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STUDY ON THE EFFECT OF DITHANE ON N. MUSCORUM IN DIFFERENT MEDIA UNSUPPLEMENTED WITH 500 PPM GLUCOSE

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INTRODUCTION:

There agrochemicals affects the metabolic processes of the target organism, thereby killing them to be eradicated. The metabolic processes of the non target organism may also be affected by these chemicals and may produce deleterious effect.'Use of pesticides in rice field also affects the immensely significant cynobacterial flora of the field. Several reports are available in which pesticides have been found to be stimulatory at lower doses (Ahmad and Venkatraman, 1973). 2, 4-D is perhaps the first herbicide to be studied for its effects on N2 fixing blue-green algae (Venkatraman and Rajyalakshmi, 1971; Das and Singh, 1977b; Arvike/a/., 1971; Singh, 1974;Tiwarie/a/.,1982, 1984). Several other groups of pesticides have been studied well such as phenyl carbonate herbicide (Wright, 1972, 1978), anilide group (Propanil - Vaishampayan et a/., 1978; Pandey et al, 1984; Butachlor-Vaishampayan and Prasad, 1981c, and Alachior-Vaishampayan, 1984f, 1985c; Singh & Vaishampayan, 1979) Thioneb- Xia , 2005 and .-Bipyridyl group (Das and Singh,1977a, Wright, 1978; Vaishampayam 1982). Some insecticide have also been screened for their toxic as well as mutagenic role on cyanobacteria (Sevin on Nostoc muscorum Vaishampayan & Prasad, 1982b, Dichlorvos Verma and Verma,1995, Dithane - Gangawane, 1979, Vaishampayan & Prasad 1981, Blitox-Vaishampayan and Prasad 1982, Panacid- Prasad et al, 1991, 2,4 - D -Wong, 2000; Divap 100 [Organophosphorus] - Lakshmi and Annamalai, 2007).

It is evident from the aforesaid facts that pesticides are toxic even mutagenic to economically important N2 -fixers of paddy fields although they are not targeted. Such studies have not been conducted on cyanobacterial strains collected from the rice field of Manjhi block of Saran district. The present study was therefore, undertaken to screen the biological effects of two pesticides, Dithane (Carbamate group) and Rogor (organophosphate group) on two cyanobacterial strains Nostoc muscorum and Anabanena variabilis collected and isolated from the rice fields of the area. Following studies were conducted:

2. MATERIALS & METHODS:

Combined inorganic nitrogen free medium i.e. N_2 - medium was used to prepare different concentrations of the two pesticides. Doses of the pesticides were decided on the basis of their field dose. 10, 20, 50, 100, 250, 500 solutions of the two pesticides were prepared by appropriately diluting with N_2 medium.

The log phase culture growing in 5mM KNO_3 supplemented culture medium (Chu NolO as modified by Gerloff et a/., 1950) was harvested and washed thoroughly with N_2-medium.Traces of nitrate was removed by repeated washing and such cultures were homogenized with sterile glass beads. The

equal amount of homogeneous suspension of culture was used in different experiments.

Since the two algae were N_2 fixing, the survivality and Relative tolerance were studied in N_2 - free solid and liquid medium. For survivality studies equal amount of treated cultures were inoculated in petri plates containing N_2 free medium. Parallel to this, untreated cultures were also observed for their growth on into N_2 free culture media.

After 15 days of incubation the colony forming units (CPU) in control as well as petri plates containing pesticides treated cultures were counted and % survival was calculated considering no. of colony forming units developed in control plates as 100%.

For the study of relative tolerance, experiments were conducted in 10 ml liquid N_2 free medium containing pesticides at various levels. The homogenized culture was prepared as discussed earlier and equal amount of aliquot was inoculated in test tube containing 10 ml nitrogen free culture medium with various concentrations of the two pesticides. Growth was recorded in terms of optical density of the acetone extract of pigment after 24 days of incubation. Methods of measurement of growth and extraction of pigment have been discussed in chapter-2.

For further studies i.e. the toxicological studies (mode of action and biological effects), experiments were restricted to only three concentrations of the two pesticides. Three concentrations 10, 50 and 250 ppm, were taken into 'Consideration. Experimental culture was treated with three concentrations of two pesticides for 30 mts. After treatment cultures were washed thoroughly with sterile double distilled water. Equal amount of the treated cultures of the two test materials was transferred into different test tubes containing 10 ml liquid culture media with nitrogen from different sources (N_2, 5mM $KN0_3$, 3 mM $KN0_2$ and ImM NH_4C1). Two sets of experiments were designed one with exogenous supplementation of organic source of carbon (500 ppm glucose) and the other without. exogenous source of organic carbon. Growth was recorded at the interval of four days of incubation i.e. at 4 , 8^{th}, 12^{th}, 16^{th1} 20^{th} and 24^{th} day of inoculation whereas heterocyst frequency was recorded alternate day from a parallel set of experiment.

3. RESULTS:

There is slight difference in Vhe two pesticides. So far as the survivality of the two algae at different levels of pesticide is concerned, Rogor appears to produce decreased survival at lower doses in comparison to Dithane. When the two algae are compared A. variabilis appears to give more survivality % in comparison to N. mmcorum.

Relative Tolerance:

There had been little impact of the two pesticides on the two algae at lower doses. However, the toxicity increases with increase in the concentration of the pesticide. Out of the two pesticides Rogor appears to be more toxic than Dithane and so far as the two algae are concerned A.variabilis seems to be more tolerant than N. muscorum. However, no growth was recorded at SOOppm of both the pesticide in both the algae.

Toxicological Studies of the Pesticides:

Toxicological studies i.e. the impact of the two pesticides on the two algae were conducted in different media containing nitrogen from different sources and either the media were supplemented or unsupplemented with exogenous source of organic carbon in form of 500 ppm glucose. represent the impact of Dithane on the two algae in different media containing N_2, 5mM $KN0_3$, 3mM KNOs and ImM NH_4C1 without 500 ppm glucose whereas table - 5.5 and 5.6 represent the growth of the same

algal culture in same media but with exogenous supply of organic carbon in form of 500 ppm glucose.

It is evident from the result that untreated culture grew well in ammonical nitrogen medium in comparision to other media with different sources of nitrogen (N2, $KN0_3$ & KNO_2) media. Growth of treated culture is reduced with increase in the concentration of the pesticide, Dithane Toxicity of the chemical is more in ammonical nitrogen followed by nitrate, nitrite and elemental nitrogen medium. No end point (24 day of incubation) growth was recorded at 250 ppm of the pesticides. However, A.variabilis appears to be more resistant than N.muscorum. All these results were obtained in media unsupplemented with 500 ppm glucose. Results of the toxic effect of the .pesticide, Dithane in media supplemented with 500 ppm glucose It is evident that the growth inhibitory"effect of the pesticide is reversed by supplementation of 500 ppm of glucose.

Table.1 Effect of Dithane on N.muscorum in different media unsupplemented with 500 ppm glucose

Dose	Days	N_2	$KN0_3$	$KN0_2$	NH4C1
		0.18	0.18	0.18	0.18
Nil	4	0.21 ±0.009	0.23 ±0.012	0.28 ±0.012	0.30 ±0.012
	8	0.25 ±0.0 17	0.29 ±0.014	0.31 ±0.013	0.34 ±0.012
	12	0.29 ± 0.014	0.33 ±0.0 12	0.35 ±0.014	0.38 ±0.014
	16	0.35 ±0.012	0.38 ±0.015	0.41 ±0.012	0.45 ±0.016
	20	0.38 ±0.014	0.42 ±0.013	0.45 ±0.018	0.51 ±0.013
	24	0.41 ±0.012	0.46 ±0.0 15	0.49 ± 0.014	0.56 ± 0.009
10	4	0.22 ±0.013	0.24 ±0.021	0.26 ±0.016	0.24 ±0.016
	8	0.25 ±0.014	0.26 ±0.012	0.28 ±0.012	0.26 ±0.012
	12	0.27 ±0.012	0.31 ±0.016	0.29 ±0.013	0.28 ±0.014
	16	0.32 ±0.009	0.34 ±0.012	0.32 ±0.01 8	0.30 ±0.014
	20	0.35 ±0.011	0.38 ±0.015	0.34 ±0.009	0.25 ± 0.009
	24	0.38 ±0.012	0.37 ±0.013	0.31 ±0.014	0.24 ± 0.013
50	4	0.20 ±0.013	0.20 ±0.009	0.21 ±0.015	0.1 8 ±0.009
	8	0.20 ±0.014	0.21 ±0.01 8	0.18 ±0.013	0.17 ±0.016
	12	0.18 ±0.012	0.17 ±0.017	0.1 8 ±0.014	O.H ± 0.021
	16	0.1 8 ±0.024	0.16±0.013	0.17 ±0.012	0.12 ±0.017
	20	0.15 ±0.012	0.15 ±0.012	0.14 ±0.021	0.12 ±0.012
	24	0.14±0.013	0.1 5 ±0.009	0.14 ±0.012	0.12 ±0.016
250	4	0.18 ±0.016	0.15 ±0.016	0.11 ±0.012	0.08 ±0.014
	8	0.18 ±0.019	0.12 ±0.015	0.12 ±0.009	0.08 ±0.018
	12	0.16 ±0.016	0.12 ±0.014	0.09 ±0.01	0.06 ±0.015
	16	0.06 ±0.0 15	0.09 ±0.0 14	0.04 ±0.02	0
	20	0.06 ±0.009	0.06 ±0.0 14	0	0
	24	0.04 ±0.012	0	0	0

Table-2 Effect of Dithane on A. variabilis in different media unsupplemented with 500 ppm glucose

Dose	Days	N_2	$KN0_3$	$KN0_2$	NH_4C1
Nil	4	0.23 ±0.012	0.23 ±0.014	0.24 ±0.012	0.24 ±0.014
	8	0.26 ±0.012	0.27 ±0.01 3	0.28 ±0.014	0.30 ±0.015
	12	0.32 ±0.015	0.35 ±0.014	0.38 ±0.018	0.41 ±0.016
	16	0.38 ±0.016	0.41 ±0.018	0.44 ±0.014	0.48 ± 0.016
	20	0.41 ±0.015	0.42 ±0.016	0.46 ±0.016	0.52 ±0.014
	24	0.42 ±0.017	0.44 ±0.01 5	0.48 ±0.014	0.56 ±0.012
10	4	0.21 ±0.013	0.22 ±0.010	0.21 ±0.012	0.20 ±0.013
	8	0.23 ±0.013	0.25 ±0.013	0.26 ±0.013	0.24 ±0.013
	12	0.25 ± 0.012	0.27 ±0.014	0.28 ±0.016	0.26 ±0.015
	16	0.29 ±0.011	0.32 ±0.016	0.31 ±0.015	0.28 ±0.016
	20	0.35 ±0.018	0.36 ±0.012	0.32 ±0.010	0.30 ±0.013
	24	0.40 ±0.017	0.38 ±0.013	0.35 ±0.015	0.31 ±0.014
50	4	0.22 ±0.014	0.22 ±0.019	0.16 ±0.016	0,16 ±0.018
	8	0.22 ±0.019	0.20 ±0.014	-0.16 ±0.016	0.16 ±0.019
	12	0.20 ±0.018	0.18 ±0.016	0.16 ±0.014	0.15 ±0.016
	16	0.19 ±0.013	0.17 ±0.017	0.17 ± 0.013	0.14 ±0.014
	20	0.19 ±0.01	0.17 ±0.018	0.17 ±0.015	0.14 ±0.013
	24	0.18 ±.009	0.17 ±0.015	0.16 ±0.009	0.12 ±0.012
250	4	0.18 ±0.015	0.18 ±0.010	0.16 ±0.018	0.12±0.011
	8	0.16 ±0.017	0.14 ±0.019	0.14 ±0.021	0.08 ±0.013
	12	0.12 ± 0.01	0.09 ±0.0 10	0.07 ±0.012	0
	16	0.08 ±0.01	0.07 ±0.012	0.07 ±0.013	0
	20	0.08 ±0.017	0.07 ±0.012	0	0
	24	0	0	0	0

Table -3 Effect of Dithane on N. muscorum in different media supplemented with 500 ppm glucose.

Dose	Days	N_2	KNO_3	KNO_2	NH_4C1
Nil	4	0.22 ±0.01	0.24 ± 0.013	0.31 ±0.019	0.32 ±0.016
	8	0.26 ±0.007	0.30 ± 0.01 1	0.32 ±0.012	0.36 ±0.011
	12	0.32 ±0.016	0.34 ±0.016	0.38 ±0.014	0.42 ±0.018
	16	0.38 ±0.016	0.45 ± 0.018	0.49 ±0.016	0.48 ±0.013
	20	0.41 ± 0.014	0.48±P.019	0.50 ±0.018	0.56 ±0.019
	24	0.42 ±0.013	0.49 ±0.011	0.51 ±0.019	0.58 ±0.009
10	4	0.20±0.002	0.22±0.009	0.30±0.010	0.3H0.004
	8	0.30 ±0.011	0.28 ± 0.01 4	0.36 ±0.019	0.36 ±0.009
	12	0.36 ±0.013	0.38 ± 0.010	0.42 ±0.019	0.41 ±0.019
	16	0.39 ±0.0 9	0.41 ±0.01	0.48 ±0.014	0.46 ± 0.009
	20	0.40 ±0.014	0.44 ±0.014	0.49 ± 0.009	0.49 ±0.018
	24	0.40 ±0.014	0.47 ±0.017	0.50 ±0.01	0.53 ±0.013
50	4	0.20 ±0.018	0.21 ±0.018	0.28 ±0.012	0.28 ±0.016
	8	0.28 ±0.013	0.26 ±0.018	0.35 ±0.015	0.35 ±0.016
	12	0.35 ±0.018	0.35 ±0.013	0.40 ±0.014	0.38 ±0.016
	16	0.38 ±0.013	0.38 ±0.014	0.44 ±0.014	0.41 ±0.013
	20	0.38 ±0.014	0.40 ±0.014	0.46 ±0.016	0.42 ±0.012
	24	0.38 ±0.011	0.40 ±0.012	0.46 ±0.014	0.42 ±0.013
250	4	0.21 ±0.009	0.20 ±0.018	0.26 ±10.012	0.25 ±0.018
	8	0.29 ±0.015	0.28 ±0.011	0.31 ±0.013	0,35 ±0.011
	12	0.35±10.01 2	0.33±10.011	0.39 ±0.013	0.36 ±0.013
	16	0.36 ±0.017	0.36 ±0.01	0.41 ±0.011	0.39 ±0.021
	20	0.37 ±0.011	0.38 ±0.009	0.42 ±0.014	0.41 ±0.014
	24	0.38 ±0.009	0.38 ±0.01 8	0.42 ±0.012	0.41 ±0.012

Effect of Rogor treatment on the two algae N.muscorum and A. variabilis in different inorganic nitrogen media unsupplemented or supplemented with 500 ppm glucose as exogenous source of organic carbon. When the result obtained for-Roger treated cultures of the two algae are compared, one would find more or less similar pattern of toxicity. However, Rogor appears to be more toxic than Dithane as less growth and early lysis of cells have been found in Rogor treated cultures. Here also, the toxic effect of Rogor is reversed by addition of 500 ppm glucose in the medium as exogenous source of carbon. A. variabilis - appears to be more resistant to Rogor than N. muscorum. The toxicity of the chemical,Rogor is more in ammoniacal nitrogen" medium which is similar to Dithane.

Heterocyst frequency of the two test algae is adversely affected by the two pesticides. Almost similar pattern has been observed for the two pesticides in both the algae. However, Rogor produced more inlibitpry effect than Dithane and the inhibitory effect increased with the increase in concentration of the pesticide. Complete inhibition of heterocyst differentiation was noticed at 250 ppm of the two pesticides. These inhibitory effect of pesticides are reversed by supplementing the media with 500 ppm glucose as exogenous source of organic carbon.

DISCUSSION:

Pesticides, Dithane and Rogor appear to be less effective at lower concentration where as their high doses are growth inhibitory. No end point growth was recorded at higher concentration. Out of the two algae A.variabilis appears to be more tolerant to the pesticides in comparison to N.muscorum, However,the pattern of response of the two algae is more or less similar. Out of the two pesticides Rogor, an organophorus insecticide is more toxic than Dithane, a member of carbamate group of

fungicide. Similar experiments have been conducted by Venkatraman & Rajyalakshmi (1971,1972), Kaushik and Venkataraman (1983), Adhikary et. al (1977) Singh, et al (1988), Goyal et al (1991), Adhikary (1989). Such studies shall be helpful in deciding the use of N-fixers and the pesticides in rice- field.

So far as the toxicity of the two chemicals in different media is concerned, both follow almost similar pattern. They produce more toxicity in ammonical nitrogen medium than the other media. It is also evident that the ammonical nitrogen is best utilized by the two algae showing enhanced growth followed by nitrate, nitrite and elemental nitrogen medium. Therefore, it is evident that the toxicity is more in the medium in which the algae grow better. When microscopically examined lysis of cells was found earlier in ammonical nitrogen medium. Also Rogor produces early and more lysis of treated cultures in comparison to Dithane. This effect of the two pesticides may be compared with the toxic effect of bipyridyl herbicide (Dodge, 1975) and pre emergence herbicide, Machete (Vaishampayan, 1979). Growth inhibitory effect is reversed by supplementing the media with 500 ppm glucose.

Reductant is also required for carbon assimilation so, as expected DCMU also inhibits this process. DCMU treated photoheterotrops which utilizes organic carbon supplement such as glucose, grows significantly in presence of such source of carbon. In the present study the growth inhibitory effect and the adverse effect on heterocyst differentiation are reversed significantly with the supplementation of 500 ppm glucose. Therefore, it appears that the two chemicals like DCMU, affect the photosynthetic process and there by indirectly affecting the activity of nitrogenase. The derepression of heterocyst differentiation requires organic carbon either photosynthetically fixed or externally supplied. Inhibition of photosynthetic oxygen evolution in Westiellopsis prolifica on its exposure to over 500 ppm Rogor has been found by Adhikary (1989).

It is evident from the above that the two chemicals are photosynthetic inhibitor and there by adversely affect the heterocysf differentiation as well as nitrogenase activity by limiting the generation of reductant, $NADPH_2$. Thus, the target sites of the two chemicals are the two vital process photosynthesis and N_2 - fixation. Therefore, the application of these two chemicals in rice fields may result in a change in C/N ratio. Decreased availability of fixed inorganic carbon ($C0_2$) decreases the biological nitrogen fixation (Moustafa, 1969, Sloger et al, 1975). However, application of these chemicals may be suggested at lower dose as there is very little effect on the growth and heterocyst differentiation in the two algae.

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