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Study Of The Effects Of Thepesticides On


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The Selected Strains of blue Green Algae Ofpaddy Fields

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INTRODUCTION

Pesticides are manmade agrochemicals which are commonly used in crop fields in order to increase the yield. Pesticide are designed to be toxic to the target living system but some time it may affect the non target life system resulting into more harmful consequences. These agrochemicals affect 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 cyanobacterial 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 al.*, 1978; Pandey *et al.*, 1984; Butachlor-Vaishampayan and Prasad, 1981c, and Alachlor-Vaishampayan, 1984f, 1985c; Singh & Vaishampayan, 1979) Thioneb- Xia, 2005 and .-Bipyridyl group (Das and Singh, 1977a, Wright, 1978; Vaishampayan 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 *Anabaena variabilis*

collected and isolated from the rice fields of the area. Following studies were conducted:

(a) Survivability and Relative tolerance of the two strains towards two pesticides

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2. Materials & methods

Detailed description of the two test materials - *Nostoc muscorum* and *Anabaena variabilis* as well as of the two pesticides (fungicide -Dithane and insecticide - Rogor). The two test materials which are heterocystous and filamentous have been found growing consistently in the rice fields under study during paddy growing season. The two pesticides, Dithane and Rogor belong to carbamate and organophosphate group, respectively.

Combined inorganic nitrogen free medium i.e. N₂ - 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 N₂ medium.

The log phase culture growing in 5mM KNO₃ supplemented culture medium (Chu No10 as modified by Gerloff *et al.*, 1950) was harvested and washed thoroughly with N₂-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 N₂ fixing, the survivability and Relative tolerance were studied in N₂ - free solid and liquid medium. For survivability studies equal amount of treated cultures were inoculated in petri plates containing N₂ free medium. Parallel to this, untreated cultures were also observed for their growth on into N₂ free culture media.

After 15 days of incubation the colony forming units (CFU) 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 N₂ 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 (N₂, 5mM KNO₃, 3 mM KNO₂ and 1mM NH₄C1). 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 , 8th, 12th, 16th 20th and 24th day of inoculation whereas heterocyst frequency was recorded alternate day from a parallel set of experiment.

3. Results

3.1 Survival studies

Table - 1 & represent the % survival of the two algae after treatment with different concentrations of the two pesticides for 30 mts. It is evident that the two pesticides have little or no impact on the survivality at lower doses where as the survivality decreases with increase in the concentration. 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. mncorum*.

Table -.1

**Effect of Dithane and Rogor on survival of *Anabaena variabilis*
and *Nostoc muscorum***

No. of Colony Forming Units (CFU) in control - 224 ± 3.055

Pesticide	Concentration	% Survival	
		<i>N. muscorum</i>	<i>A. Variabilis</i>
Dithane	10	97.32 \pm 0.576	98.21 \pm 0.768
	20	80.35 \pm 1.154	88.39 \pm 1.891
	50	58.03 \pm 2.629	66.96 \pm 1.123
	100	41.07 \pm 2.789	45.53 \pm 0.682
	250	14.28 \pm 1.239	20.08 \pm 1.329
	500	0	3.57 \pm 1.4921
		<i>N. muscorum</i>	<i>A. Variabilis</i>
Rogor "	10	94.64 \pm 1.201	97.32 \pm 2.576

	20	76.78 ±1.576	49.46 ±0.693
	50	52.67 ±0.612	47.14 ±1.230 [^]
	100	34.82 ±1.132	37.94 ±0.549
	250	8.03 ±1.239	11.16 ±0.851
	500	0	0

3.2 Relative Tolerance

Table - 2 & represent the relative tolerance of the two algae towards the two pesticides Dithane & Rogor. 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 500ppm of both the pesticide in both the algae.

Table-.2

Relative tolerance of the algal culture in combined inorganic nitrogen free medium.

Pesticide	Dose	Growth (OD on 24 th day)

		<i>N.muscorum</i>	<i>A. variabilis</i>
Dithane	10	0.48	0.52
	20	0.45	0.49
	50	0.38	0.43
	100	0.22	0.29
	250	0.08	0.12
	500	0	0
Rogor	10	0.44	0.48
	20	0.38	0.42
	50	0.29	0.35
	100-	0.18	0.26
	250	0	0.08

	500	0	0

3.3 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 N₂, 5mM KNO₃, 3mM KNO₂ and 1mM NH₄Cl 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 comparison to other media with different sources of nitrogen (N₂, KNO₃ & KNO₂) 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.3

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

Dose	Days	N ₂	KNO ₃	KNO ₂	NH ₄ Cl
		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	0.15 ±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 ±0.015	0.09 ±0.014	0.04 ±0.02	0
	20	0.06 ±0.009	0.06 ±0.014	0	0
	24	0.04 ±0.012	0	0	0

Table-4

Effect of Dithane on *A. variabilis* in different media unsupplemented with 500 ppm glucose.

Dose	Days	N ₂	KN ₃	KN ₂	NH ₄ C1
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 ±0.013	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 ±0.015	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 -5

Effect of Dithane on *N. muscorum* in different media supplemented with 500 ppm glucose.

Dose	Days	N ₂	KNO ₃	KNO ₂	NH ₄ C1
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
	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

50	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±10.012	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

Table -.6

Effect of Dithane on *A. variabilis* in different media supplemented with 500 ppm glucose.

Dose	Days	N ₂	KNO ₃	KNO ₂	NH ₄ C1
Nil	4	0.23 ±0.013	0.23 ±0.014	0.24 ±0.016	0.26 ±0.016
	8	0.29 ±0.012	0.28 ±0.015	0.29 ±0.018	0.32 ±0.013
	12	0.36 ±0.013	0.36 ±0.016	0.38 ±0.013	0.41 ±0.014
	16	0.39 ±0.019	0.38 ± 0.017	0.39 ±0.019	0.48 ±0.018
	20	0.41 ±0.010	0.44 ±0.014	0.48 ±0.010	0.56 ±0.015
	24	0.42 ±0.014	0.45 ±0.015	0.48 ±0.014	0.58 ±0.015
10	4	0.20 ± 0.012	0.21 ±0.011	0.21 ±0.012	0.22 ± 0.012
	8	0.26 ± 0.014	0.28 ±0.018	0.28 ±0.02	0.26 ±0.013
	12	0.32 ±0.015	0.38 ±0.018	0.32 ±0.018	0.31 ±0.016
	16	0.39 ±0.013	0.39 ±0.013	0.36 ±0.013	0.32 ±0.014
	20	0.41 ±0.011	0.39 ±0.013	0.37 ±0.017	0.34 ±0.013
	24	0.42 ± 0.014	0.40 ±0.014	0.38 ±0.013	0.35 ±0.012
50	4	0.22 ± 0.012	0.20 ±0.012	0.18 ±0.014	0.18 ±0.011
	8	0.26 ±0.016	0.26±OX)16	0.20 ±0.012	0.20 ±0.014
	12	0.31 ±0.013	0.29 ± 0.019	0.29 ±0.014	0.26 ±0.018
	16	0.35 ±0.015	0.31 ±0.011	0.31 ±0.03	0.29 ± 0.019
	20	0.37 ±0.017	0.35 ±0.013	0.35 ±0.01	0.30 ±0.011

	24	0.38 ±0.013	0.36 ±0.016	0.35 ±0.015	0.32 ±0.014
250	4	0.20 ±0.019	0.18±0.011	0.16 ±0.016	0.16 ±0.016
	8	0.22 ±0.012	0.19 ±0.012	0.14 ±0.021	0.16 ±0.018
	12	0.29 ±0.019	0.30 ±0.02	0.28 ±0.012	0.26 ±0.014
	16	0.31 ±0.013	0.32 ±0.013	0.30 ±0.014	0.28 ±0.013
	20	0.34 ±0.014	0.32 ±0.013	0.30 ±0.015	0.30 ±0.003
	24	0.35 ± 0:015	0.34 ±0.013	0.32 ±0.016	0.30 ± 0.011

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-Rogor 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.

Table-7

Effect of Rogor on *N. muscorum* in different media unsupplemented with 500 ppm glucose.

Dose	Days	N ₂	KNO ₃	KNO ₂	NBUCl
Nil	4	0.18 ±0.019	0.19 ±0.012	0.20 ± 0.014	0.19 ±0.014
	8	0.25 ±0.018	0.26 ±0.011	0.28 ±0.014	0.29 ± 0.016
	12	0.31 ±0.016	0.32 ±0.014	0.34 ±0.016	0.36 ±0.014
	16	0.39 ±0.013	0.40 ±0.014	0.42 ±0.014	0.44 ±0.015
	20	0.40 ±0.015	0.43 ±0.016	0.45 ±0.016	0.52 ±0.016
	24	0.42 ±0.018	0.45 ±0.016	0.48 ±0.018	0.59 ±0.014
10	4	0.20 ±0.016	0.20 ±0.014	0.21 ±0.018	0.22 ± 0.014
	8	0.22 ±0.014	0.27 ±0.016	0.29 ±0.019	0.30 ±0.02
	12	0.35 ±0.011	0.35 ±0.008	0.31 ±0.010	0.41 ±0.012
	16	0.38 ±0.012	0.35 ±0.017	0.34 ±0.016	0.45 ±0.013
	20	0.39 ±0.015	0.36 ±0.017	0.36 ±0.016	0.46 ±0.013
	24	0.40 ±0.017	0.38 ±0.016	0.36 ±0.012	0.48 ±0.014
50	4	0.20 ±0.016	0.19 ±0.012	0.19 ±0.013	0.18 ±0.04
	8	0.22 ±0.014	0.24 ±0.011	0.22 ±0.013	0.20 ±0.012
	12	0.25 ±0.013	0.26 ±0.014	0.24 ±0.013	0.21 ±0.016

	16	0.29 ±0.012	0.28 ±0.016	0.26 ±0.016	0.21 ±0.016
	20	0.30 ±0.014	0.30 ±0.017	0.29 ± 0.017	0.20 ±0.017
	24	0.34 ±0.016	0.30 ±0.016	0.29 ±0.015	0.20 ±0.019
250	4	0.19 ±0.017	0.19 ±0.014	0.14 ±0.014	0.12 ±0.011
	8	0.16 ±0.018	0.16 ±0.013	0.14 ±0.014	0.12 ±0.011
	12	0.12 ±0.011	0.14±0.013	0.09 ±0.016	0.05 ±0.012
	16	0.09 ±0.014	0.05±0.014	0.09 ±0.012	0
	20	0.06 ±0.012	0.05 ±0.016	0.04 ±0.012	0
	24	0.06 ±0.014	00 ±0.014	0	0

Table.8

Effect of Rogor on *A. variabilis* in different media unsupplemented with 500 ppm glucose.

Dose	Days	N ₂	KNO ₃	KNO ₂	NBUCI
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Nil		0.20 ±0.012	0.20 ±0.014	0.22 ±0.019	0.22 ±0.004
	8	0.26 ±0.014	0.28 ±0.014	0.30 ±0.016	0.32 ±0.004
	12	0.34 ± 0.014	0.35 ±0.016	0.39 ±0.016	0.41 ±0.002
	16	0.36 ±0.016	0.37 ±0.013	0.40 ±0.014	0.48 ±0.005
	20	0.37 ±0.018	0.39 ±0.016	0.47 ±0.014	0.54 ±0.002
	24	0.38 ±0.019	0.42 ±0.014	0.49 ±0.014	0.56 ±0.003
10	4	0.20 ± 0.012	0.20 ±0.018	0.22 ±0.013	0.18 ±0.004
	8	0.24 ±0.011	0.26 ±0.019	0.31 ±0.013	0.32 ±0.004
	12	0.30 ±0.012	0.29 ±0.011	0.31 ±0.012	0.32 ±0.009
	16	0.32 ±0.012	0.36 ±0.012	0.38 ±0.012	0.44 ±0.010
	20	0.31 ±0.013	0.39 ±0.014	0.40 ±0.014	0.46 ±0.009
	24	0.37 ±0.04	0.40 ±0.015	0.44 ±0.013	0.49 ±0.010
50	4	0.19 ±0.016	0.19 ±0.016	0.20 ±0.013	0.16 ±0.01
	8	0.20 ±0.009	0.18 ±0.018	0.19 ±0.012	0.19±t).004
	12	0.20 ±0.014	0.16 ±0.019	0.14 ±0.012	0.15 ±0.002
	16	0.18 ±0.016	0.14 ±0.014	0.12 ±0.019	0.12 ±0.004
	20	0.14 ±0.012	0.14 ±0.013	0.10 ±0.018	0.08 ±0.006
	24	0.09 ±0.014	0.12 ±0.013	0	0
250	4	0.20 ± G.G16	0.18 ±0.014	0.16 ±0.016	0.14 ±0.004

	8	0.18 ±0.015	0.16 ±0.016	0.14 ±0.014	0.09 ±0.009
	12	0.11 ±0.014	0.09 ±0.012	0.06 ±0.012	0.08 ±0.004
	16	0.11 ±0.013	0.09 ±0.011	0.05 ± 0.02	0.02 ± 0.002
	20	0.06 ±0.012	0.07 ±0.012	0.04 ±0.014	0
	24	0.04 ±0.013	0.06 ±0.011	0	0

Table-9

Effect of Rogor on *N. muscorum* in different media supplemented with 500 ppm glucose.

Dose	Days	N ₂	KNO ₃	KNO ₂	NH ₄ C1
Nil	4	0.18 ±0.016	0.19 ±0.012	0.20 ± 0.012	0.20 ±0.012
	8	0.24 ±0.015	0.28 ±0.014	0.29 ± 0.012	0.34 ± 0.014
	12	0.29 ±0.015	0.31 ±0.012	0.33 ±0.014	0.39 ±0.012
	16	0.34 ±0.013	0.36 ±0.013	0.38 ±0.016	0.48 ±0.015
	20	0.36 ±0.014	0.39 ±0.014	0.42 ±0.016	0.52 ±0.016

	24	0.40 ±0.016	0.42 ±0.016	0.46 ±0.01 8	0.55 ±0.013
10	4	0.20 ±0.018	0.21 ±0.016	0.21 ±0.018	0.21 ±0.019
	8	0.26 ±0.019	0.26 ±0.018	0.24 ±0.019	0.26 ±0.01
	12	0.29 ±0.011	0.30 ±0.019	0.32 ±0.016	0.35 ±0.012
	16	0.32 ±0.012	0.34 ±0.015	0.33 ±0.017	0.37 ±0.014
	20	0.35 ±0.016	0.35 ±0.014	0.42 ±0.015	0.47 ±0.016
	24	0.36 ±0.018	0.35 ±0.014	0.44 ±0.014	0.48 ±0.013
150	4	0.20 ±0.015	0.19 ±0.013	0.19 ±0.012	0.18 ±0.013
	8	0.22 ±0.014	0.24 ±0.013	0.25 ±0.012	0.22 ±0.015
	12	0.26 ±0.014	0.27 ±0.014	0.28 ±0.014	0.28 ±0.015
	16	0.29 ±0.012	0.29 ±0.016	0.35 ±0.016	0.36 ±0.011
	20	0.32 ±0.013	0.30 ±0.014	0.36 ±0.018	0.37 ±0.012
	24	0.34 ±0.014	0.35 ±0.016	0.40 ± 0.009	0.45 ±0.013
250	4	0.20 ±0.0 13	0.19±0.018	0.18±0.011	0.16 ±0.014
	8	0.22 ±0.016	0.24 ± 0.01	0.24 ±0.01	0.16 ±0.014

12	0.25 ±0.018	0.24 ±0.019	0.26 ±0.019	0.26 ±0.014
16	0.29 ±0.019	0.25 ± 0.012	0.28 ±0.016	0.32 ±0.019
20	0.32 ±0.012	0.26 ±0.012	0.30 ±0.012	0.36 ±0.018
24	0.32 ±0.014	0.32 ±0.013	0.36 ±0.013	0.40 ±0.011

Table-10

Effect of Rogor on *A. variabilis* in different media supplemented with 500 ppm glucose.

Dose	Days	N ₂	KNO ₃	KNO ₂	NftjCl
Nil	4	0.20 ±0.012	0.20 ±0.012	0.22 ±0.013	0.22 ±0.012
	8	0.24 ±0.015	0.26 ±0.014	0.27 ±0.013	0.28 ±0.014
	12	0.30 ±0.014	0.29 ±0.015	0.31 ±0.013	0.36 ±0.016
	16	0.32 ±0.014	0.35 ±0.016	0.37 ±0.014	0.39 ±0.016
	20	0.36 ±0.016	0.38 ±0.017	0.40 ±0.016	0.48 ±0.018
	24	0.36 ±0.013	0.40 ±0.018	0.45 ±0.009	0.52 ±0.01
10	4	0.20 ±0.014	0.21 ±0.016	0.20 ±0.017	0.20 ±0.009
	8	0.24 ±0.012	0.25 ±0.013	0.29 ±0.018	0.28 ±0.013
	12	0.28 ±0.014	0.29 ±0.014	0.32 ±0.01	0.38 ±0.014
	16	0.31 ±0.013	0.32 ±0.014	0.38 ±0.02	0.42 ±0.016

	20	0.32 ±0.019	0.36 ±0.016	0.40 ±0.013	0.47 ±0.015
	24	0.34 ±0.019	0.38 ±0.019	0.42 ±0.013	0.48 ±0.015
150	4	0.19 ±0.01	0.20 ±0.019	0.20 ±0.011	0.19 ±0.014
	8	0.24 ±0.012	0.22 ± 0.08	0.26 ±0.011	0.24 ±0.013
	12	0.26 ±0.013	0.28 ±0.016	0.30 ±0.012	0.36 ±0.016
	16	0.29 ±0.014	0.29 ±0.013	0.36 ±0.014	0.37 ±0.016
	20	0.31 ±0.015	0.36 ±0.013	0.38 ±0.013	0.40 ±0.018
	24	0.32 ±0.016	0.36 ±0.013	0.38 ±0.014	0.46 ±0.019
250	4	0.20 ±0.016	0.18 ±0.014	0.19 ±0.015	0.18 ±0.015
	8	0.23 ±0.018	0.24 ±0.015	0.22 ±0.016	0.22 ±0.011
	12	0.26 ±0.018	0.28 ±0.015	0.26 ±0.016	0.28 ±0.012
	ljU	0.32 ±0.018	0.31 ±0.014	0.32 ±0.018	0.32 ±0.013
	20	0.32 ±0.019	0.32 ±0.012	0.34 ±0.018	0.36 ±0.014
	24	0.33 ±0.01	0.34 ±0.013	0.36 ±0.014	0.38 ±0.016

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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 inhibitory 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.

4. 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 photoheterotrophs 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 heterocyst differentiation as well as nitrogenase activity by limiting the generation of reductant, NADPH₂. Thus, the target sites of the two chemicals are the two vital process photosynthesis and N₂ - 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 (CO₂) 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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