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Foliar application of red seaweed (*Kappaphycus alvarezii*) bioformulations increased the levels of chlorophyll content in rice

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Abstract

Rice is an important food crop cultivated all over the world. India occupies first and second position in area and production respectively. This study aimed at revealing the role of red seaweed (*Kappaphycus alvarezii*) bioformulations (LBS-6 and LBD-1) in increasing the chlorophyll content of rice. Results have shown that seaweed bioformulations improves the efficiency when used in combination with fungicides. physiological parameter like chlorophyll content was elevated in leaves which were sprayed with LBS6 (38.50 SPAD value) which was T_{10} (carbendazim 50 %WP @ 0.6 g + LBS6 @ 1ml/lit).

Keywords: LBS-6, LBD-1, chlorophyll content, SPAD meter

Introduction

Agricultural growing practices have been evolving towards organic, sustainable or environmental friendly systems. The aim of modern agriculture is to reduce inputs without reducing the yield and quality. Organic molecules like seaweed extracts are able to activate plant metabolism and allow an improvement in plant performance in a short period of time and in a cheaper way. Seaweed extracts are macroalgae extracts and contain a wide range of bioactive compounds that are mostly still unknown. Many of these extracts are also able to counteract the effect of biotic and abiotic stresses, enhancing quality and crop yield by stimulating plant physiological processes (Ziosi *et al*. 2013) [14].

Rice (*Oryza sativa* L.) is an important cereal crop and staple food for large population of the world especially in South and Southeast Asia. The global annual demand for rice will be around 800 million tons by 2025 in view of expected increase in population. More than 90 per cent of the world's rice is grown and consumed in Asia. In this region it represents a high-value commodity crop. The global production of rice has been estimated to be 697.22 million tons with productivity of 4.4 tons ha^{-1} is being harvested from 158.43 million ha annually, producing 21 per cent of world's food calorie supply (FAO, 2012) [7].

Biotic and abiotic stresses are the primary constraints in rice production worldwide. These stresses are responsible for large-scale crop loss each year and with the predicted climate change, such losses are expected to increase. Therefore, research for sustainable approaches to alleviate these stresses is a high priority (Bird *et al.*, 2016) [2].

Seaweed extracts as organic molecules are emerging as commercial formulations for use as plant growth promoting factors and a method to improve tolerance to stress. These extracts target a number of pathways to increase tolerance under stress. Seaweeds are red, green and brown macroalgae that represent 10 per cent of marine productivity (Van Oosten *et al.*, 2017) [12]. Currently, there are over 47 companies producing and marketing various algal extracts for agricultural use. The majority of the formulations are from the red and brown algae. Tropical red seaweeds are macro algae, able to activate plant metabolism and improve plant performance in a short period of time which is a cost effective.

The effects of seaweed extracts have generally been attributed to the presence of phytohormones and a range of organic molecules (Battacharyya *et al.*, 2015) [1] acting as compatible solutes, including betaines (Blunden *et al.*, 2009) [3]. Seaweed extracts are rich in carbohydrates and amino acids (Khan *et al.*, 2009) [8] as well as bioactive secondary metabolites such as vitamins and their precursors (Stasio *et al.*, 2018) [11].

The research of red seaweed extracts and their formulated products on rice is very limited in India and mainly done in the plain land rice ecosystems (Devi and Mani, 2015) [5]. However, the effects of *K. alvarezii* on rice with respect to induction of increased levels of chlorophyll content not been studied.

Therefore, the current investigations were carried out to know the biological effects of seaweed bioformulations (LBD1 and LBS6) obtained from tropical red seaweed (*Kappaphycus alvarezii*) in increasing the levels of chlorophyll content.

Material and Methods

Experimental design of field trial

In order to determine the possible effect of seaweed bioformulations in increasing the levels of chlorophyll content, field experiment was conducted during the *Kharif* 2016-17 and at ZARS, V.C Farm, Mandya. Rice variety IR-64 susceptible to blast disease was used for field trial. The net plot size of 5 m^2 was maintained for each replication with 20 cm distance between rows and 15 cm between plants. A total of 13 treatments were selected and experiment was laid out as per Randamised Compleate Block Design (RCBD).

Stress induction in field

Fungicidal stress was induced by spraying of selected fungicides at an interval of 15 days to rice in field condition. Observations pertaining to chlorophyll content was assessed at different intervals of the treatments (before the spray, 4 and 8 days after the spray). A total three sprays were taken, observations were analysed from the leaf samples collected after each spraying.

Table 1: Treatment details of field trial

Treatments	Treatment details	Dosage/l
T_1	Tricyclazole 75 %WP	0.6g
T_2	Carbendazim 50 %WP	1.0 g
T_3	LBS6	1.0 ml
T_4	LBD1	2.0 ml
T_5	Tricyclazole 75 %WP + LBS6	0.6g + 1.0 ml
T_6	Tricyclazole 75 %WP + LBS6	0.4g + 1.0 ml
T_7	Tricyclazole 75 %WP + LBD1	0.4g + 1.0 ml
T_8	Tricyclazole 75 %WP + LBD1	0.4 g + 2.0 ml
T_9	Carbendazim 50 %WP + LBS6	1.0g + 1.0 ml
T_{10}	Carbendazim 50 %WP + LBS6	0.6g + 1.0 ml
T_{11}	Carbendazim 50 %WP + LBD1	0.6 g + 1.0 ml
T_{12}	Carbendazim 50 %WP + LBD1	0.6 g + 2.0 ml
T_{13}	Untreated check	Water spray

Estimation of Chlorophyll Content

Chlorophyll content of stressed and unstressed plants was measured using SPAD (Konica minolta) chlorophyll meter before and after the treatments impose. Younger leaves were selected and tagged for estimation of chlorophyll at different interval. Randomly five leaves were tagged and observed for chlorophyll difference at 0, 4 and 8 days after the spray in each sprayings.

Results and Discussion

Effect on chlorophyll content

Chlorophyll content was estimated at different intervals of spraying. In the first season chlorophyll was estimated using SPAD meter for all the three sprays at different interval. There was a significant difference in the chlorophyll content before and after spray. Seaweed treated and/or combinations of fungicides and seaweed treated plots had higher chlorophyll content in all the three sprays compared to the untreated control (Table 2).

Chlorophyll content was reduced after 3 and 6 days after spray. Chlorophyll content of different treatments were compared with before the spray. After first spray highest chlorophyll content was recorded in T_{10} (Carbendazim+LBS6 (0.6g+1ml/l (40.67SPAD value**)** treated plants. LBD1@ 2 ml/l sprayed treatment showed 40.42 SPAD value after 8 days of spray.

However chlorophyll content was low in leaves which were treated with fungicide alone. In 2^{nd} spray, higher chlorophyll content was recorded in LBS6 treated leaves (35.14 SPAD value) after 8 days of spray, whereas 33.59 SPAD value was recorded before the spray in same treatment. Whereas LBD-1 has increased the chlorophyll content when sprayed with tricyclazole (after 8 days 35.22) compared to before spray (30.74).

In 3^{rd} spray chlorophyll content in LBS6 treated leaves was 38.50 SPAD value which was on par with T_{10} (carbendazim 50 %WP @ 0.6 g + LBS6 @ 1ml/lit). LBS 6 treated plot had significantly higher chlorophyll content in all the sprays compared to the untreated control. In LBD1@ 2 ml/l treated plants recorded the SPAD value of 36.45 which was higher than chlorophyll content before the spray (35.70 SPAD value).

Foliar spray of seaweeds bioformulations with combination of fungicides decreased the stress induced by increasing and stabilizing the chlorophyll content. The increased chlorophyll content is due to increased sugar biosynthesis and it was also associated with net photosynthesis and quantum efficiency of photosystem II (Ertani and Nardi, 2013) [6]. Several studies have been reported the effect of seaweeds on increasing the chlorophyll content (Michalak *et al.*, 2017; Wang *et al.*, 2017) [9, 13].

The The seaweed *Ulva lactuca* extracts with high carbohydrate content stimulated the seedling growth and increased chlorophyll a content in mung bean (Castellanos-Barriga *et al.*, 2017) [4].

Rayirath *et al.* (2009) [8] reported the factor for the reduction in chlorophyll degradation rate which was attributed to reduced expression of the chlorophyll degrading gene (AtCLH1 and AtCLH2) chlorophyllase in seaweeds treated plants. Plants exhibited reduced chlorophyllase enzyme activity in cold temperatures, and the plants had three times the chlorophyll content when compared to water treated plants.

Table 2: Effect of seaweed bioformulations on chlorophyll in rice during *Kharif*-2016-17

Treatments	Treatment details	1st spray			2nd spray			3rd spray		
		Chlorophyll(SPAD value)			Chlorophyll (SPAD value)			Chlorophyll (SPAD value)		
		Before	4 DAS	8 DAS	Before	4 DAS	8 DAS	Before	4 DAS	8 DAS
T_1	Tricyclazole @ 0.6 g/l	42.20	38.38	39.40	35.16	32.38	33.60	34.55	31.79	36.75
T_2	Carbendazim @ 1 g/l	40.92	38.35	39.40	33.26	31.89	33.05	35.43	33.88	36.89
T_3	LBS6@ 1ml/l	41.37	39.25	39.66	33.59	32.45	35.14	34.81	32.65	38.50
T_4	LBD1@ 2 ml/l	39.74	38.97	40.42	32.50	30.62	34.14	35.70	33.54	36.45
T_5	Tricyclazole+LBS6 (0.6g+1ml/l)	39.11	37.28	38.90	29.01	28.42	32.53	35.47	33.97	35.71
T_6	Tricyclazole+LBS6 (0.4g+1ml/l)	38.51	36.13	40.48	30.05	28.69	32.13	35.50	34.11	37.77
T_7	Tricyclazole+LBD1 (0.4g+1ml/l)	39.16	37.05	40.40	30.74	32.99	35.22	35.72	34.42	37.34
T_8	Tricyclazole+LBD1 (0.4g+2ml/l)	38.85	33.67	39.86	31.51	29.03	32.72	35.50	35.53	36.91
T_9	Carbendazim+LBS6 (1g+1ml/l)	37.44	34.10	40.70	28.98	27.44	33.87	34.66	34.62	37.29
T_{10}	Carbendazim+LBS6 (0.6g+1ml/l)	36.37	32.95	40.67	30.63	28.86	32.40	35.53	34.99	37.90
T_{11}	Carbendazim+LBD1 (0.6g+1ml/l)	40.50	36.20	41.33	30.21	29.00	32.82	36.64	35.52	35.82
T_{12}	Carbendazim+LBD1 (0.6g+2ml/l)	41.54	35.07	39.92	32.03	30.30	32.66	34.49	34.47	35.41
T_{13}	Untreated check	36.20	36.12	36.80	29.57	29.65	30.52	35.22	33.81	35.99
	SEm±	0.92	0.75	1.01	1.38	0.82	1.35	0.91	0.74	0.82
	C.D at 5%	2.82	2.20	2.95	4.02	2.41	3.94	2.81	2.10	2.41

DAS: Days after spray

Conclusion

The findings of current study clearly showed that seaweed bioformulations can act as anti-stress agents in relieving fungicidal stress by regulating the chlorophyll content. These seaweed bioformulations can be included in organic farming. Seaweed bioformulations not only reduce the usage of pesticides but also counteract the disease and maintain good plant health.

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