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Agrochemicals influencing nitrogenase, biomass of N_2-fixing cyanobacteria and yield of rice in wetland cultivation

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ABSTRACT

Cyanobacteria maintain soil fertility by performing N_2-fixation and act as a key biocatalyst in nitrogen cycle. Chemical N-fertilizers and pesticides as agrochemicals are intensively being used in rice farming to boost rice production, this work deals with the first hand information on their influence on native N_2-fixing cyanobacteria, which play an important role in maintaining soil health. A field study was conducted for three consecutive seasons in water logged rice field to observe the influence of agrochemicals, urea, benthiocarb and carbofuran in isolation and in combinations on biomass, acetylene reduction activity (ARA) and N-yield of native cyanobacteria as well as, on growth and yield of rice. The ARA and N-yield followed almost same trend. It is discernible that both urea and benthiocarb had deleterious effects whereas, carbofuran was promoting effects on cyanobacterial growth, ARA and N-yield. The combination of all the three above agrochemicals was found inhibitory, but inhibition was comparatively less than that of urea or benthiocarb in isolation or urea plus benthiocarb treatments. It is concluded that the combination of agrochemicals was toxic, in comparison to the control, but was better than application of urea N or benthiocarb alone or with their combinations. It was recorded that along with rice straw and gain yields, panicle numbers were the maximum at the combination with treatments of benthiocarb+carbofuran. Adverse effects of used agrochemicals on cyanobacteria in wetland rice cultivation could be avoided by a prudent use of chemical N-fertilizers and pesticide(s) in combination.

1. Introduction

Rice is widely cultivated in wetlands having high temperature, low soil organic carbon and high humidity. Such environmental conditions are favorable for cyanobacteria mainly due to their ability to fix atmospheric nitrogen and carbon. Cyanobacteria are considered as key biocatalysts in the N_2 cycle (Vitousek et al., 2002; Latysheva et al., 2012). Cyanobacteria were reported as first agent to fix atmospheric nitrogen in flooded rice soils (Singh, 1961) and maintenance of natural soil fertility in such ecosystem was attributed mainly due to these organisms (De, 1939; Singh, 1961). Before introduction of chemical fertilizers, rice has been cultivated year after year without losing soil fertility which was considered mainly due to cyanobacteria and thus role of these organisms in N- economy of rice fields has been widely advocated (Singh, 1961; Venkataraman, 1972; Roger and Kulasooriya, 1980; Swarnalakshmi et al., 2006). With the introduction of high yielding rice varieties, agrochemicals are invariably being used to obtain high yields. Thus, simultaneous or sequential application of agrochemicals such as herbicides, insecticides and nitrogenous fertilizers is indispensible in modern rice farming. But these chemicals affect other plants, animals and microorganisms which prevail in such habitat (Chen et al., 2007; Aktar et al., 2009; Casida, 2009; Geisseler and Scow, 2014). When two or more chemicals are applied together as a mixture or one after another during a cropping season, they interact and cause synergistic, additive or antagonistic responses (Tammes, 1964; Akobundu et al., 1975; Magnusson et al., 2010; Padhy and Rath, 2015). Therefore, information on the interactions of these chemicals or their degradation products with rice field ecosystem is very important. It is particularly relevant in case of cyanobacteria, which are the dominating flora of the tropical rice fields and are known to play important role in building soil organic carbon and nitrogen in the flooded rice ecosystem (Venkataraman, 1972; Singh, 1978; Singh and Bisyoi, 1989; Whitton, 2000; Nayak et al., 2004; Swarnalakshmi et al., 2006). Effects of pesticides on cyanobacteria in laboratory cultures

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were reported earlier (Singh, 1973; Das and Singh, 1978; Tiwari et al., 2001; Chen et al., 2007; Padhy and Rath, 2015). Recently, Das et al. (2015) reported from pot experiment conducted with planted rice that herbicide (butachlor) application adversely affected native and inoculated cyanobacteria whereas insecticide (metacid) application was favorable to them.

The information on the interaction of different agrochemicals with cyanobacteria in paddy fields is inadequate. Therefore, in present investigation, an experiment was performed to analyse the impact of commonly used agrochemicals (i.e. herbicide, insecticide and N-fertilizer) in isolation and in combination for three consecutive seasons on native cyanobacterial biomass, nitrogenase activity in terms of acetylene reduction assay (ARA), cyanobacterial N-yield as well as growth and yield of rice.

2. Materials and methods

2.1. Experimental design and treatments

A field experiment was carried out for three consecutive seasons (two dry seasons and one wet season) with native cyanobacteria in plots of 5 m×2 m size using urea (CH_4N_2O), Benthiocarb (trade name Saturn) (S-(4-Chlorobenzyl) N, N- diethylthiolcarbamate; $C_{12}H_{16}CINOS$) and carbofuran (trade name furadan) (2,2-dimethyl-3H-1-benzofuran-7-yl) N-methylcarbamate; $C_{12}H_{15}NO_3$) as N fertilizer, herbicide and insecticide, respectively. The experiment had a completely randomized block design with three replications and had the following treatments: T_1 control (no urea, benthiocarb, carbofuran); T_2-Urea (60 kg N ha^{-1}); T_3-benthiocarb (1 kg active ingredient (a.i.) ha^{-1}); T_4-carbofuran (1 kg a.i. ha^{-1}); T_5-T_2+T_3; T_6-T_2+T_4; T_7-T_3+T_4; T_8-T_2+T_3+T_4. The treatments T_5, T_6, T_7 and T_8 had the same doses as used in T_2, T_3 and T_4. The soil of experimental plots was classified as coastal alluvium sandy clay loam (Aeric Endoaquept) (Soil Survey Staff, 2010) with 0.68% organic C, 0.07% total N (C: N ratio 9.7), 11 μg g^{-1} available P (Olson), 59.9% P-fixing capacity and pH 6.4. A high yielding rice variety (*Oryza sativa* L.) IR-36 of 120 days duration was used in the experiment. Other experimental details and methodologies adopted in the experiment are given below.

2.2. Season and weather conditions

The experiment was carried out at the farm of Central Rice Research Institute, Cuttack, India. It is situated on the bank of river Mahanadi in Orissa state (Eastern India) at latitude of 25.5°N, longitude of 86°E, altitude of 23.48 m above the mean sea level and is 80 km in the west from the Bay of Bengal in the main rice growing tract of India. During the period of experimentation, average annual rainfall was 1553.2 mm. Mean maximum temperature was 35.3–36.5 °C during May and June, whereas the mean minimum temperature was 13.6–15.1 °C during January and December and relative humidity was 86–97% during July-September months.

2.3. Field preparation and transplanting

The fields were ploughed, cross ploughed, puddled, levelled and thick bounds were raised to avoid mixing of treatments. The healthy and sprouted rice seeds were sown in seed beds and 25–30 days old healthy seedlings were transplanted at the rate of 2 seedlings $hill^{-1}$ with a spacing of 15×10 cm.

2.4. Biocides applications

The half of the N fertilizer (urea at the rate of 60 kg N ha^{-1}) was applied as a basal dose during puddling and a quarter each at 25 and 45 days after transplanting (DAT). The pre-emergence herbicide benthiocarb (1 kg a.i. ha^{-1}) having intergenic selectivity between rice plants and graminaceous weeds was applied in one dose at the time of puddling whereas insecticide carbofuran (1 kg a.i. ha^{-1}) a systematic and contact poison acts as miticide was applied in two equal splits at 25 and 45 DAT. The P fertilizer (20 kg P ha^{-1}) was applied in 3 equal split doses at a weekly interval after transplantation (Bisyoi and Singh, 1988a).

2.5. Growth of native cyanobacteria in experimental field

The rice fields of CRRI farm harbour N_2-fixing cyanobacteria abundantly and the dominating species mostly belonged to genera *Aulosira, Aphanothece, Gloeotrichia, Anabaena* and *Nostoc*. To promote their growth superphosphate at a rate of 20 kg P ha^{-1} was applied in three equal splits at 7 days interval (Bisyoi and Singh, 1988a).

2.6. Collection and processing of cyanobacteria samples and measurement of their growth

Ten cyanobacterial samples were randomly collected from each plot with a metallic quadrate (25 cm×25 cm×25 cm) having both ends open (Singh, 1961). The cyanobacterial biomass inside the quadrate was collected by passing the flood water through two layers of cheese cloth. The samples were then washed several times with water to remove the contaminating materials and blotted with the help of blotting paper to remove excess water and utmost care was taken to remove green algae and other contaminants. However, in such field studies, total contaminants cannot be removed and dominating biomass has to be taken into consideration for further study. The fresh weight of the cyanobacterial biomass was recorded immediately after collection and 20 g fresh material was dried in an oven at 80 °C for 24 h for determination of dry weight. From this, the total dry weight was computed and the dry weight of the sample was expressed in kg ha^{-1} (Bisyoi and Singh, 1988a, 1988b).

2.7. Measurement of nitrogenase activity (acetylene reduction activity; ARA)

The nitrogenase activity (ARA) was measured following the method of Reddy and Roger (1988). Ten soil-water core samples were randomly collected within each plot with the help of glass tubes of 1.8 cm diameter and 10 cm length. The flood water along with top 0.5 cm of the soil core suspension was adjusted with the distilled water to make 254 mL, a volume that equals to 10 times (in cm^2) of the soil surface cored by 10 samples, giving a dilution of 10^{-1} on the surface basis. The suspension was then stirred at 400 rpm for 30 min to disrupt the cyanobacterial clumps and serially diluted to 10^{-3} dilution. 5 mL of the diluted sample was incubated in the field with 10% acetylene (v/v) for 1 h. After incubation, 0.5 mL of the gas mixture was injected into the gas chromatograph (AIMIL-5500 series, Nucon Engineers, New Delhi). The amount of ethylene produced was expressed in nmole C_2H_4 cm^{-2} h x 10^{-3} (Schollhorn and Burris, 1966; Stewart et al., 1968).

2.8. Measurement of cyanobacterial N-yield

The total N content (%) of cyanobacteria was estimated by the micro-kjeldahl method (Jackson, 1973). 100 mg of dried cyanobacterial samples were taken in duplicate in 100 mL kjeldahl flasks to which a pinch of digestion mixture (K_2SO_4, $CuSO_4$ and SiO_2 in a 100:10:1 ratio) and 2 mL of concentrated H_2SO_4 (36N) were added. The flasks were heated in the digestion chamber for 2–3 h and the digested samples were used for determination of N content. The N-yield was calculated by multiplying the N content with the dry weight and expressed in kg N ha^{-1}.