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Green Algae Diagnosis and Management in Low Land Paddy Fields of Cauvery Delta Zone, Tamil Nadu, India

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A B S T R A C T

Rice is a prime food crop for Asian countries. Wet land rice cultivation contributes maximum grain yield than dry land rice. Cauvery delta is a predominant area for rice cultivation in Tamil Nadu. Green algae growth during Kuruvai (June -August) season is a serious problem in wet land rice. Laboratory and field experiments were conducted to find out the remedial measures. The results of soil and water analyses showed that use of bore well water and dumping of phosphatic fertilizers leads to salt accumulation which favours the algal growth. The results of the laboratory experiment revealed that the $CuSO_{4,}$ londox power, propiconazole and hexaconazole showed moderate inhibition on 5th day after treatment. The findings from field experiment indicated that use of conoweeder and $CuSO_4$ drenching at the rate of 2.5 kg/ha when green algae appearance has just noticed or 5.0 kg/ha when severe growth occurred is effective in managing the green algae. Biofertilizers application, crop rotation, green manure trampling to be practised to control the algae growth not rectified by $CuSO_4$ application.

Introduction

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population. Globally, rice is grown on 161 million hectares, with an average annual production of 678.7 million tonnes (Bargali *et al.*, 2009; Vibhuti *et al.*, 2015). India is the largest rice growing country, accounting for about one-third of the world acreage under the crop (Bargali *et al.*, 2007 & 2009; Rajkumar, 2013). It is one of the most widely grown crops in Tamil Nadu state of India under million hectares.

Green algae or slime is a perpetual problem in wet land rice cultivation. It is prevalent in the water as a large slab of algae, that covers the surface and smothers the emerging rice crop. It thrives well where there is plenty of nutrients in the water and plentiful sunlight encourages photosynthesis (Coptrol, Rural chemicals industries (Australia) private limited, 1957). Green algae (singular: green alga) are a large, informal, grouping of algae consisting of the chlorophyte and charophyte/Streptophyta, which are now placed in separate divisions, as well as the more basal Mesostigmatophyceae and Chlorokybophyceae (Sánchez-Baracaldo *et al.*, 2017). Embryophytes or green algae have emerged from the charophytes (Jeffrey *et al.,* 2004). Green algae have chloroplasts that contain chlorophyll *a* and *b* giving them a bright colour as well as the accessory pigments *beta* carotene and xanthophylls in stacked thylakoids (Hoek *et al.,* 1995).

The cell walls of green algae usually contain cellulose and they store carbohydrate in the form of starch (Judd *et al.,* 2002). Like land plants, green algae undergo open mitosis without centrioles (Raveen *et al.,* 2005).

Algae are commonly present in irrigation water and generally the rate they multiply is balanced by natural degeneration. However, when the balance is upset through increased sunshine or by the introduction of extra nutrients, the rate of growth increases and the algae blooms become a problem in rice growing.

Before algae are visually detected a lot of damage has already been done to the crop at its most vulnerable stage of growth (Coptrol, Rural chemicals industries (Australia) private limited, 1957). With this background the current investigation was focused to find out the reason behind the algae growth and to device management practices to control algae growth in wet land rice fields.

Materials and Methods

Laboratory experiment

Laboratory experiments were carried out at Tamil Nadu Rice Research Institute, Aduthurai. Green algae were collected from infected rice fields. Ten gram of algae was placed in the Petri dish. Fungicides, weedicides and algicides were added at the rate of 2ml/plate each separately, closed with lid and left for 7 days. The observations were recorded every day after incubation.

Preliminary field experiment

A field experiment was conducted at Tamil Nadu Rice Research Institute, Aduthurai during June to August 2015 (*Kuruvai* season) to determine green algae management practices under wet land rice ecosystem. Experiments were laid out in a randomized block design with 11 treatments and 3 replications. Fertilizers were applied at the rate of 150: 50: 50 kg NPK/ha. N was applied 50% as basal and two top dressings of 25% each at the time of active tillering and panicle initiation stages. The size of the experimental plot adopted with 3 x 4m and 2 seedlings / hill. Soil sample (50g) was drawn at 30, 60 and 90 days after planting (DAP) for enumeration of bacteria (Allen, 1953), fungi (Martin, 1950) and actinomycetes (Allen, 1953).The survival of *Azospirillum* (Okon *et al.,* 1977) and Phosphobacteria (Sperber, 1958) in the rhizosphere of ADT 43 was estimated by MPN and standard plate count method (Parkinson, 1971) respectively. The *Pseudomonas* population was estimated using King's B (Kings *et al.,* 1954) medium. Available N, P and K were estimated by alkaline permanganate method (Subbian and Asija, 1956), Olsen's method (Olson *et al.,* 1954) and flame photometer method (Standford and English, 1949) respectively. The grain yield was recorded at the time of harvest (Fig. 1 and 2).

a Total bacteria were enumerated by serial dilution plating method on soil extract agar medium (James, 1958).

b Total culturable fungi were enumerated by serial dilution plating method as described by Parkinson *et al.,* (1971)

c Total diazotrophs were enumerated by the procedure as described by Rennie (1981).

Confirmatory field experiment

Field experiments were conducted during *Kuruvai* season in two places *viz.,* Aduthurai and Maharajapuram village located at Thanjavur district during June to August 2016. Fertilizers were applied as in the case of preliminary field experiment. The experiments were laid out in a randomized block design with 12 treatments and 3 replications. The plot size was 4m x 3m with 20 x 15 cm spacing having 2 seedlings / hill. ADT 45 variety was used in this experiment

Soil and water physical parameter analysis

Soil and water sample were collected from algae noticed fields for analysing the reason behind the over algae growth. Available N, P and K were estimated as described earlier. Micronutrients *viz*. HCO_3, S, Cl, Ca, Mg, Na and K were estimated using Atomic Adsorption Spectrophotometer (AAS) method and expressed in ppm. The electrical conductivity (EC) and pH were estimated using EC and pH meter respectively.

Results and Discussion

Effect of fungicides, weedicides and algicides on algae growth under laboratory and field conditions

The laboratory experiments revealed that the application of propiconazole, hexaconazole, $CuSO_4$ and londox power showed moderate inhibition on algae growth at 5^{th} day of incubation. The algae became shrunken and black in colour. Other chemicals don't have negative impact on the algae growth as given below (Table 1). Field experiment results revealed that $CuSO_4$application inhibited algal growth 100%, whereas, londox power exhibited 50% inhibition of algal growth (Table 2) while, other chemicals don't have inhibition on algal growth. Chemicals réaction on algae under *in vitro* and *in vivo* conditions were significantly different. Eventhough $CuSO_4$ inhibit algae growth under field conditions, it was not observed in labortory conditions. This might bé due to the change in the soil pH due to $CuSO_4$application.

Effect of fungicide, weedicide and algicide application on nutrient availability and microbial population under field conditions

Bispyriphos sodium and butachlor application increased soil available N content at the rate of 831.25 and 650 kg/ha respectively. On the other hand, butachlor and $CuSO_4$ + CaOapplication increased soil available P (142 kg/ha). Bispyriphos sodium and propiconazole application increased soil available K (775 kg/ha) at 90 DAT as given in the Table 3. This might be due to the changes in soil acidic and alkali status by the application of the chemicals. There is no sufficient data related to the effect of this fungicide, weedicide and algicides on soil nutrient availability, hence it needs to be studied in future.

The results revealed that the application of all the chemicals significantly reduced the beneficial soil microbe's *viz*., bacteria, fungi, actinomycetes, phosphobacteria, *Azospirillum* and *Pseudomonas*. This might be due to the inhibitory effect of chemicals on soil microbes (Table 4 and 5).

Ponnuswamy *et al.,* (1997) reported that the application of various weedicides in soybean affected the bacterial population in soil. All the plots treated with weedicides gave depressing fungal population in first few days of application and regained its population after long period. Similar observations were recorded by Anonymous (1971) and Mukhopadhyay (1980).

Fungicides are used extensively in modern agriculture for the control of fungal pathogens. The chemicals alter the number and activity of microorganism and thus affect biochemical processes and fertility of the soil (Wainwright, 1978). Majority of biochemical transformation in soil results from microbial activity. Fungicides treated soil harboured less population of fungi in comparison to control. This is in conformity with earlier reports of several authors (Colinas *et al.*, 1994; Sukla *et al.*, 1987).

Investigations were carried out on the effects of Cu and other heavy metals on soil microorganisms, invariably indicated the adverse effects of these elements on the soil microbial activity, number and population as reported earlier by various workers (Olson and Thornton, 1982; Duxbury and Bicknell, 1983; Maliszewska *et al.*, 1985; Hiroki, 1992; Chander and Brookes, 1991, 1993; Hattori, 1992; Huysman *et al.*, 1994; Doelman and Haanstra, 1984; Aoyama and Nagumo, 1996; 1997). For example excessive heavy metal concentration in the soil has been reported to cause a decrease in microbial population (Hicks *et al.*, 1990; McGrath *et al.*, 1995), changes in populations structure (Chaudri *et al.*, 1993; Bardgett *et al.*, 1994; Huysman *et al.*, 1994) and physiological activity (Bitton and Dutka, 1986; Cotrufo *et al.*, 1995; Valsecchi *et al.*, 1995).

Copper is known to be very toxic to microorganisms in the free ionic form especially Cu^{2+} and $CuOH^{+}$ (Zevenhuizen *et al.*, 1979). The addition of copper to soil was reported to significantly decrease the amount of microbial biomass and exert a pronounce toxic effect on the size of biomass compared to certain metals such as Pb and As when compared on a molarity basis (Aoyama and Nagumo, 1997).

Effect of fungicide, weedicide and algicide application on plant growth parameters under field conditions

Application of $CuSO_4$ significantly increases the root length (22 cm/plant), shoot length (108 cm/plant) and grain yield (4962.5 kg/ha) than the other treatments (Table 6). Horii *et al.*, (2007) reported that the germination percent of fungicide treated seeds got increased with increase in concentration of fungicide. Various other studies also found that seed germination was stimulated by thiamethoxam in soybean, pea and corn (Cataneo *et al.*, 2010).The shoot length was increased with increase in concentration of fungicide. Windham & Windham (2004) reported that systemic fungicides, which are based on sterol biosynthesis inhibitor, are closely related to plant growth regulators, the use of which at higher than labeled rates shorten the internodes which may lead to slow shoot growth. Vigor index of fungicide treated seedlings got increased with increase in concentration of fungicide. The increase in vigor index was significant at almost all the concentration of fungicide as compared to control. These present findings supports Doyle *et al.*, (2001) proved that seedlings treated with thiamethoxam had a particular advantage of improved seedling vigor. Csinos (2004) revealed that mefenoxam improved vigor index of tobacco. All herbicidal treatments significantly increased yield and yield components like seed yield, test weight, pod dry weight, number of pods / plant, number of seeds / plant, harvest index (Amarogouda *et al.*, 2013).

Copper is one of the essential micronutrients for plant growth and involved in numerous physiological functions as a component of several enzymes, mainly those which participate in electron flow, catalyze redox reactions in mitochondria and chloroplasts (Lolkema and Vooijs, 1986; Harrison *et al.*, 1999; Hansch and Mendel, 2009). However, in large amount copper becomes toxic as it interferes with photosynthetic and respiratory processes, protein synthesis and development of plant organelles (Agarwala *et al.*, 1995; Upadhyay and Panda, 2009). Specifically, excess copper can cause chlorosis, inhibition of root growth and damage to plasma membrane permeability, leading to ion leakage (Ouzounidou *et al.*, 1992; Berglund *et al.*, 2002; Bouazizi *et al.*, 2010).

Pankaj Giri *et al.*, (2014) findings suggest that Cu fluxes affect the growth and yield of wheat plants. Application of Cu in low amount may promote growth, pigments synthesis, protein and sugar contents while in excess amount it adversely affects the yield and other growth parameters.

Table.1 The physicochemical and microbiological populations of the experimental field soil

Soil properties	Mean ± SE
Soil type	Clay loam
pH	7.8± 0.09
EC (dSm^{-1})	0.04± 0.15
Organic carbon (%)	0.50 ± 0.01
Available N (%)	162± 2.88
Available P (%)	35± 0.57
Available K (%)	250± 1.45
Total bacteria (cfu x 10^5 /g drwt of soil)[a]	46 ± 1.15
Fungi (cfu x 10^3 /g drwt of soil)[b]	2 ± 0.16
Diazotrophs (cfu x 10^4 /g drwt of soil)[c]	21 ± 1.15

Table.2 Effect of weedicides, fungicides and algicides application on algae growth under laboratory/field conditions

Treatments	Recommended dose /ha	Laboratory conditions (dosage: 2ml/plate)	Field conditions
T_1-Bispyriphos sodium	20 g	No inhibition	No inhibition
T_2 – Almix	20 g	No inhibition	No inhibition
T_3 –Pyrosulfuran	200 g	No inhibition	No inhibition
T_4 - Londox power	10 Kg	Moderate inhibition	Moderate inhibition
T_5 -$CuSO_4$	2.5 Kg	Moderate inhibition	Inhibition
T_6 –CaO	25 Kg	No inhibition	No inhibition
T_7 -$CuSO_4$ + CaO	2.5 kg + 25 Kg	Moderate inhibition	Moderate inhibition
T_8 –Butachlor	2.5 lit	No inhibition	No inhibition
T_9 –Propiconazole	250 ml	Moderate inhibition	No inhibition
T_{10} -Hexaconazole	250 ml	Moderate inhibition	No inhibition
T_{11}– control	-	No inhibition	No inhibition

Table.3 Effect of weedicides, fungicides and algicides application on available N, P and K content of the soil infected with green algae under wet land rice (ADT 43) ecosystem

Treatments	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
T_1–Bispyriphossodium	831.25	75	775.0
T_2 – Almix	385.0	58	462.5
T_3 –Pyrosulfuran	317.5	117	287.5
T_4 - Londox power	332.5	95	400.0
T_5 -$CuSO_4$	280.0	64	437.5
T_6 –CaO	202.5	104	262.5
T_7 -$CuSO_4$ + CaO	177.5	142	350.0
T_8 –Butachlor	650.0	181	237.5
T_9 –Propiconazole	332.5	72	512.5
T_{10} -Hexaconazole	280.0	83	225.0
T_{11} -control	260.0	34	400.0
SEd	26.13	6.25	25.69
CD(P =0.05)	54.89	13.15	53.97

Table.4 Effect of weedicides, fungicides and algicides application on microbial population in the rhizosphere of soil cropped with rice (ADT 43) under wet land rice ecosystem

Treatments	Bacteria (x 10^4cfu/g)			Fungi (x 10^3cfu/g)			Actinomycetes (x 10^2cfu/g)		
	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP
T_1 - Bispyriphos sodium	27	15	8	5	3	0	24	15	7
T_2 - Almix	34	28	20	20	12	5	18	12	5
T_3 -Pyrosulfuran	30	24	12	12	8	3	13	9	4
T_4 - Londox power	32	27	20	22	15	7	10	7	5
T_5 -$CuSO_4$	40	32	15	27	17	11	27	19	8
T_6 -CaO	32	25	17	25	15	8	25	15	6
T_7 -$CuSO_4$ + CaO	35	28	22	18	12	4	27	18	12
T_8 -Butachlor	34	26	15	25	16	9	23	14	11
T_9 -Propiconaole	28	1 7	8	22	14	6	21	11	7
T_{10} -Hexaconazole	32	23	11	20	12	7	19	8	6
SEd	1.93	1.49	0.93	1.23	0.79	0.41	1.20	0.75	0.44
CD (P =0.05)	4.06	3.13	1.97	2.59	1.66	0.85	2.53	1.59	0.92

Table.5 Effect of weedicides, fungicides and algicides on beneficial microbial population in the rhizosphere of soil cropped with rice (ADT 43) under wet land rice ecosystem

Treatments	Phosphobacteria ($x\ 10^4$cfu/g)			*Azospirillum* ($x10^5$ MPN/g)			*Pseudomonas* ($x10^4$cfu/g)		
	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP
T_1 - Bispyriphos sodium	15	10	4	1.2	0.9	1.0	18	7	5
T_2–Almix	25	18	10	1.4	0.8	0.1	25	20	18
T_3 -Pyrosulfuran	18	12	6	2.5	1.4	0.5	25	20	12
T_4 - Londox power	26	20	10	2.5	1.8	0.6	23	18	11
T_5 -$CuSO_4$	30	25	12	2.8	1.8	0.9	40	32	22
T_6 -CaO	28	20	12	2.2	1.5	0.1	30	25	16
T_7 -$CuSO_4$ + CaO	27	20	12	1.4	0.8	0.1	36	30	22
T_8 -Butachlor	20	14	7	2.5	1.6	0.6	35	28	15
T_9 -Propiconaole	17	12	6	1.8	1.5	1.0	24	22	11
T_{10} -Hexaconazole	12	6	2	1.4	0.8	0.5	20	15	8
SEd	1.35	1.02	0.52	0.12	0.08	0.042	1.66	1.33	0.86
CD (P =0.05)	2.84	2.14	1.11	0.26	0.017	0.087	3.49	2.80	1.81

Table.6 Effect of the fungicides, weedicides and algicides application on root length, shoot length and grain yield of rice (ADT 43) under wet land rice ecosystem

Treatments	Root length (cm/plant)			Shoot length (cm/plant)			Yield (Kg/ha)
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	
T_1 - Bispyriphos sodium	11.0	19	9.0	53.5	73.5	93.5	3568.0
T_2–Almix	12.5	17.0	8.0	54.0	74.0	94.0	3913.0
T_3 -Pyrosulfuran	14.0	18.0	10	52.5	72.5	92.5	4887.5
T_4 - Londox power	12.0	16.0	8.0	53.5	73.5	93.5	3762.5
T_5 -$CuSO_4$	18.0	22.0	14	68.0	88.0	108.0	4962.5
T_6 -CaO	15.0	19.5	11	52.5	72.5	92.5	4837.5
T_7 -$CuSO_4$ + CaO	16.5	21.0	11	61.5	81.5	101.5	4487.5
T_8 -Butachlor	14.0	17.0	8.0	60.5	80.5	100.5	4775.0
T_9 -Propiconazole	13.0	18.0	9.0	57.5	77.5	97.5	3468.0
T_{10} -Hexaconazole	11.0	16.0	8.0	48.5	68.5	92.5	4712.5
SEd	0.81	1.08	0.57	3.35	4.54	5.75	3.807
CD (P =0.05)	1.71	2.27	1.21	7.05	9.54	12.089	7.999

Table.7 Physical parameters of the soil sample at Maharajapuram field

Date of sampling	EC (dSm^{-1})	pH	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)
16.10.15	0.63	8.2	63.0	25	90
05.11.15	0.16	8. 1	32.2	50	75
25.11.15	0.14	8.0	46.2	38	155

Table.8 Effect of $CuSO_4$ application on algae growth under field conditions

Treatments	Dosage/ha	Aduthuraifield condition	Maharajapuram field condition
T_1 -Alternate wetting and thawing the algal growth with conoweeder if algal growth occurred	-	Inhibition	Moderate inhibition
T_2 - $CuSO_4$	Soil application– 1 Kg	Inhibition	Moderate inhibition
T_3 - T_1 + T_2	As indicated Above	Inhibition	Moderate inhibition
T_4 - $CuSO_4$	Soil drenching-0.5%	Moderate inhibition	Moderate inhibition
T_5 -$Cu(OH)_2$	Soil application – 1 Kg	Moderate inhibition	No inhibition
T_6 -T_1 + T_5	As indicated Above	Inhibition	No inhibition
T_7 -$Cu(OH)_2$	Soil Drencing-0.5%	Moderate inhibition	No inhibition
T_8 -Londox power	Soil application – 1 Kg	Moderate inhibition	No inhibition
T_9 -T_1 + T_8	As indicated Above	Inhibition	No inhibition
T_{10} -Londox power	Soil drenching -0.5%	Moderate inhibition	No inhibition
T_{11} -Soil extract with cell free extract containing Rhamnolipids from *Pseudomonas chlororamphis*	10%	No inhibition	No inhibition
T_{12} -Control	-	No inhibition	No inhibition

Table.9 Physical parameters of the water sample at Maharajapuram field

Date of sampling	pH	EC (dSm^{-1})	HCO_3 (ppm)	S (ppm)	Cl (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	K (ppm)
16.10.15	8.2	1.40	5.2	0.138	7.4	3.4	4.5	5.2	0.1
05.11.15	8.0	1.38	4.8	0.125	7.1	3.1	4.2	5.4	0.1
25.11.15	7.9	1.11	4.4	0.63	4.7	2.9	2.8	5.0	0.1

Fig.1 Green algae infected field

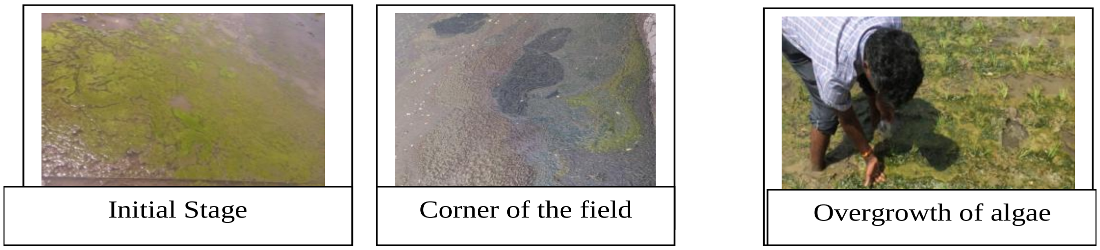
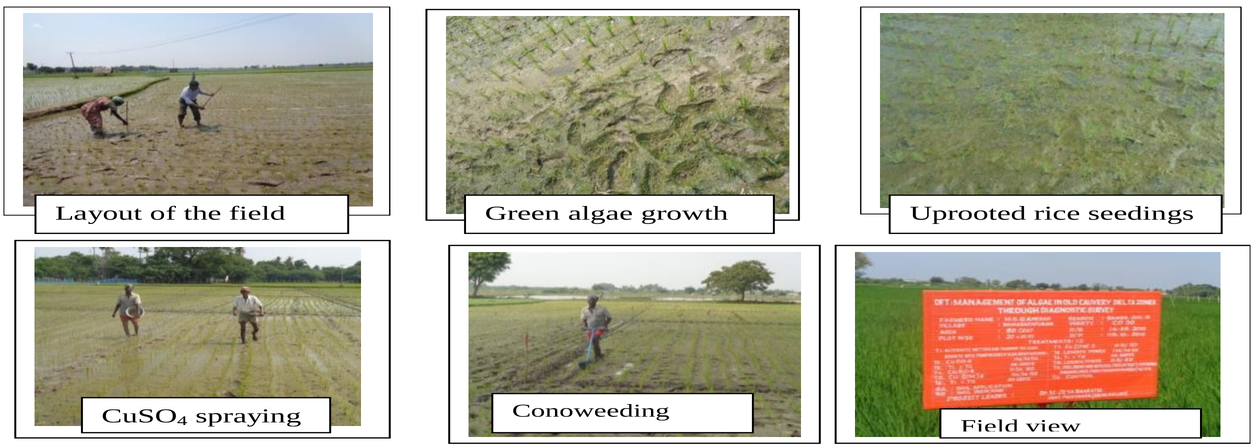
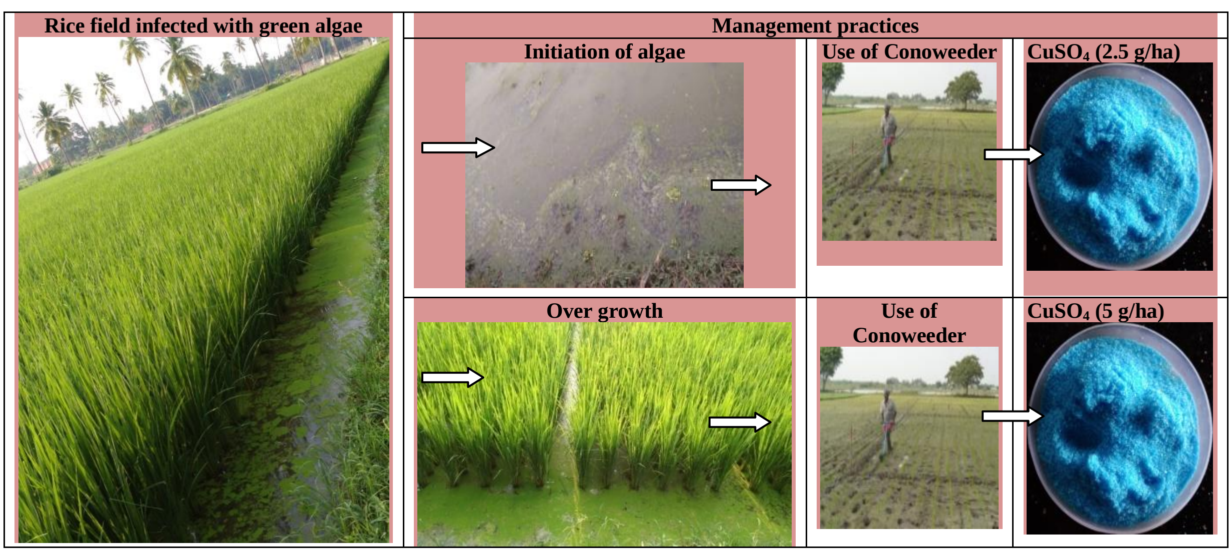


Fig.2 Overview of the field experiment



Graphical Abstract



Effect of $CuSO_4$ application on green algae growth at Aduthurai

The results of the confirmatory field experiment at Aduthurai revealed that by simple physical management practices like alternate wetting and drying, manual trampling of algae and use of conoweeder reduces the green algae growth (Table 6). Repeated stagnation of bore well water increases the phosphorus content in soil leads to the stimulation of algae spore. When stimulation of algae spores observed, water was completely drained, $CuSO_4$ drenching at the rate of 2.5 kg/ha was done. But during tremendous algae growth, $CuSO_4$ drenching at the rate of 5.0 kg/ha was done. In most cases action to eradicate green algae is taken only when filaments are present in huge numbers and the treatments available are limited. Generally Australian farmers use water control, bluestone ($CuSO_4$) and coptrol (2. 5 lit/ha), both of which are drastic answers to a problem where a lot of damage has already been done (Hrudey *et al.*, 1999, Jones and Burch, 1997; Jones and Orr, 1994 and http//www.affa.gov.au/nra/welcome.html accessed January 2002).

PAK™27 (BioSafe Systems, 2008 and Hazardous Substances Data Bank, 2009) is a granular product that attacks planktonic and filamentous algae on contact. The active ingredient is sodium carbonate peroxyhydrate and creates a powerful oxidation reaction that destroys algal cell membranes and chlorophyll providing immediate control of algae. Fast acting within 60 seconds of application and leaves behind no harmful residues and adds 13% bio-available oxygen to the water.

Effect of $CuSO_4$ application at Maharajapuram rice field

The results of confirmatory field experiment at Maharajapuram showed that the algae growth was not completely arrested even after 3rd day of $CuSO_4$ application. Hence, the soil and water sample were collected from the Maharajapuram field to know the physical parameters of the soil. Maharajapuram soil and water consists of alkaline pH (8.2) and more HCO_3ions (5.2). This is the main cause of overgrowth of green algae which was not controlled by recommended quantity of$CuSO_4$ (5 kg/ha) application. This was supported by the following findings. In culture media, the optimal pH for the growth of cyanobacteria ranges from 7.5 – 10, with a lower limit of 6.5 – 7.0. However, in soil culture experiments, soils having slightly alkaline reaction were more favourable, while in natural environments cyanobacteria prefer neutral to alkaline pH (De, 1939 and Roger and Reynaud, 1979).

In this regard pH is one of most important factor which affects the presence of BGA in a habitat. Saline and Sodic (alkali) nature of soils significantly reduce the value and productivity of affected lands. Salt affected soil are divided into three groups depending on the total soluble salts (measured in the terms of Electrical Conductivity E. C.), soil pH and Exchangeable Na %. Usar soils are grouped into two divisions Saline (Solanchak) and Alkalines (Solonetz). (Pandey *et al.*, 2005)

It is concluded that soil is consider as a buffer that can tolerate the pH change. Even though continuous dumping of DAP and other chemical fertilizers changes the soil pH, innate capacity and fix more amount of the salt in the soil, which indeed difficult by reclamation practices ($CuSO_4$). In this situation continuous treatments like gypsum (bring the pH in acidic), infiltration with water (leach excess ions *viz*. Na, HCO_3), biofertilizers treatment (to move the accumulated P) and periodical soil and water sample analysis are important. It is always advisable to prevent the excess algae growth by fertilizer and water management practices rather than control, since chemicals are toxic to soil microorganisms. However, when situation warrants, application of 2.5 kg of $CuSO_4$/ha when the growth of algae is mild and 5.0 kg/ha when severe algae growth is noticed is the best management practices.

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