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Bioefficacy and Residue Studies of Fantac (Biostimulant) in Rice Crop under Sub-Tropical Conditions

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ABSTRACT

Field investigations were conducted for three seasons from July 2006-2007 *to test the efficacy of Fantac* (*N-ATCA*) *in transplanted rice and also determine its harvest time residues in paddy grain*, *straw*, *husk and soil by HPLC. It was applied at the rate of* 25, 50, 75 *and* 100 *mL*/100 *L respectively. The results revealed that foliar spray of this agrochemical on rice crop did not produce any significant effect on the general growth parameters but increased the number of grains/panicle. The residues of fantac were found to be* < 0.05 *μg/g at the time of harvest which are below MRL and thus Fantac can be considered as environmentally safe.*

***Keywords*:** *Fantac*, *Biostmulant*, *Harvest*, *Residues*, *HPLC*

1. Introduction

Biostimulants are referred to as positive plant growth regulators or metabolic enhancers which when applied in small quantities enhance the growth and overall development of plants. One such biostimulant is Fantac, a mixture of 5% N-Acetyl thiazolidine carboxylic acid (N-ATCA) and 0.1% folic acid has been recently introduced by Coromandel Fertilizers, Secunderabad, India for attaining higher values of yield attributes in rice. It is a stabilizer buffer which on application to plants helps them to sustain stresses more effectively and enhance crop yields both in quality and quantity [1-3]. It also produces specific effects on the physiological processes of plants [4].

Though biostimulants serve to be very effective in plant growth and enhancing crop yield, but since soil acts as an ultimate sink for all the chemicals applied on it, therefore even slightly higher concentrations of any chemical can harm the next crop in rotation or may also leach down to contaminate ground water resources. Recommendations for the use of agrochemicals on a crop cannot be made until its residue studies have been carried out [5]. Hence a detailed study was therefore conducted to evaluate the effect of Fantac on rice and also estimate the harvest time residues of this biostimulant in paddy (grain, straw, husk) and soil with a view to ensure human and environmental safety.

2. Materials and Methods

2.1. Procurement of Chemicals

The technical grade Fantac (99.9% purity) was obtained from M/S Coromandel Fertilizers Pvt. Ltd., Secunderabad, India. All the other reagents and chemicals were procured from Merck, India Ltd.

2.2. Field Experiment

Bioefficacy field trials were conducted for three seasons during the year 2006-2007 on rice (cv. Pant Dhan-4) in randomised block design at Crop Research Centre, G.B. Pant University, Pantnagar with three replications and five treatments including untreated control. The crop was was sowed in 6 m × 6 m plot in June 2006 and the transplanting was done after 30 days of sowing. Application of Fantac at the rate of 25, 50, 75 and 100 mL/100 L water was done using a knapsack sprayer at tillering stage (I^{st} application), panicle initation stage (2^{nd} application) and 15 days after the second application (last application).

The observations for the plant height, tillers per plant, panicle length, grains per panicle, grain and straw yield were taken. The phytotoxicity of fantac was also determined as per CIB guidelines on 0-10 scale, by comparing the toxicity symptoms on fifty days old plants from the treated and untreated plots.

For residue studies the samples of paddy grain, straw, husk and soil treated with Fantac at the rate of 75 and 100 mL/100 L water were collected from each plot at the time of harvest and extracted for its residues.

2.3. Extraction and Cleanup

Paddy samples: The well ground rice grain, chopped straw and husk samples (50 g each) in triplicate were extracted twice with methanol and filtered. The combined filterate was partitioned with isooctane and 0.01% t-butanol in dichloromethane mixture. The aqueous phase was extracted twice and passed through anhydrous sodium sulfate to remove any traces of water. The organic layer was concentrated in a Rotavapor and the residue was dissolved in methanol: acetonitrile mixture (1:1). The dissolved residue was cleaned up by column chromatography using activated silica gel as an adsorbent. The column was eluted with acetonitrile: methanol mixture (1:1) and again evaporated to dryness followed by reconstitution of the residue in the mobile phase.

Soil: Soil samples were air dried and sieved through a 2 mm sieve. Representative samples (50 g) were extracted twice with methanol and filtered. The combined filterate was subjected to liquid partitioning with isooctane and 0.01% t-butanol in dichloromethane mixture and then cleaned up by passing through a column packed with silica gel. The column eluent was dried and dissolved in the mobile phase for HPLC analysis.

Recovery studies: Recovery studies were also performed by spiking all the samples with two levels of fortification of Fantac (0.05 μgg^{-1} and 0.5 μgg^{-1}) and adopting the same procedure as discussed above in order to validate the efficiency of the method of the analytical method. The samples were extracted and cleaned up following the procedure described in preceding section.

2.4. Residue Analysis

A Water's HPLC system with varying wavelength detector was used for chromatographic analysis. The operating parameters were column: C-18, mobile phase acetonitrile sodium dihydrogen orthophosphate buffer (6:94 v/v), pH 2.5 in isocratic mode at a flow rate of 1.0 mL/min. and detection at 210 nm.

3. Results and Discussion

No phytotoxic symptoms necrosis, epinasty, hyponasty, leaf tip injury, leaf surface injury, wilting and vein clearing were observed in any of the treated plots even for 10 d after the foliar spray of this chemical. The pooled data on the effect of Fantac and other growth promoters on growth, yield attributes and grain and straw yields of rice (var. PD-4) are presented in **Table 1.** It is clearly evident from the data that foliar spray of this agrochemical on rice crop did not have any significant effect on the general growth parameters like plant height and number of tillers/plant. The yield attribute like the average panicle length was also not significantly influenced by the foliar sprays of this chemical. However, foliar spray of Fantac at the rate of 25, 50 and 75 mL/100 L water increased the number of grains/panicle by 4.8, 6.2 and 6.6 percent over control, respectively but Fantac at the rate of 50 and 100 mL/100 L water decreased the number of chaffy grains/ panicle significantly by 8.1 and 13.6 percent over control, respectively. As regards the grain yield of rice, foliar spray of fantac at the rate of 25, 50, 75 and 100 mL/100 L water increased it significantly by 11.3, 17.3, 16.6 and 16.2 percent over control, respectively. Fantac alone or in combination with trace element mixture has been reported for the highest grain/seed yields in wheat [6]. In case of fruits, Fantac has been reported to enhance the photosynthetic activity and produce a significant increase in yield and size of apples [7]. Dubravec has reported an increase in the cluster number and yield of grapevine [8]. In strawberries too, an increase in the berry weight and yield has been reported [9]. The straw yield of rice in this experiment was however not significantly influenced by different treatments.

Recovery studies were done by fortifying known amount of Fantac standards and estimation of the residues left, as per the method outlined earlier. The percent recovery values of Fantac from all the samples were found to be 90.2 to 92.0 percent at higher fortification rate and 82.6 to 85.0 percent at lower rate of fortification, respectively. Standard deviation associated with the determinations ranged from 2.6 to 4.8% and the LOQ and LOD values for fantac were 0.05 and 0.02 μgg^{-1} respectively. The study revealed that the mean recovery percentages were 87.0 for soil, 90.8 for paddy grain and 92.0 for rice straw respectively, which indicates that the method adopted for estimation of Fantac is quite suitable for extraction.

The fantac residues were also estimated in the harvested samples of paddy grain, straw and soil (**Table 2)**. The fate and behavior of Fantac in soil has also been observed and since it is easily degraded by microorganisms in the soil its half life is very short [10,11]. The residue levels in the present study at harvest time were 0.05 μgg^{-1} which is below the toxicity level in all *i.e.* paddy grain, straw and soil. Hence it can be considered safe both for

Table 1. Effect of foliar spray of growth promoters on growth parameters and yield of rice (pooled data of three season).

Treatments*	Plant height (cm)	Tillers/ plants	Panicle length (cm)	Grain/ panicle	Chaffy gain/ panicle	Grain yield (q/h)	Straw yield (q/h)
T1	80.2	11.3	24.7	112	12	50.78	67.08
T2	80.4	11.5	23.7	114	11	53.54	63.09
T3	79.8	11.5	23.7	114	12	53.22	63.20
T4	79.4	12.2	23.3	111	10	53.04	66.32
T5	79.3	11.5	24.2	107	13	45.63	61.87
S.Em.	0.7	0.3	0.4	1.8	0.8	1.06	1.44
CD (p = 0.05)	NS	NS	NS	5	2	3.02	NS
C.V. (%)	3.6	8.81	4.31	4.8	19.06	6.16	6.69

*(T1, T2, T3, T4 and T5 are treatments of Fantac @ 25, 50, 75, 100 and 0 mL/100 L of water)

Table 2. Harvest time residues of Fantac in rice.

Treatment	Rice grain	Rice straw	Soil
Fantac @ 75 mL/100 L	BDL	BDL	BDL
Fantac @ 100 mL/100 L	BDL	BDL	BDL

BDL: Below detection limit (< 0.05 ppm)

the present as well as the succeeding crop.

4. Conclusions

It can be concluded from the present investigation that the growth promoter, Fantac helped in better seed setting which resulted in higher grain yields of rice. The simple extraction and clean up procedure developed for Fantac analysis in paddy grain, straw and soil was found to yield good recovery of the biostimulant and it is safe from environmental and health point of view as its residues do not persist both in crop and soil at the harvest time.

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