarni, S., and Basvarajaiah, A.B. Toxic effect of the culture filtrate of Phaeophelospora indica. Indian Phytopath, 1979; 32: 291.
29. Sonadiya, B.S. Isolation, Purification and Characterization of Secondary Metabolites of Alternaria alternata (FGCC#101) for the Management of Parthenium hysterophorus, Dissertation thesis, R.D. University, 2006.
30. Templeton G.E., Te Beest D.O and Smith R.J. Jr, Biological control with mycoherbicides. Annu. Rev. Phytopathol, 1979; 17: 301-310.
31. Thakur, G.S. Isolation, Purification and Characterization of Herbicidal Compounds of Curvularia lunata (FGCC # 25) for the management of Parthenium hysterophorus, Dissertation thesis, R.D. University, 2006.
32. Thapar, Riti., A.K. Singh., Archana, Pandey and A.K. Pandey Bioactivity of CFCF of Curvularia lunata in Parthenium hysterophorus L. J. Basic Appl. Mycol, 2002; 1: 126-129.
33. Verma,BK and Mishra BK. Flora of Allahbad district UP India, 1992.
34. Vikrant P., Verma K.K., Rajak R.C. and Pandey A.K. Characterization of a phytotoxin from

Phoma herbarum for management of Parthenium hysterophorus L. J. Phytopathology, 2006; 154: 1-8.
35. Walter, H. Uber die Stickstoffanspruche der Ruderalpflanzen. Mitt. Florist-Soziol.Arbeitsgem.NF10, 1963; 56-96.
36. Wilson M.E., Norman D. Davis and Diener Urben L. A toxic metabolite of Nigrospora oryzae (Berk and Br.) petch. Mycopathologia Alabama Agricultural Exp. Station J., 1986; 6-84744.