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ALLELOPATHIC EFFECT OF CYANOBACTERIAL STRAINS ON PHYTOPATHOGENIC FUNGI

RICHA MISHRA[1] and ASIT KUMAR[2]

[1]Department of Microbiology, A.P. S.G. M. N. S. Govt. P.G, College, KABIRDHAM (C.G.) INDIA
[2]Department of Zoology, Govt. R. V. R. S. Kanya Mahavidyalaya Kawardha , (C.G.) INDIA
***Corresponding Author:** E. mail.richa.micro10@gmail.com

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Allelochemicals are subsets of secondary metabolites that no longer required for metabolism of the allelopathic organism and their poor allelopathic results are vital part of organism defense against antagonists. Allelopathic interactions involving Cyanobacterial flora are being explored for their pharmaceutical and environmental importance. Cyanobacterial allelopathy may be appeared as one of the massive elements influencing their dominance in numerous habitats and as particular producers of a selection of allelochemicals that can be applied as green biocontrol marketers. In present work detrimental (dreadful allelopathy) effects of locally isolated Cyanobacterial strains had been evaluated in opposition to plant pathogenic three fungal isolates (*Fusarium oxysporum, Sclerotium rolfsi and Rhizoctonia solani*). It turned into discovered that the crude extracts of 4 Cyanobacterial isolates (*Microcystis aeruginosa, Oscilatoria boryana, Anabaena sphaerica and Nostoc calcicola*), had been able to diminishing the boom and similarly development of phyto-pathogenic fungal isolates. While *M. Aeruginosa* showed greater allelopathic interest compared to different cyanobacterial strains. Methanol crude extracts have been extra efficient. Crude extract (100 % in Methanol) of *Microcystis aeruginosa* changed into determined extra efficient towards neighborhood isolates *Fusarium oxysporum* (% MI – 88.Forty six ± 0.24) and *Sclerotium rolfsii* (83.42), in comparison to *Rhizoctonia solani* (78.33). Allelopathic potentiality of cyanobacteria have need to be further investigated which could provide promising answers in bio-control in opposition to pathogenic Fungi.

Keywords: Allelopathy, Cyanobacteria, Phyto-pathogens, Crude extracts, Biocontrol agents.

Introduction:

An expanding international population and urgency of removing hunger and malnutrition call for decided rules and powerful actions to make certain sustainable growth in agricultural productiveness. India is a biggest Country in South Asia that includes 70 percent of the total nearby populace. After green Revolution big progress with cutting-edge irrigation and fertilizer software has been made. The concern of pesticide use with respect to human fitness and surroundings has added increasing hobby in options by using fending off terrible results on the environment. Secondary metabolites from cyanobacteria are related to poisonous, hormonal, antineoplastic and antimicrobial effects (Shweta et al., 2011). Currently algae are one of the leader organic dealers which have been studied for the manipulate of plant pathogenic fungi, especially soil-borne disorder (Hewedy, 2000). Cyanobacteria and eukaryotic algal produce biologically active compounds which have antifungal and antibacterial pastime (Kulik, 1995) against plant pathogens. Allelopathy is a unique adaptation to gain a aggressive gain over different organism inhabiting the equal microbial network, Allelopathic impact of Cyanobacteria on pathogenic fungi was also studied (Tiwari and Kaur, 2014). Cyanobacteria are known to supply intracellular and extracellular metabolites with diverse biological activities consisting of antialgal, antibacterial, antifungal and antiviral pastime (Mohamed El-anwar H. Osman, 2011; Shrivastava et al., 2012; Shrivastava et al., 2014., Shrivastava et al., 2017). The antimicrobial substances may additionally target diverse sorts of microorganisms. Secondary metabolites influence different organisms inside the location and are idea to be of phylogenetically crucial. Kulik, (1995), stated some of reasons, cyanobacteria and algae are suitable candidates for exploitation as biocontrol agents of plant pathogenic fungi. The ability for the use of Cyanobacteria is the biocontrol of plant pathogenic bacteria and fungi. Tiwari & Sharma (2013), has found the antifungal interest of *Anabaena variabilis* in opposition to plant pathogens. Screening of cyanobacteria for antibiotics has opened a new horizon for discovering new capsules. Exploring allelopathic efficacy of cyanobacteria to govern plant pathogenic fungi can prove to be a fantastic option as they're easy to develop with minimum vitamins, price effective, no aspect results and surroundings friendly. This work describes the alleopathic impact of cyanobacterial strains towards phytopathogenic microorganisms.

MATERIALS AND METHODS:

Isolation and identification of microbes:

For the choice of the experimental materials distinct species of fungi and cyanobacteria have been remoted applying right media and methods. Identification of remoted microbes became finished with the aid of microscopic statement and biochemical checks.

Cyanobacteria:

Soil samples had been amassed from diverse agro-fields of Bilaspur division. The samples had been introduced to laboratory in plastic vials /zipped polythene bag and washed with distilled water to save potential contaminants. Isolation of cyanobacterial strains became carried out in particular nutrient media (Allen & Arnon, BG-one hundred ten and Chu-10) and changed into saved as suspension way of life in boom medium below prescribed situation (Shrivastava, 2000 and Shrivastava et al., 2005). Isolated strains have been recognized by way of microscopic observation with the help of Key standardized by means of Desikachary (1959) and Anand (1989).

Fungal lines:

The fungal strains have been remoted from agronomic soil samples. They were grown in the laboratory in suitable media, for fungal evaluation media used which includes Potato dextrose agar media (PDA) and Sabouraud’s agar

media and were identified on the basis of their morphological traits. Taxonomic guides (Alexopoulus, C. J., et al., 1996) have been used to become aware of the remoted fungal culture.

Extraction:

The bloom samples were collected from various zones of Bilaspur division. The bloom samples had been accumulated the use of plankton net of 20µm mesh size. Samples were saved in sterile zipped polythene luggage. Cyanobacterial cells had been focused by way of the usage of centrifugation. A portion of the concentrated samples turned into filtered thru a 0.45 µm glass fiber clear out (What's men-41) and air dried in an oven at 60 ^{0}C. Dried cellular mass-2g/25ml (w/v) of Cyanobacteria had been extracted with 75% methanol (HPLC grade), for one hour then centrifuged at 5000 rpm for 7 min. The supernatant changed into separated in clean glass vials and filtered with 0.45 µm pore length. Specific Dilutions have been organized for toxicity check in-vitro.

Toxicity assessment:

For checking out the allelopathic activities of crude extract of cyanobacteria against diagnosed fungal isolates with the help of special methods and Carbendazim and Mancozeb (as fungicides) had been used as control. The following techniques had been hired to confer the toxicity of crude extract of various Cyanobacterial isolates on identified micro organism & fungi.

Percent mycelial inhibition:

Petri plates with medium were aseptically inoculated on the centre with mycelial disc of 5mm diameter taken from ninety six hrs vintage way of life. Plates were then incubated at 26 ± 1°C in BOD incubator and mycelial growth pattern became found. Radial diameter turned into recorded twice perpendicularly after 96 hrs incubation using a transparent millimeter ruler. Percent inhibition of mycelia boom changed into calculated.

RESULTS AND DISCUSSION:

Through exam of samples overall sixteen species of cyanobacteria have been remoted, out of which 9 heterocystous shape and 7 non-heterocystous bureaucracy have been recognized as nitrogen fixing and non nitrogen fixing traces respectively (table -1, PLATE -1; Fig. – I to XVI).

Table - 1: Morphologically distinct Cyanobacteria isolated at some point of the survey.

TABLE - 1: Morphologically dissimilar Cyanobacteria isolated during the survey.

	Non Nitrogen fixing	Nitrogen fixing
Unicellular	*1. Aphanocapsa elachista* *2. Aphanothece saxicola*	NIL
Colonial	*3. Microcystis aeruginosa*	NIL

Filamentous	*4. Lyngbya birgei* *5. Lyngbya shackletoni* *6. Oscillatoria boryana* *7. Oscillatoria cortiana*	*8. Anabaena oryzae* *9. Anabaena sphaerica* *10. Anabaena unispora* *11. Aulosira prolifica* *12. Calothrix elenkinii* *13. Cylindrospermum indicum* *14. Gloeotrichia echinulata* *15. Nostoc calcicola* *16. Nostoc ellipsosporum*

Simultaneously total 6 fungal species (***Aspergillus niger, Penicillum notatum, Mucor sp., Sclerotium rolfsii, Fusarium oxysporum*** and ***Rhizoctonia solani)*** were isolated and identified on the basis of their morphological features (TABLE -2, PLATE -2; Fig. – I to VI).

TABLE-2: Characteristic features of fungal isolates.

NAME OF FUNGI	TAXONOMIC STATUS	COLOUR	MICROSCOPIC OBSERVATION
Aspergillus niger	Ascomycetes	dark to pale yellow in early stage while became black at maturity	Jet black conidia, reverse usually grey, spherical conidia, rough with maturity.
Penicillium notatum	Ascomycetes	velvety or woolly colonies initially white and become green	Conidiophore green in colour, repeatedly branched, brushy head.
Mucor sp.	Zygomycetes	filamentous colonies, fluffy appearance, white in colour	Globular sporangia round black in colour that was supported by elevated column like columella. Non-septate or partially septate hyphae .
Sclerotium rolfsii	Basidiomycetes	It was characterized as felty white appearance	Silky white hyphae tend to aggregate into rhizomorphic cord as well as characteristic sclerotium – fan shape was found.
Fusarium oxysporum	Ascomycetes	It was characterized as white to pink colony	Moist appearance, red with cottony and orange brown mycelium, with light brown exudates hyphen septet, small conidia in chain, sickle shaped long conidia were also observed.
Rhizoctonia solani	Basidiomycetes	Buff to black colony	The mycelium is generally hyaline, irregular in shape. Hyphae produce branches at right and acute angles to the main hyphae. specialized hyphae composed of monilioid cells, that fuse together to produce hard structure called sclerotia.

The efficacy of Cyanobacterial allelopathy changed into screened in opposition to the increase of E. Coli (ATCC-25922).The effect of crude extracts of all Cyanobacterial isolates was tested on growth pattern of test strain via measurement of optical density (600nm). And located information had been referred to in table–four and PLATE -four.

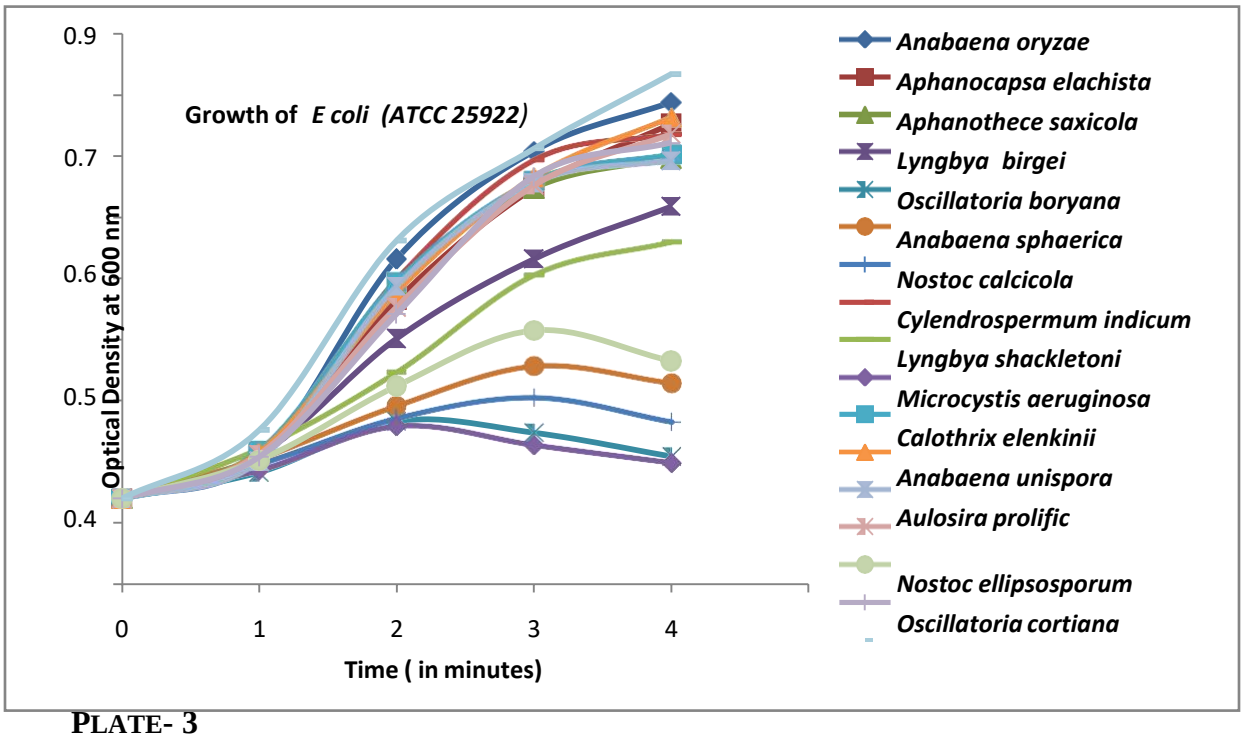
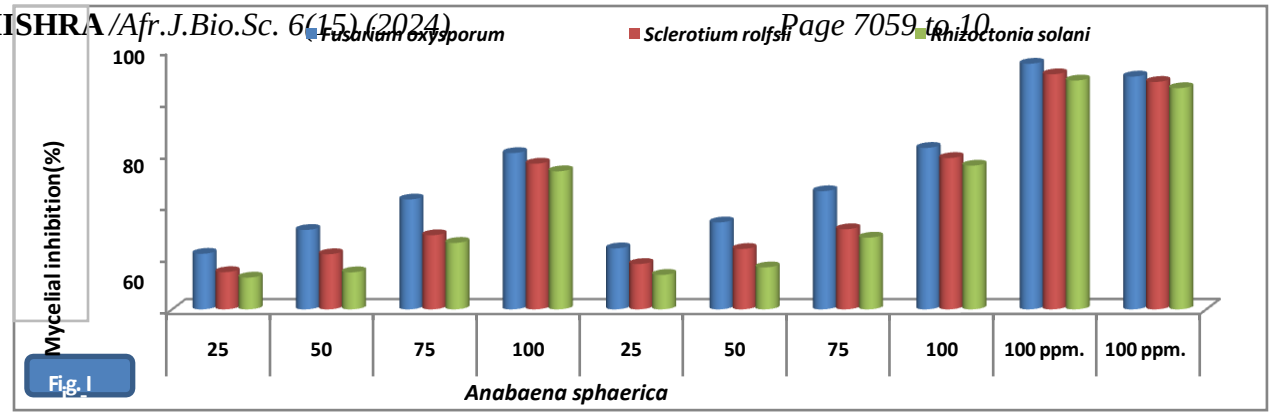


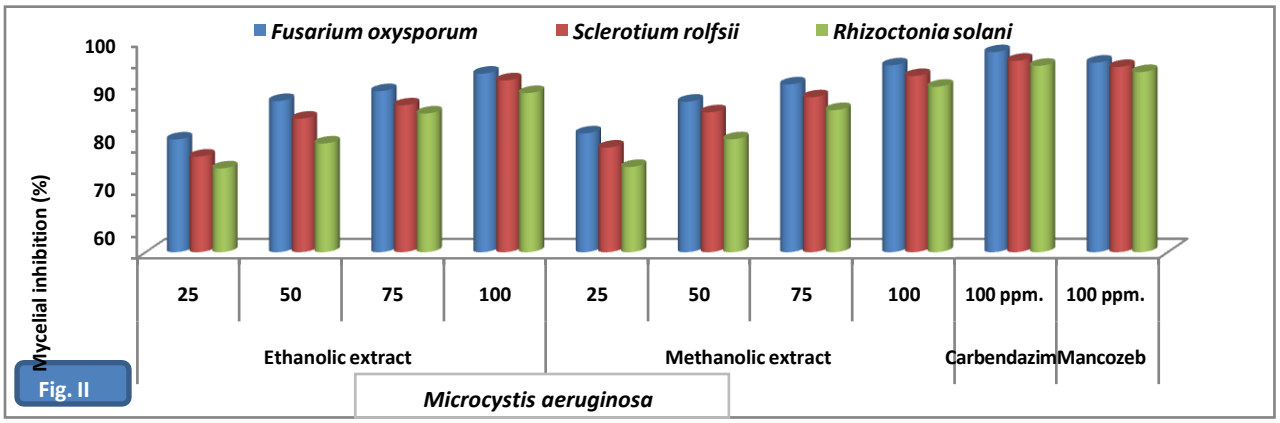
PLATE- 3

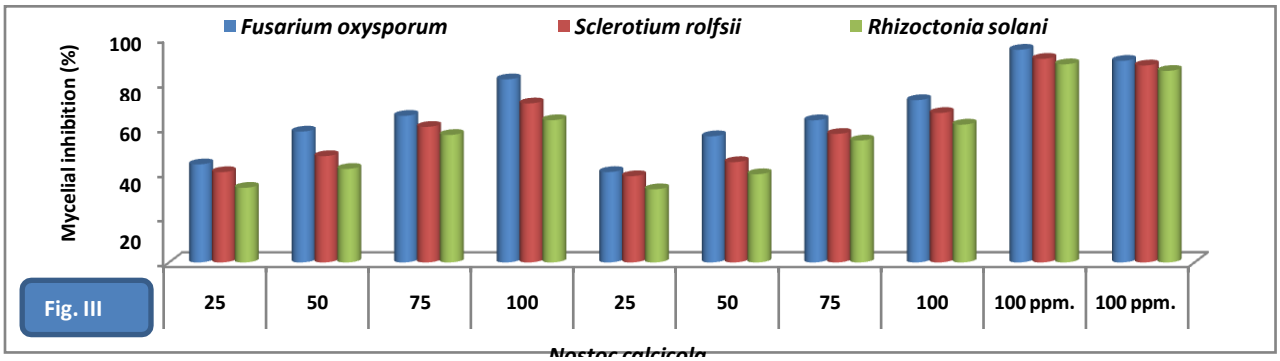
On the idea of toxic potentiality of crude extracts non- heterocystous (*Microcystis aeruginosa & Oscillatoria boryana*) and two heterocystous (*Nostoc calcicola & Anabaena sphaerica*) cyanobacterial isolates had been selected for similarly test in view of the evaluation of the allelopathic effect of cyanobacterial isolates on phytopathogenic Fungal isolates. To assess the Allelopathic impact of cyanobacterial became evaluated through percent mycelia inhibition and involved graph indicated that the extract of *Microcystis aeruginosa* have great potential as fungicides Carbendazim and Mancozeb compound against pathogenic fungi which turned into examined (desk – five, PLATE -five; Fig. I - IV.). Poisonous potentiality of *Nostoc calcicola* was observed lesser than *Oscilatoria boryana* however extra than different cyanobacterial isolates, whereas minimum doubtlessly of growth inhibition turned into discovered in case of *Anabaena sphaerica.*

TABLE – 5 Allelopathic effect of crude extracts of Cyanobacterial isolate on Fungal isolates (*Fusarium oxysporum, Sclerotium rolfsii* and *Rhizoctonia solani).*

Cyanobacterial Isolates	Crude extracts	Conc. of crude extract	Percent of Mycelial Inhibition (Mean value ±SD)		
			Fusarium oxysporum	*Sclerotium rolfsii*	*Rhizoctonia solani*
Anabaena sphaerica	Ethanolic extract	25	21.42 ±0.25	14.35 ±0.18	12.23 ±0.22
		50	30.61 ±0.32	21.25 ±0.43	14.27 ±0.52
		75	42.37 ±0.27	28.58 ±0.26	25.62 ±0.35
		100	60.25 ±0.22	56.16 ±0.15	53.24 ±0.28
	Methanolic extract	25	23.51 ±0.24	17.48 ±0.35	13.26 ±0.26
		50	33.52 ±0.22	23.28 ±0.43	16.17 ±0.52
		75	45.56 ±0.26	30.88 ±0.36	27.65 ±0.41
		100	62.25 ±0.33	58.35 ±0.38	55.41 ±0.29
Microcystis aeruginosa	Ethanolic extract	25	53.37 ±0.32	45.25 ±0.24	39.67 ±0.25
		50	71.56 ±0.16	63.21 ±0.23	51.45 ±0.21
		75	76.24 ±0.34	69.51 ±0.28	65.66 ±0.19
		100	84.52 ±0.35	81.34 ±0.42	75.35 ±0.28
	Methanolic extract	25	56.23 ±0.42	49.52 ±0.34	40.34 ±0.27
		50	71.24 ±0.36	66.23 ±0.45	53.45 ±0.28
		75	79.43 ±0.27	73.34 ±0.35	67.28 ±0.32
		100	88.46 ±0.24	83.42 ±0.28	78.33 ±0.45
Nostoc calcicola	Ethanolic extract	*25*	43.65 ±0.35	40.18 ±0.43	33.21 ±0.56
		50	58.25 ±0.32	47.32 ±0.24	41.62 ±0.29
		75	65.28 ±0.32	60.35 ±0.46	56.75 ±0.34
		100	81.52 ±0.25	70.81 ±0.21	63.33 ±0.33
	Methanolic extract	25	40.25 ±0.17	38.4 ±0.25	32.56 ±0.21
		50	56.18 ±0.42	44.67 ±0.16	39.28 ±0.32
		75	63.3 ±0.32	57.23 ±0.18	54.28 ±0.15
		100	72.26 ±0.26	66.53 ±0.34	61.36 ±0.42
Oscillatoria boryana	Ethanolic extract	25	43.65 ±0.23	41.56 ±0.28	35.57 ±0.23
		50	62.21 ±0.35	48.26 ±0.27	43.55 ±0.26
		75	72.24 ±0.44	64.51 ±0.35	62.66 ±0.45
		100	80.32 ±0.35	78.24 ±0.42	71.44 ±0.28
	Methanolic extract	25	48.35 ±0.15	42.51 ±0.35	36.23 ±0.22
		50	64.25 ±0.12	52.56 ±0.17	43.45 ±0.25
		75	76.27 ±0.27	68.33 ±0.35	59.23 ±0.32
		100	83.34 ±0.34	79.36 ±0.28	73.31 ±0.45
Solvent control			0	0	0
Fungicides					
Carbendazim		100 ppm.	94.70 ±0.13	90.64 ±0.16	88.23 ±0.17
Mancozeb		100 ppm.	89.76 ±0.21	87.70 ±0.24	85.29 ±0.36







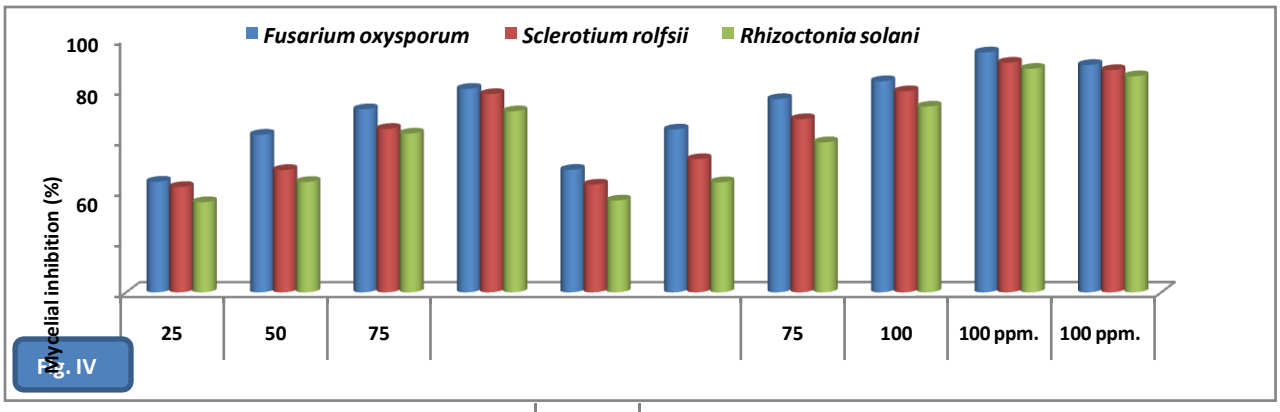


PLATE- 5: Fig I - IV

Discussion:

The consequences obtained all through present course of research, concerning Cyanobacterial toxicity, revealed that the boom of fungi. Fungal isolates have been inhibited once they had been subjected to crude extracts (Ethanol or Methanol) on solid nutrient media. Boom inhibition potency of crude extracts of cyanobacterial isolates have additionally been as compared with wellknown fungicides towards fungal boom respectively. The end result of toxic potentiality as acquired in opposition to fungal pathogens has additionally been supported the findings. Cyanobacteria produce biologically energetic compounds that have allelopathic and toxic hobby in opposition to plant pathogens (Bonjouklian, R. Et al. 1991; Kiviranta, J., et al. 2006). Kim and Kim, 2008, said inhibition of F.Oxysporum f. Sp. Lycopersici by way of extracts of N. Commune FA-103. Biological control of Fusarium oxysporum f. Sp. Lycopersici (FOL) inflicting wilt disorder of tomato was studied in vitro as well as beneath pot situations and became concluded that N. Linkia, can be utilised for the organic manage of wilt ailment of tomato which may additionally help to gain a higher yield and suitable fitness in agriculture (Hend, A. 2012). Mohamed et al. 2011, investigated the suppression effect of cyanobacterial species- Nostoc endophytum and Nostoc muscurum against, the causal agent of soyabean root rot Rhizoctonia solani.

The prevailing studies work correlates the findings of various people as suggested earlier concerning concerned research paintings. On this have a look at Cynobacteria confirmed tremendous allelopathic activities. This type of investigation produce a miles generalized view that cynobacteria are able to inhibiting phytopathogens. This work makes a speciality of the ability of Canobacteria to be used as allelopathic biocontrol agent further research on Cyanobacterial metabolites are vital for clinical employees to pick out the poisonous effect on phytopathogenic fungi which might also lead to the formula of considerable bio-energetic compounds of organic origin.

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