

Partial Characterization and Phytotoxicity Assessment of Secondary Metabolite of Fungi Isolated From *Hyptis suaveolens*.L

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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 their 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*.

Key words: *Hyptis suaveolens*, *Nigrospora* SP Secondary metabolite, Phytotoxin, Bioassay, *Alternaria Alternata*, CFCF, *Fusarium Oxysporum*, *Acremonium* SP.

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 occurrence 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 forceps and under aseptic condition transferred to petridishes containing presterilized PDA medium. The petridishes were later incubated at 28±1°C in BOD (Yorco, India) incubator and examined regularly.

Production of CFCF

150 ml Erlenmeyer flasks containing 50 ml of Richard's broth (KNO₃-10gm, KH₂PO₄-5 gm, MgSO₄ 7 H₂O-2.8gm, Sucrose-35 gm, FeCl₃-100µg(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±1°C in BOD (Yorco, India) inoculated flask were incubated at 28±1°C in BOD incubator for 7, 14, 21, 28 days.

Extraction of CFCF

Extraction 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 vacuum dessicator (Rotary evaporator, Buchi) at 45°C (Templeton, 1979).

Phytotoxic activity of various fractions

All fractions separated by solvent extraction were subjected to *in vacuo* dessication at 45°C to remove any trace of solvents and to obtain the final residue. Residue 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).

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

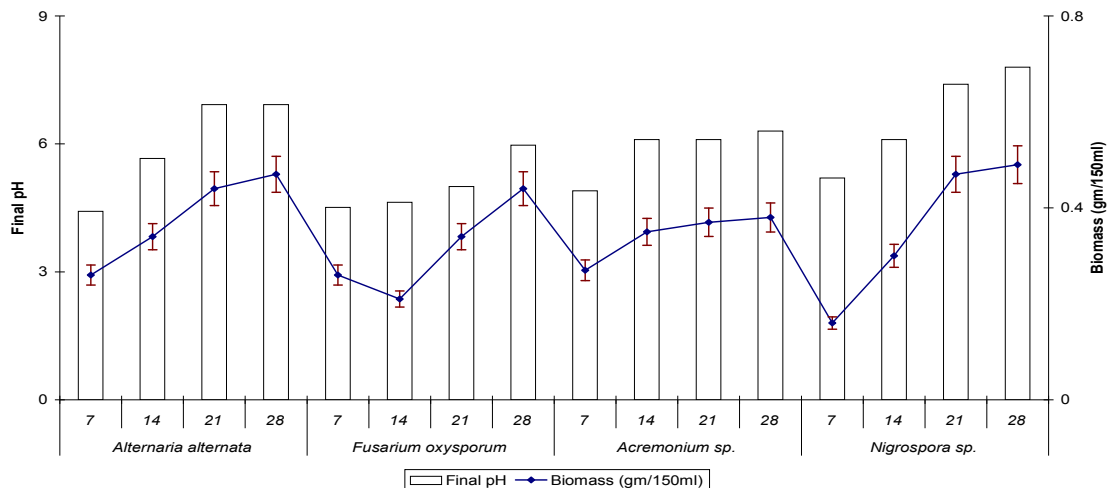


Figure 2: Phytotoxic damaging rating of *Hyptis* shoot on treatment with different CFCF's of various fungi after different hours post treatment

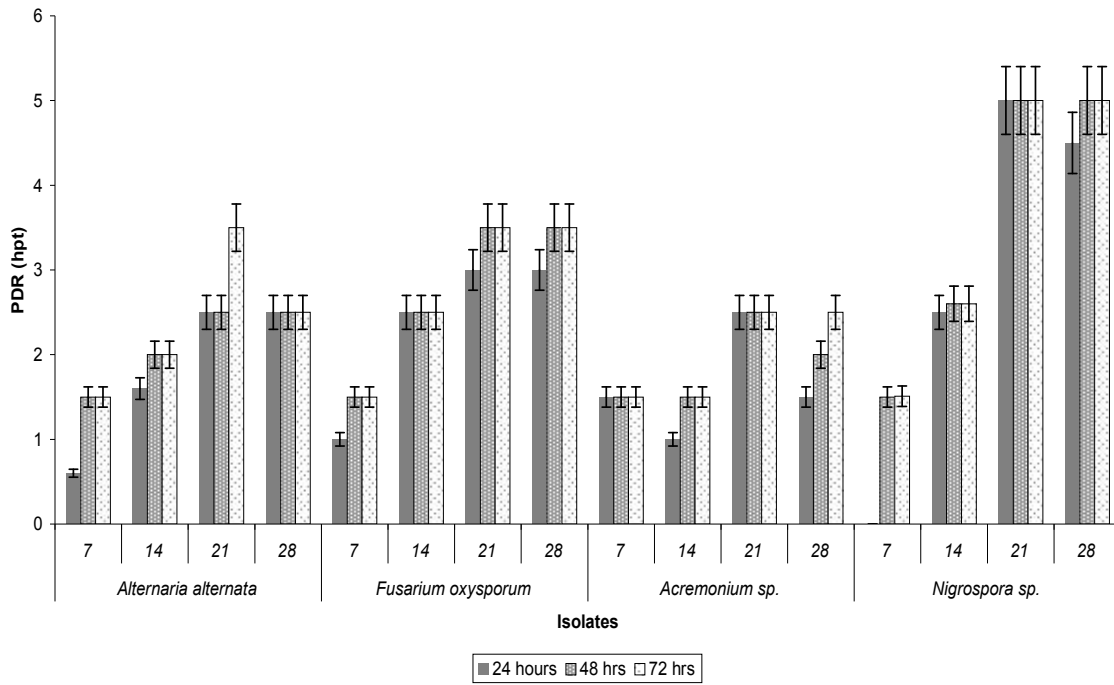


Figure 3: Assessment of phytotoxic damage rating of *Hyptis* treated with different days old CFCF of *Nigrospora sp.* FGCC#74 by detached leaf Bioassay

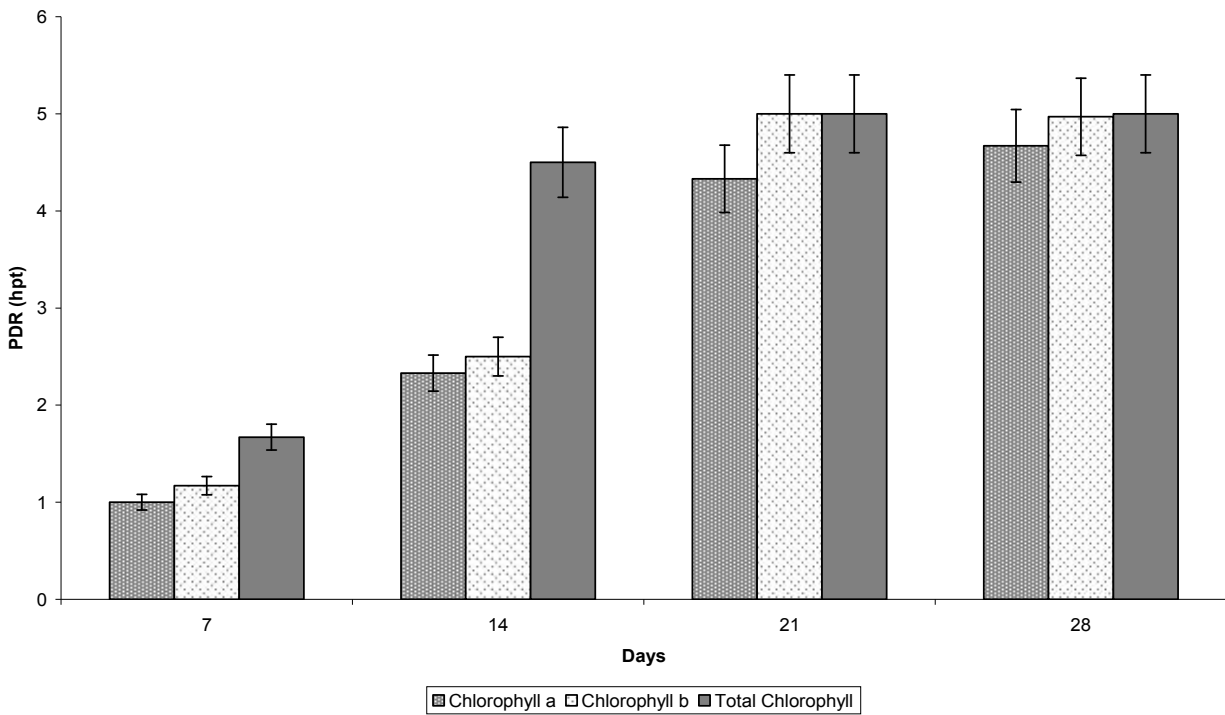


Figure 4: Herbicidal potential of Cell Free Culture Filtrate of *Nigrospora* sp. against *Hyptis suaveolens*

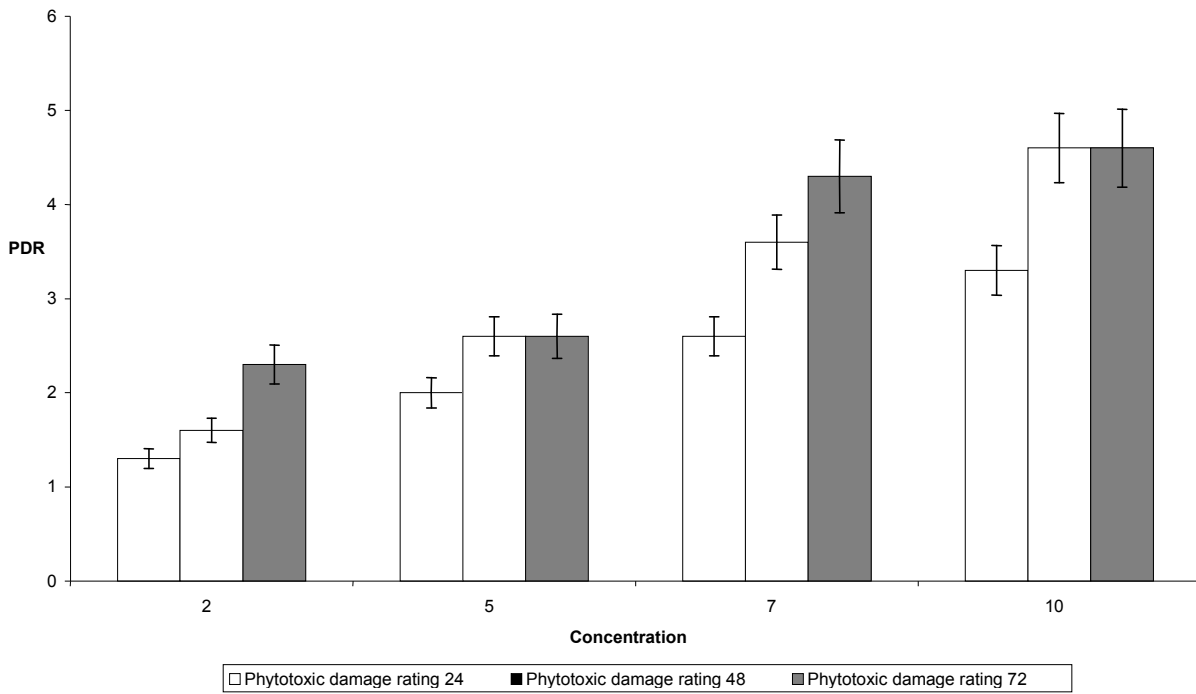


Figure 5: Percentage reduction in biological contents of *Hyptis suaveolens* leaves treated with different concentrations of old CFCF of *Nigrospora* sp.(FGCC#74)

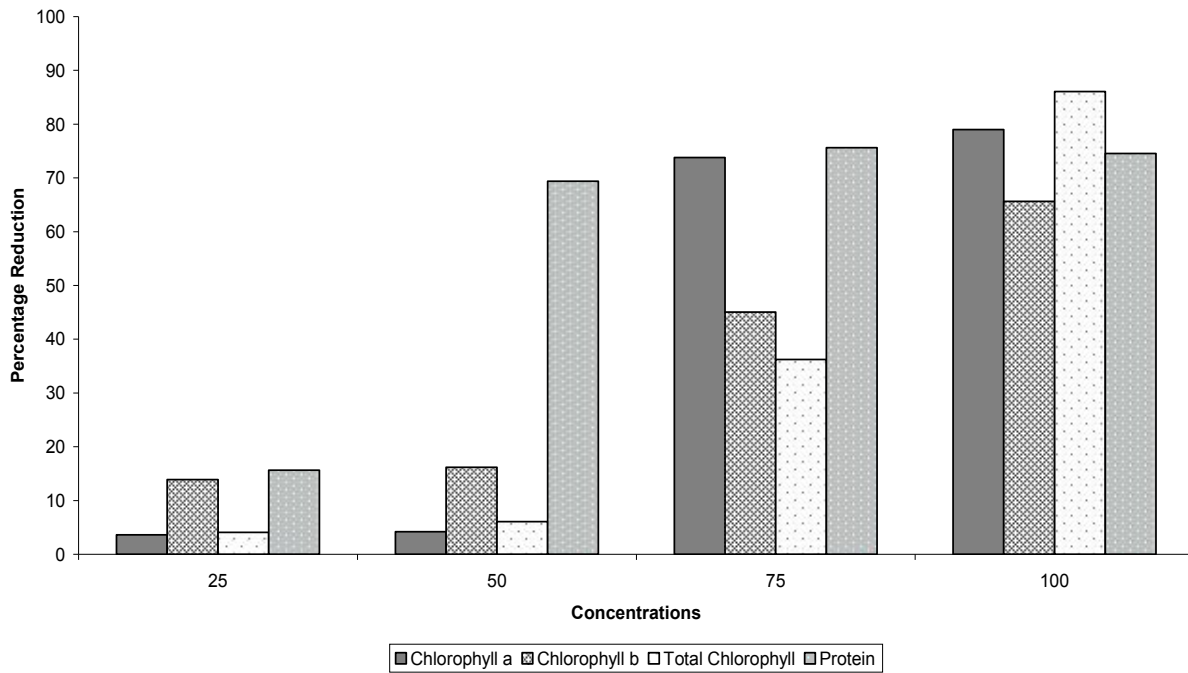


Figure 6: Percentage reduction in biological contents of *Hyptis suaveolens* leaves treated with different days old CFCF of *Nigrospora* sp. (FGCC#74)

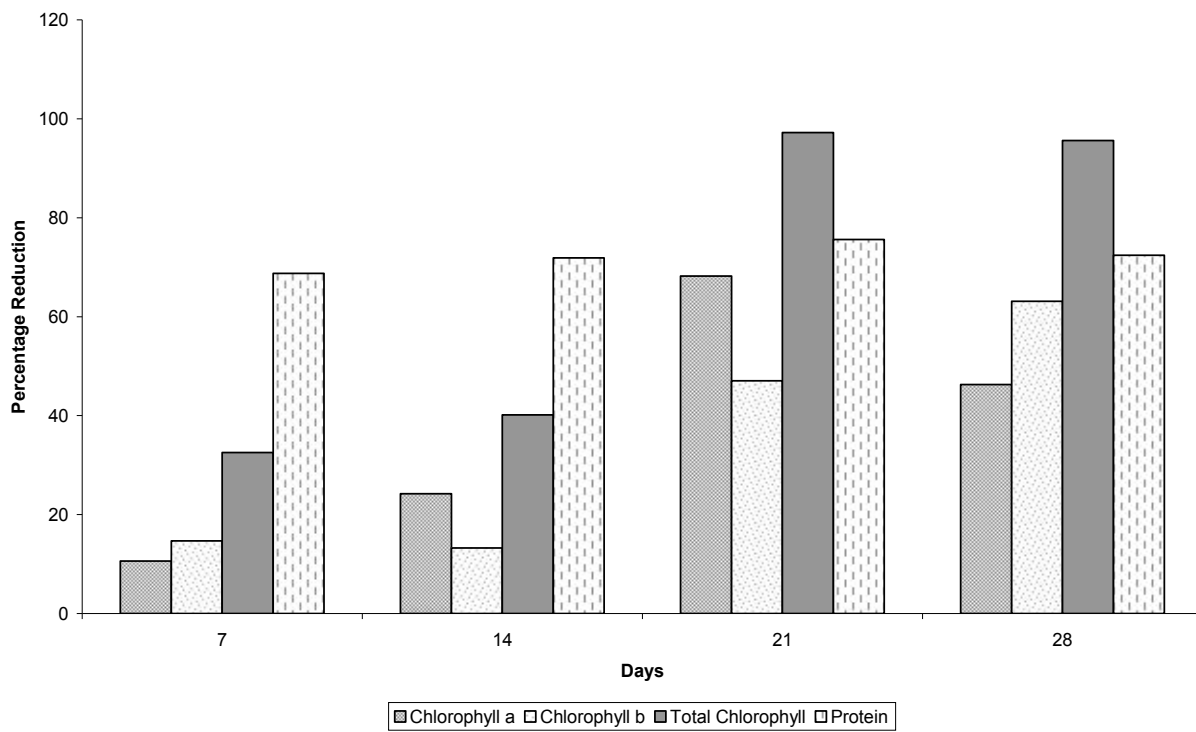


Figure 7: Thermal stability of phytotoxin from *Nigrospora* sp.(FGCC#74) against *Hyptis suaveolens* by shoot cut bioassay.

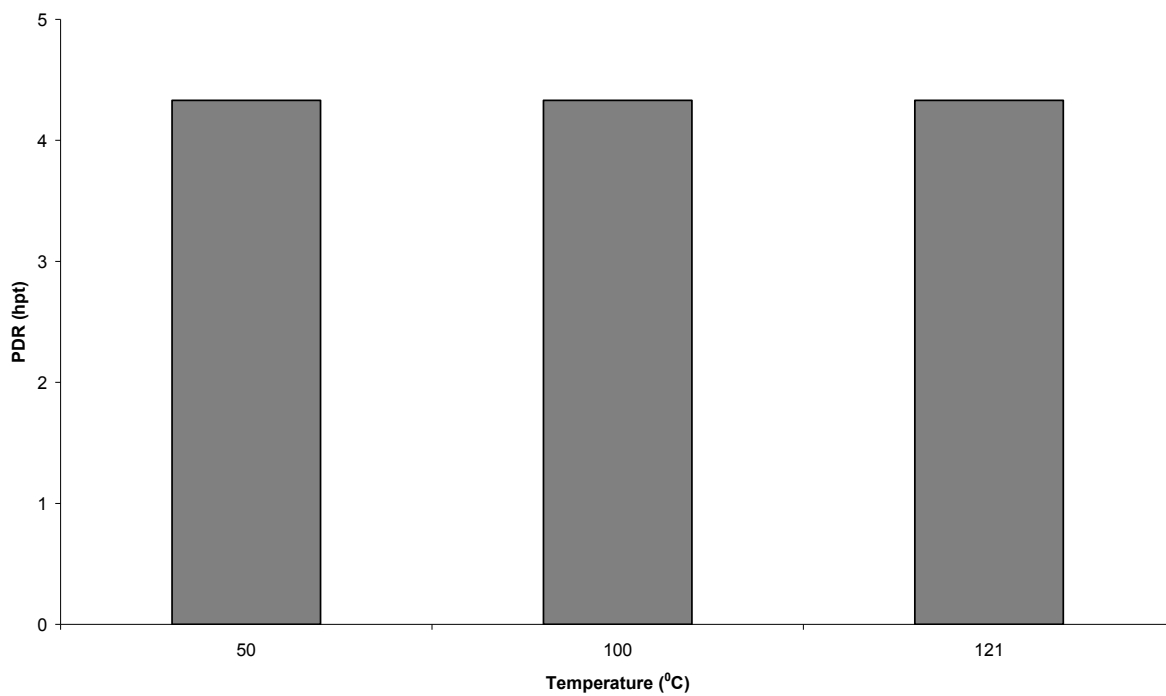


Figure 8: Phytotoxic damage rating of solvent extracted fraction of CFCF of *Nigrospora* sp. (FGCC#74) against *Hyptis suaveolens* (DLB).

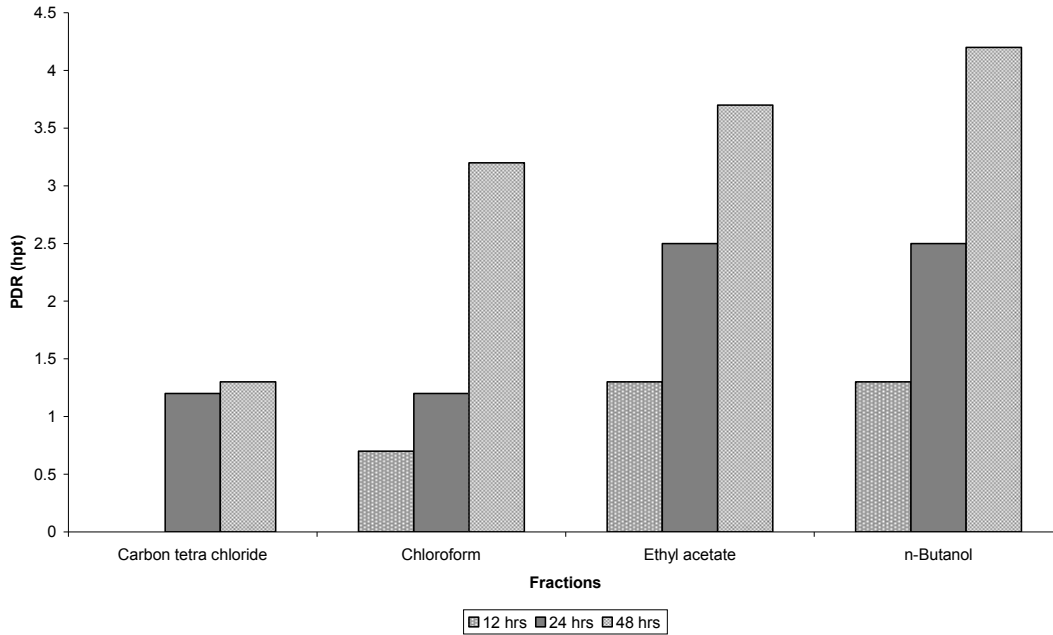
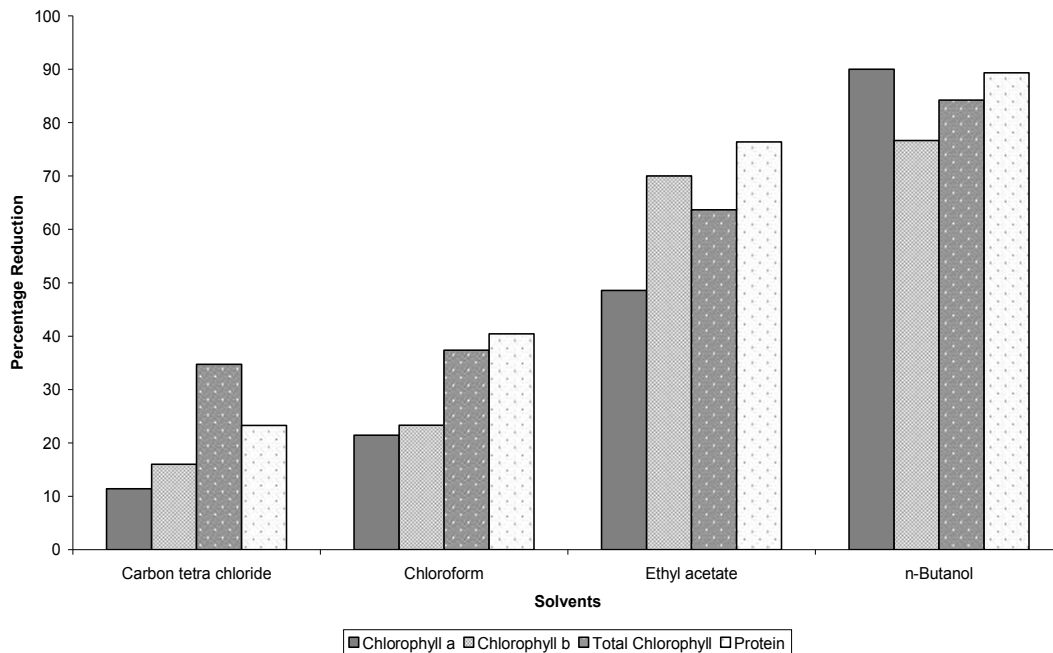


Figure 9: Percentage reduction in biological contents of *Hyptis suaveolens* leaves treated with partially purified CFCF of *Nigrospora* sp. (FGCC#74)



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°C, 100°C and 121°C (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 *Phaeophleospora 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 proteinaceous nature of toxin produced by *Cristulariella pyramidalis*.

Fig.8 shows phytotoxic damage rating when *Hyptis* leaves were treated with different solvent extracted fraction of CFCF. Maximum phytotoxic damage was observed 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 of *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 (Orsenigo, 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 rats at 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 phytotoxicity 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).

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