Excecutive Summary

Title of Project: "A comparative study on effects of synthetic and botanical pesticides on physiology and biochemistry of some cyanobacteria"

UGC Major Project { F.No. 39-406/2010 (SR)}

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Objectives of Project:

1- A survey on synthetic pesticides and Neem based products used for different purposes in various soil ecologies of Varanasi will be done.

2- Cyanobacterial diversity of Soil ecologies of Varanasi will be studied.

3- Impact of Aqueous and Alcoholic preparations from Neem parts used for agricultural purposes and commercial formulations of Neem on growth, biochemical composition and enzymes of nitrogen metabolism of cyanobacteria will be studied.

4- Impact of some of synthetic pesticides, currently used in fields, on physiology and biochemistry of cyanobacteria will be done.

5- Impact of bioactive compounds of Neem on cyanobacteria will be evaluated.

Cyanobacteria improve the soil fertility by increasing organic content, water holding capacity, nitrogen content, phosphate solubilization, secretion of polysachharide and through various kinds of secretions. These properties of cyanobacteria prove it suitable and eco-friendly biofertilizer. Paddy fields favor luxuriant growth of cyanobactria and most of biological nitrogen fixation of these ecosystem is done by N_2-fixing cyanobacteria. Many nitrogen fixing strains of cyanobacteria have been isolated and used in biofertilizer consortia in South east Asian countries. Our country is one of largest consumer of pesticides and highest among south Asian counties. The adverse environmental impact of pesticides have become a major concern, from the

mid-1970s. Pesticides affect non-target microorganisms including cyanobacteria. Tolerant strains of cyanobacteria to regularly used pesticides and potential to degrade them are desirable qualities for cyanobacterial biofertilizer. Varanasi is located in the Indo-Gangetic Plains of North India, the land is very fertile because low level floods in the Ganges continually replenish the soil. According to statistical report of Varanasi the Wheat and Rice is major crop of district. Varanasi have diversity of agroecosystem / soil ecologies which support rich diversity of cyanobacteria. Huge amount of pesticides are being poured regularly in agricultural fields of Varanasi. In this research plan ecological details of sampling locations, cyanobacterial diversity and effect of some common pesticides (CM-75, Malathion and Butachlor) on growth, biochemical composition and activity of some enzymes (NR & GS) of *Fischerella muscicola* NDUPC001 and *Nostoc ellipsosporum* NDUPC002.was done.

Followings are conclusion of over all work.

1. Fifteen Locations of Varanasi district was selected to study the generic diversity of cyanobacteria.
2. 27 cyanobacterial genera were identified from fifteen selected locations of Varanasi. The genus *Nostoc* was most abundant (26%, 51 strains), followed by *Phormidium* (12%, 24) and *Calothrix* (12%, 23).
3. Shannon diversity index was highest in Jolhupur site (1.129) and lowest in Nowpur-1 (0.301) site. Margaleff diversity index ranges from highest, 86.37 (Nowpur-1) to lowest 18.599 (Jalhupur).
4. Clay loam medium up land soil with pH 7.32 and EC 0.0246 of Jolhupur site supports the maximum number of cyanobacterial strains.
5. Cyanobacteria *Fischerella muscicola* NDUPC001 and *Nostoc ellipsosporum* NDUPC002 were isolated from agricultural fields of Varanasi and characterized by morphological as well molecular methods.
6. LC_{50} conc. of CM-75 for *Fischerella muscicola* NDUPC001 was 6 ppm. and 2 ppm for *Nostoc ellipsosporum* NDUPC002.
7. Effect of 3ppm, 6ppm and 9 ppm conc. of CM-75 on *Fischerella muscicola* NDUPC001 growth behavior of was studied. All concentrations of fungicide inhibited the growth of cyanobacteria.

8. All treatment concentrations of fungicide CM-75 on *Fischerella muscicola* NDUPC001 decreased the Chl.-a, carbohydrate and protein content of cyanobacteria with maximum inhibition of 29.29%, 29.9% and 19.26% respectively in 9ppm treatment.
9. All treatment concentrations of fungicide CM-75 on *Fischerella muscicola* NDUPC001 inhibited the activity of Nitrate reductase and Glutamine synthetase with maximum inhibition of 35.11% and 21.74% respectively in 9 ppm treatment.
10. LC_{50} conc. of Malathion for *Fischerella muscicola* NDUPC001 was 100 ppm. Effect of 50 ppm, 100 ppm and 150 ppm conc. of CM-75 on *Fischerella muscicola* NDUPC001 growth behavior of was studied. All concentrations of fungicide inhibited the growth of cyanobacteria. Maximum inhibition was observed in 150 ppm treatment.
11. 50 ppm treatment conc. of malathion on *Fischerella muscicola* NDUPC001 induced the chl.-a and protein content 4.33% and 8.83% respectively where as other treatments decreased the chl.-a and protein content with max. decrease of 41.14% and 24.19% respectively in 150 ppm treatment. Carbohydrate content decreased in all treatments with maximum decrease of 41.71% in 150 ppm concentration.
12. Nitrate reductase activity was slightly induced (0.31%) in 50 ppm treatment conc. of malathion on *Fischerella muscicola* NDUPC001 and decreased in other two treatments with maximum decrease of 27.83% in 150 ppm treatment. All treatment concentrations of insecticide inhibited the activity of Glutamine synthetase with maximum inhibition of 34.98% in 150 ppm treatment.
13. LC_{50} conc. of butachlor for *Fischerella muscicola* NDUPC001 was 400 ppm. Effect of 200 ppm, 400 ppm and 600 ppm conc. of butachlor on *Fischerella muscicola* NDUPC001 growth behavior of was studied. All concentrations of fungicide inhibited the growth of cyanobacteria. Maximum inhibition was observed in 600 ppm treatment.
14. 200 ppm treatment conc. of butachlor on *Fischerella muscicola* NDUPC001 induced the chl.-a and protein content 2.11% and 3.57% respectively where as other treatments decreased the chl.-a and protein content with max. Decrease of 33.85% and 31.38% respectively in 600 ppm treatment. Carbohydrate content decreased in all treatments with the maximum decrease of 33.09% in 600 ppm concentration.

15. All treatment concentrations of herbicide on *Fischerella muscicola* NDUPC001 inhibited the activity of Nitrate reductase and Glutamine synthetase with maximum inhibition of 37.48% and 35.96% respectively in 600 ppm treatment.
16. LC_{50} conc. of CM-75 for *Nostoc ellipsosporum* NDUPC002 was 2 ppm. Effect of 1ppm, 2ppm and 4 ppm conc. of CM-75 on growth behavior of *Nostoc ellipsosporum* NDUPC002 was studied. All concentrations of fungicide inhibited the growth of cyanobacteria. Maximum inhibition was observed in 4ppm treatment.
17. All treatment concentrations of fungicide on *Nostoc ellipsosporum* NDUPC002 decreased the Chl.-a and carbohydrate content of cyanobacteria with maximum inhibition of 23.63% and 19.98% respectively in 4ppm treatment. Total protein content was slightly increased (1.57 %) in 1ppm treatment and decreased in other treatments with maximum inhibition of 15.47 % in 4 ppm treatment.
18. All treatment concentrations of fungicide on *Nostoc ellipsosporum* NDUPC002 inhibited the activity of Nitrate reductase and Glutamine synthetase with maximum inhibition of 58.93% and 41.15 % respectively in 4ppm.
19. LC_{50} conc. of Malathion for *Nostoc ellipsosporum* NDUPC002 was 10 ppm. All treatments of malathion inhibited the growth of cyanobacteria. Maximum inhibition was observed in 15ppm treatment.
20. Chl.-a was induced (12.33%) in the 5ppm treatment of Malathion on *Nostoc ellipsosporum* NDUPC002 and decreased in other two treatments with a maximum reduction of 15.41% in 15ppm treatment. Total protein content was increased in all treatments with maximum induction of 5.72% in 10ppm treatment. Total carbohydrate was also induced in all treatments with maximum induction of 8.08% in 10ppm treatment.
21. Nitrate reductase activity was induced in 5ppm (12.47%) treatment of Malathion on *Nostoc ellipsosporum* NDUPC002 and reduced in other two treatments with maximum inhibition of 46.56% in 15ppm treatment. The activity of glutamine synthetase increased in 5ppm treatment (10.74%) and decreased in other two treatments with maximum inhibition of 32.29% in 15ppm treatment.
22. LC_{50} conc. of Butachlor for *Nostoc ellipsosporum* NDUPC002 was 40 ppm. All treatments of butachlor inhibited the growth of cyanobacteria. Maximum inhibition was observed in 60 ppm treatment.
23. Chl.-a was slightly induced (0.122%) in the 20ppm treatment of butachlor on *Nostoc ellipsosporum* NDUPC002 and decreased in other two treatments with a maximum

reduction of 31.01% in 60ppm treatment. Total protein content was decreased in all treatments with the maximum reduction of 36.96% in 60ppm treatment. Total carbohydrate was also decreased in all treatments with the maximum decrease of 59.27% in 60ppm treatment.

24. Effects of treatments of butachlor on the activity of Nitrate reductase and Glutamine synthetase of *Nostoc ellipsosporum* NDUPC002 was studied. All treatment concentrations of fungicide inhibited the activity of Nitrate reductase and Glutamine synthetase with maximum inhibition of 42.29% and 36.4% respectively in 4ppm.
25. Effect of Neem seed kernel water extract (NSKWE) and Neem seed fertilizer water extract (NSFWE) on growth, biochemical composition and some enzymes of two most common cynobacteria i.e. *Nostoc ellopsosporum* NDUPC002 and *Fischerella muscicola* NDUPC001 was studied.
26. HPLC analysis of NSKWE and NSFWE for azadirachtin content was done . A negligible amount of azadirachtin (1.86ppm/ 5ml) was reported in NSKWE.
27. LC_{50} conc. of NSKWE and NSFWE for *Nostoc ellipsosporum* NDUPC002 was 2.0% and 10.00% respectively. Lower conc. of NSKWE (1.0%)and NSFWE (5.0%) induced the growth of cyanobacteria whereas LC_{50} and above concentrations inhibited the growth.
28. All the treatment conc. of NSKWE and NSFWE induced the chl-a and protein content of *Nostoc ellipsosporum* NDUPC002 but decreased the carbohydrate content.
29. All the treatment conc. of NSKWE and NSFWE decreased the Nitrate reductase (NR) activity whereas lower conc. increased the Glutamine synthetase (GS) activity but LC_{50} and above concentrations decreased the GS activity of *Nostoc ellipsosporum* NDUPC002.
30. LC_{50} conc. of NSKWE and NSFWE for *Fischerella muscicola* NDUPC001was 6.0% and 9.00% respectively. Lower conc. of NSKWE (1.0%)and NSFWE (5.0%) induced the growth of cyanobacteria whereas LC_{50} and above concentrations inhibited the growth.
31. All the treatment conc. of NSKWE and NSFWE induced the chl-a and protein content of *Fischerella muscicola* NDUPC001 but decreased the carbohydrate content.
32. All the treatment conc. of NSKWE and NSFWE decreased the Nitrate reductase (NR) activity whereas lower conc. increased the Glutamine synthetase (GS) activity but

LC_{50} and above concentrations decreased the GS activity of *Fischerella muscicola* NDUPC001.

Over all findings of this study suggest *Nostoc* is most abundant cyanobacterial genera in agricultural fields of Varanasi. Clay loam medium up land soil with pH 7.32 and EC0.0246 supports maximum number of cyanobacterial strains. Jalhupur study site have highest cyanobacterial diversity and Nowpur-1 have lowest cyanobacterial diversity. LC_{50} and above concentrations of all the three pesticides, NSKWE and NSFWE were inhibitory to growth, biochemical composition and enzymes (NR & GS) of *Fischerella muscicola* NDUPC001 and *Nostoc ellipsosporum* NDUPC002. *Fischerella muscicola* NDUPC001 is more tolerant to all the three pesticides, NSKWE and NSFWE in comparison to *Nostoc ellipsosporum* NDUPC002. This study draw attention against over use of pesticides as they are harmful for beneficial micro biota of soil and also helps to screen the tolerant strains of cyanobacteria for selection of potential cyanobacterial inoculam for region.

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