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Impact of individual and combined application of tetracycline and streptomycin on rice seed germination plant performance

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ABSTRACT

Rice seeds are recommended to be pre-treated with antibiotics before sowing to eradicate bacterial diseases. A phytotoxicity test was conducted to evaluate the effects of tetracycline (TC) and streptomycin (STR) individually or in combination as Streptocycline (STC) at three different doses (half the recommended dose; 0.5RD, recommended dose; RD and double the recommended dose; 2RD) on rice seed germination and related parameters. The percentage of germination, mean germination time (MGT), vigour index I, vigour index II and shoot length were significantly affected by types of antibiotics and their dosages. However, root length, fresh weight of roots and shoots of rice seedlings was not significantly affected by the antibiotics or their dosages. The germination parameters were significantly affected in combined application as compared to their single applications. In all cases higher doses (2RD) reduced the germination parameters in comparison to RD and 0.5RD treatments. Combined application of TC and STR at higher doses may inhibit the rice germination parameters and early vigour, thereby could pose serious issues in plant establishment in main rice field. The antibiotics did not caused any marked change in the OJIP fluorescence transients of the fully grown rice plants but there was a reduction of fluorescence at all levels. The primary photochemistry and the performance of plants for photosynthetic energy conversion decreased with the increase in the concentrations of antibiotics.

1. Introduction

Rice (*Oryza sativa* L.) is the most important leading food crops and is known as the grain of life and is synonymous with food for Asians. Rice attained its premier position in a balanced diet by virtue of its unique complex carbohydrate, low fat, low salt, no cholesterol, rich source of iron and zinc, high proportion of lysine and B-complex vitamins, especially thiamine, riboflavin and niacin content (Chaudhari *et al.*, 2018). Rice crops are infested by many pathogens causing a huge crop loss affecting the livelihood. The most important of all the bacterial diseases is bacterial leaf blight (BLB). The causal organism of the disease is a gram-negative bacterium, *Xanthomonas oryzae pv. oryzae (Xoo)*. Under favourable conditions, the disease can cause 6-60% of crop loss within a year (Saha *et al.*, 2015). The yield loss depends on severity of infection, type of rice variety, environmental conditions prevailed in the area as well as cultural practices. The disease may show three different types of symptoms such as leaf blight, wilt or Kresek and yellow or pale-yellow leaf.

Management of BLB can be done through the use of resistant cultivars, alteration of cultivation methods, use of bio-control agents, use of natural products or plant extracts and also by the use of chemicals. The Central Insecticide Board and Registration Committee (CIB & RC), Government of India, recommended a mixture of two antibiotics-streptomycin sulphate (90%) and tetracycline hydrochloride (10%), which was registered under the section 9(3) of the Insecticides Act, 1968. The antibiotic can be applied in three ways on rice plants to prevent the disease, viz. seed

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treatment, seedling dip and foliar spray. For seed treatment, the rice seeds are recommended to be soaked in 40 mg/l antibiotic solution for 12 hours at room temperature before sowing.

There are several reports showing the seeds are negatively affected by antibiotics exposure. The effects include delay in seed germination, inhibition of seedling growth, etc in crops other than rice. Seed germination and early growth of *Brassica campestris* was negatively affected with chlorotetracycline and erythromycin application (Cheong *et al*., 2020; Cheong *et al*., 2021). Oxytetracycline and enrofloxacin at concentrations of more than 10 mg/l affected seed germination, seedling growth and root elongation in wheat plants (Li *et al*., 2023). Similar effects have been observed in other crops like lettuce, carrot, cabbage, tomato, alfalfa, cucumber, maize, sorghum, etc. (Bellino *et al*., 2018; Hillis *et al*., 2011; Pan and Chu, 2016; Ghava *et al*., 2015; Wang *et al*., 2017). However, the physiological changes and growth characteristics of rice under antibiotic treatment have not been properly studies.The present study presents the effects of tetracycline (TC), streptomycin (STR) and their mixture, streptocycline (STC) on seed germination, growth characteristics, fluorescence transients and early vigour of rice seedlings.

2. Materials and methods

2.1 Chemicals and seeds

The certified reference material (CRM) of Tetracycline Hydrochloride (TC, purity: 96.7%) and Streptomycin Sulphate (STR, purity: 95%) were purchased from Sigma Aldrich, Merck, India. Taichung Native 1 (TN1) rice seeds were obtained from ICAR- National Rice Research Institute (NRRI) gene bank and stored in sealed paper bags at 5°C until use.

2.2 Germination test

Laboratory evaluation for the effects of TC, STR and STC on rice seed germination was carried out using the filter paper method according to the International Seed Testing Association (ISTA) test protocols (ISTA, 1985). Seeds were soaked in distilled water overnight before the germination experiment. The water-soaked seeds (25) were placed on a filter paper (9 cm diameter) kept in each petri-dish (9 cm diameter). For each antibiotic compound, the filter papers in petri-dishes were treated with 5 mL of antibiotic solution and covered before placing in an incubator maintained at 26± 2ºC and 80±5 % relative humidity. Different doses of TC were 2 mg/L (half the recommended dose; 0.5RD), 4 mg/L (recommended dose; RD) and 8 mg/L (double the recommended dose; 2RD), and of STR were 18 mg/L (0.5RD), 36 mg/L (RD) and 72 mg/L (2RD). In case of STC, combination of TC and STR were used for different doses. All the antibiotic treatments along with control were tested in five replicates each.

2.3 Early growth parameters

At the end of the experiment, seed germination (%), length of root and shoot (cm), total length, fresh weight of root and shoot (mg), and dry weight of root and shoot (mg) were measured. Germination rate was calculated as the number of seeds that germinated per petri dish divided by the total number of seeds taken. Five seedlings were selected for the measurement of length and weight. The seedlings were weighed immediately to obtain the fresh weight to avoid any drying of the seedlings. Root length was measured from the tip of the primary root to the hypocotyls using a standard centimetre scale. Total length was measured from the tip of primary root to the tip of shoot. For the measurement of dry weight of shoot and root, the samples were kept in an oven (at 55/ ±/ 1/ °C for 72/ h) to achieve constant weight.

The vigour index I and II of the seedlings were calculated as suggested by Abdul-Baki and Anderson (1973) through the formulae:

Vigour index I= seedling length x germination percentage

Vigour index II= seedling dry weight x germination percentage

Mean germination time (MGT) is the average time taken for the seeds to germinate, and was calculated as per the formula given by Ellis and Roberts (1981):

MGT= “ (n*d)/ N

Where, n = number of seeds germinated on each day, d = number of days from the beginning of the test and N = total number of seeds germinated at the end of the experiment.

2.4 Chlorophyll a fluorescence

The measurement of chlorophyll *a* fluorescence from the adaxial surface of leaf (fourth from the apex) was made using a plant efficiency analyzer (Handy PEA, Hansatech Instruments, Norfolk, UK) following the method described by Chhotaray *et al.* (2014). The fluorescence parameters, viz., variable fluorescence (Fv), 300 µs relative variable fluorescence (V_K), 2 ms relative variable fluorescence (V_J), net rate of PS II closure (M_0), quantum yield of primary photochemistry (jP_0), rate of trapped exciton movement beyond Q_A (y_0), quantum yield of electron transport (jE_0), quantum yield of energy dissipation (jD_0), effective antenna size of active RC (ABS/RC) and performance index of primary photochemistry (PI_j) were calculated using the fluorescence equations of Force et al. (2003) and Stirbet and Govindjee (2011). The chlorophyll *a* (chl *a*) fluorescence was measured from the fully grown (45 days old) potted rice plants. After spray application of the antibiotics, the plants were placed in green house for 7 days at ambient temperature and the fluorescence responses of the plants were taken as a measure of their photosynthetic performance.

2.5 Statistical analysis

The effect of antibiotics, and their doses on seed germination parameters was analysed by using two-way analysis of variance (ANOVA) using SPSS software (IBM Corporation, 2016). The treatments were compared based on by Tukey's HSD (Honestly Significant Difference) at P = 0.05. Percent seed germination data was arcsine transformed before analysis.

3. Results

The germination percentage, mean germination time (MGT), Vigour index I, Vigour index II and other parameters are presented in Table 1. Germination was noticed after 12 h of incubation. Germination percentage (94.6%) was found highest in TC at recommended dose, where as in control the germination percentage was 85.6%, indicating the enhancement of germination by the antibiotics Such enhancement was found significantly altered by type of antibiotics ($F_{2,44}$=12.55) and antibiotic doses ($F_{3,44}$=13.63) (Table 2). Individual application of antibiotics improved the germination percentage as compared to control. The gain in germination percentage was at par with RD and 0.5RD doses of TC and STR. Higher doses (2RD), however, reduced the germination in comparison to RD and 0.5RD treatments. The germination percentage was the least at any comparable dote of STC. Effect of TC and STR; and 0.5RD and RD on seed germination did not have any statistical differences.

The mean germination time (MGT) followed the similar trend as that of germination percentage and ranged from 2.16-2.56 days. It was found that germination efficiency was significantly affected by type of antibiotics ($F_{2,44}$= 7.04) and antibiotic doses ($F_{3,44}$= 6.54). STC was found to increase the mean germination time as compared to TC and STR. Among the doses, 2RD was found to negatively affect MGTas compared to other doses and the untreated control.

The antibiotics type ($F_{2,44}$= 4.12) and doses ($F_{3,44}$= 7.57) caused marked changes on the shoot length of rice seedlings. In comparison to RD and 0.5RD, the 2RD treatment shortened the shoot length thus affecting its growth. The effects of the lower doses were, however, insignificant. When TC and STR were applied together, the shoot length was more affected in comparison to individual applications. However, root length, fresh weight of roots and shoots of rice seedlings was not significantly affected by the antibiotics or their doses applied at 0.5RD but there was significant increase in the toxicity with increase in the concentration. At 2RD, the growth parameters were significantly affected as compared to other treatment doses as well as to the control. Antibiotic treatments had also an impact on the dry weight of shoot and root of rice seedlings. Antibiotic dosages had a substantial impact on shoot dry weight ($F_{3,44}$= 5.94) resulting in dose dependent inhibition. In comparison to 2RD, there was a greater amount of shoot dry weight in the 0.5RD and RD dosages but at all treated doses there was more or less a reduction in the dry weight of the shoot. The similar trend was also observed in the root dry weight ($F_{3,44}$= 5.67).

The types of antibiotics and their dosages had a significant impact on both the vigour indices. Antibiotic type (F2,44= 8.79) and dosage (F3,44= 6.98) had a significant impact on Vigour index-I. Vigour index-II was also significantly affected both by antibiotic type (F2,44= 4.40) and antibiotics doses (F3,44= 10.11)(Table 2). Among the antibiotics applied, STC caused the highest decrease of the vigour of rice seedlings in comparison to TC and STR. However, there was no significant difference between the two antibiotics with regard to the reduction of vigour index. Never the less, vigour indices decreased with increase of antibiotic dose in each treatment. In contrast to the 0.5RD and RD treatments, the 2RD dosage reduced the vigour of rice seedlings in all cases. The effects of the 0.5RD, RD, and control did not differ significantly from one another in case of separate application of the antibiotics but the combined application doses were always inhibitory.

We observed no significant change in the shape of the transients was noticed with antibiotics treatment but there was a decrease in FM without any change in OJIP rise. Such decrease was insignificant with TC treatment at 0.5 RD and RD but significant with STR treatment ? RD and at all doses of STC (Fig. 1). Significant variation in the fluorescence transients at all inflections was not seen with TC treatment up to RD level but at 2 RD the decrease in the fluorescence rise was observed in all inflections. With STR and STC treatments there was variation in the intensity of rise at the peak as well as in intermediate inflections.

Table 1

Effect of different antibiotics and their doses on rice seed germination parameters

Seed germination parameters	Control	TC			STR			STC		
		0.5 RD	RD	2 RD	0.5 RD	RD	2 RD	0.5 RD	RD	2RD
Germination percentage (%)	92.6 ± 1.67	92.4 ± 2.88	94.6 ± 2.96	87.8 ± 3.63	93.2 ±1.78	92.8 ±1.92	88.8 ± 4.14	89.0 ±1.58	84.6 ±5.17	85.0 ± 3.31
Germination time (days)	2.18 ± 0.11	2.18 ±0.02	2.19 ±0.09	2.27 ±0.10	2.22 ±0.11	2.16 ±0.07	2.22 ±0.03	2.21 ±0.18	2.33 ±0.06	2.56 ±0.19
Shoot length (cm)	4.86 ±0.45	4.79 ±0.20	4.60 ±0.14	4.55 ±0.36	4.86 ±0.45	4.31 ±0.50	4.16 ±0.44	4.42 ±0.44	4.11 ±0.69	3.83 ±0.52
Root length (cm)	3.62 ±0.48	3.70 ±0.41	3.40 ±0.42	3.31 ±0.24	3.98 ±0.35	3.62 ±0.48	3.62 ±0.40	3.70 ±0.45	3.45 ±0.26	3.19 ±0.46
Shoot fr wt (mg)	4.85 ±0.49	5.11 ±0.59	5.10 ±0.54	5.02 ±0.50	5.12 ±0.38	4.90 ±0.43	4.85 ±0.49	5.03 ±0.36	4.78 ±0.87	4.71 ±0.50
Root fr wt (mg)	1.61 ±0.15	1.70 ±0.12	1.67 ±0.16	1.61 ±0.15	1.70 ±0.19	1.67 ±0.11	1.63 ±0.14	1.69 ±0.18	1.59 ±0.29	1.56 ±0.16
Shoot dry wt (mg)	2.27 ±0.06	2.35 ±0.40	2.28 ±0.10	1.98 ±0.36	2.25 ±0.22	2.17 ±0.36	2.00 ±0.68	2.19 ±0.52	2.13 ±0.59	2.04 ±0.07
Root dry wt (mg)	1.18 ±0.03	1.23 ±0.11	1.17 ±0.19	0.99 ±0.18	1.32 ±0.20	1.24 ±0.12	1.09 ±0.26	1.06 ±0.29	1.05 ±0.06	0.99 ±0.03
Vigour index I	8.26 ±0.57	7.84 ±0.47	7.58 ±0.67	6.90 ±0.73	8.23 ±0.72	7.35 ±0.75	6.91 ±0.48	7.22 ±0.64	6.98 ±0.99	5.97 ±0.50
Vigour index II	2.56 ±0.09	3.30 ±0.40	3.26 ±0.34	2.60 ±0.39	3.56 ±0.20	3.44 ±0.47	2.74 ±0.16	2.89 ±0.73	2.70 ±0.76	2.57 ±0.11

Table 2

The coefficients of two factor ANOVA as a measure of the effects of antibiotics and doses.

Parameter	Antibiotics	Doses	Combination
Seed germination	12.55*	13.63*	2.95*
Shoot length	4.12*	7.57*	0.76
Root length	2.13	2.01	1.05
Shoot fr. Wt.	0.62	0.72	0.25
Root fr. Wt.	0.64	0.36	0.45
Shoot dry wtr.	1.48	5.94*	0.64
Root dry wt.	1.54	3.67*	2.63*
Mean germination time	7.037	6.535	2.742
Vigour index I	8.79*	6.98*	1.00
Vigour index II	4.40*	10.11*	1.24

Note: * significant at $P \leq 0.05$.

Even though some variations in the magnitude of V_K was observed with the treatments, there was no specific trend in the relative fluorescence at this inflection. Same trend was seen in the level of V_J with TC and STR treatment. However, with STC treatment, V_J was significantly high at 2RD (Table 3). In all treatment a decrease in φP_0 was observed, which was significant 2RD with TC or STR treatment but at ≥ RD with STC treatment. Similar trend was also observed for the exciton (ψ_0) and electron movement beyond Q_A (φE_0). Dissipation (φD_0), on the other hand, increased with increase in the antibiotic concentration. Such increase was significant at 2RD in all cases. There was no marked change in M_0 with TC treatment but a decreasing trend was observed with STR and STC treatments. ABS/RC did not vary significantly with STR or TC treatment but increased significantly with STC treatment. PI_φ showed continuous decrease with increase in the concentration of each antibiotic.

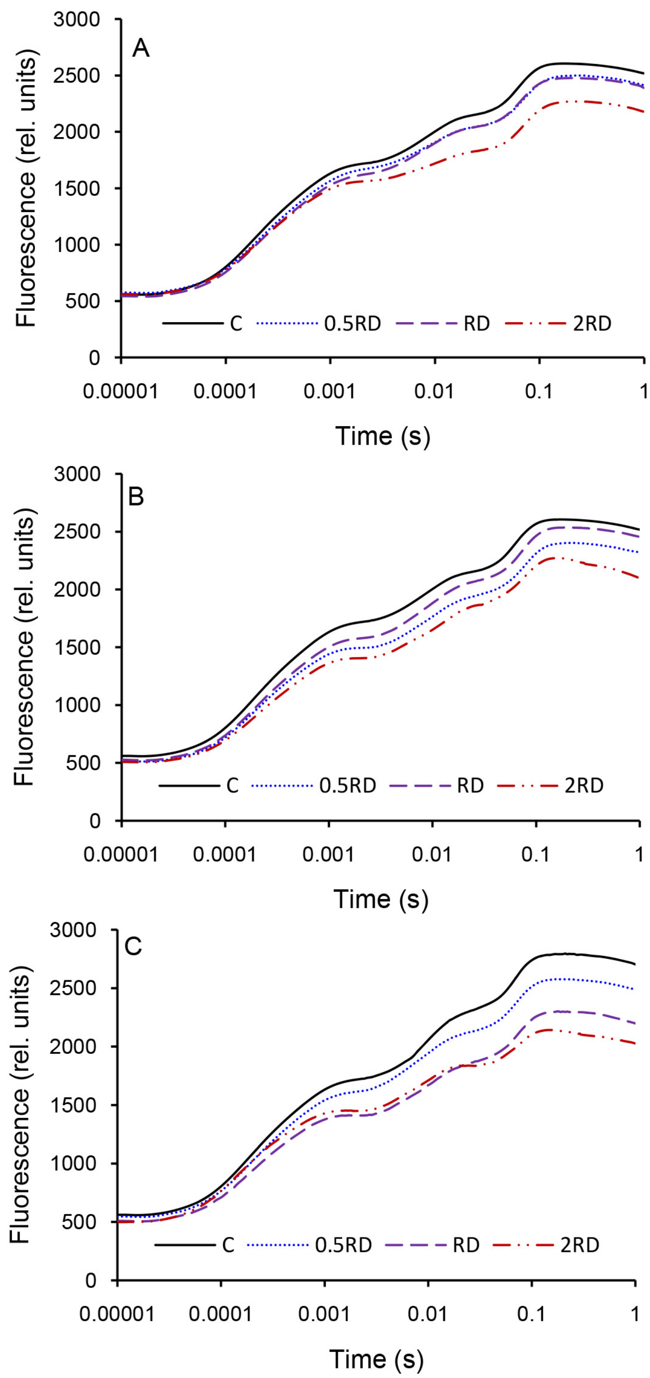

Figure 1: The OJIP fluorescence transients of fully grown rice plants on treatment with (A) tetracycline, (B) streptomycin and (C) streptocyclin. The measurement of the transients was made 7 days after treatment.

Table 3

The bioenergetic attributes of OJIP transients of fully grown rice plants, 7 days after foliar application of antibiotics.

Appl. Conc.	V_K	V_J	jp_0	y_0	jE_0	jD_0	M_0	Abs/RC	PIj
					Tetracyclin				
0	0.336^{ab} ±0.012	0.566^{ab} ±0.021	0.785^{a} ±0.045	0.434^{a} ±0.021	0.341^{a} ±0.013	0.215^{b} ±0.008	1.305^{a} ±0.062	1.867^{a} ±0.063	3.647^{a} ±0.117
0.5RD	0.320^{b} ±0.011	0.568^{ab} ±0.033	0.771^{a} ±0.036	0.432^{a} ±0.011	0.333^{a} ±0.017	0.229^{b} ±0.008	1.281^{a} ±0.049	1.739^{a} ±0.091	3.362^{a} ±0.128
RD	0.322^{b} ±0.016	0.556^{b} ±0.035	0.781^{a} ±0.028	0.444^{a} ±0.009	0.347^{a} ±0.009	0.219^{b} ±0.009	1.286^{a} ±0.058	1.807^{a} ±0.102	3.373^{a} ±0.104
2RD	0.349^{a} ±0.018	0.584^{a} ±0.022	0.742^{b} ±0.018	0.401^{b} ±0.018	0.313^{b} ±0.021	0.258^{a} ±0.007	1.397^{a} ±0.063	1.798^{a} ±0.104	3.036^{b} ±0.113
					Streptomycin				
0	0.326^{a} ±0.009	0.525^{a} ±0.022	0.785^{a} ±0.033	0.444^{a} ±0.017	0.351^{a} ±0.016	0.215^{b} ±0.009	1.345^{b} ±0.066	1.867^{a} ±0.092	3.647^{a} ±0.102
0.5RD	0.315^{a} ±0.016	0.520^{a} ±0.019	0.786^{a} ±0.028	0.440^{a} ±0.014	0.368^{a} ±0.017	0.214^{b} ±-.008	1.262^{b} ±0.048	1.909^{a} ±0.068	3.677^{a} ±0.167
RD	0.308^{a} ±0.011	0.524^{a} ±0.015	0.783^{a} ±0.042	0.446^{a} ±0.021	0.327^{b} ±0.022	0.207^{b} ±0.011	1.331^{b} ±0.029	1.863^{a} ±0.071	3.629^{a} ±0.182
2RD	0.328^{a} ±0.019	0.538^{a} ±0.017	0.746^{b} ±0.023	0.408^{b} ±0.023	0.308^{c} ±0.014	0.254^{a} ±0.014	1.582^{a} ±0.072	2.066^{a} ±0.038	3.257^{b} ±0.104
					Streptocyclin				
0	0.308^{b} ±0.014	0.518^{b} ±0.017	0.799^{a} ±0.042	0.482^{a} ±0.044	0.386^{a} ±0.011	0.201^{b} ±0.008	1.231^{b} ±0.052	1.901^{b} ±0.065	3.986^{a} ±0.102
0.5RD	0.311^{b} ±0.011	0.526^{b} ±0.016	0.789^{ab} ±0.023	0.474^{a} ±0.021	0.374^{a} ±0.019	0.211^{b} ±0.009	1.244^{b} ±0.038	1.867^{b} ±0.038	3.736^{b} ±0.143
RD	0.318^{b} ±0.016	0.504^{b} ±0.028	0.779^{b} ±0.029	0.486^{a} ±0.039	0.326^{b} ±0.009	0.221^{ab} ±0.007	1.272^{b} ±0.043	1.966^{ab} ±0.093	3.529^{c} ±0.214
2RD	0.402^{a} ±0.022	0.582^{a} ±0.027	0.733^{c} ±0.034	0.418^{b} ±0.026	0.319^{b} ±0.012	0.267^{a} ±0.011	1.608^{a} ±0.038	2.111^{a} ±0.087	3.227^{d} ±0.113

Note: Means superscripted by same letter(s) are not significantly different at p=0.05 tested through LSD.

4. Discussion

Rice seeds were chosen for this experiment due to its agronomic significance. When the antibiotics were applied singly, the germination percentage was found to increase in contrast to their combined application (20-80 mg/L) which decreased the germination percentage. With the increase in antibiotic doses, the germination was negatively affected. Generally, xenobiotics including antibiotics at low doses did not have effect on seed germination, but could affect significantly on radicle elongation stage (Luo *et al.,* 2019). Hard seed coats protect the embryo of seeds (Geneve *et al.,* 2018). A study by deRopp (1948) suggests that STR is a general inhibitor of the growth of embryonic tissues. This might be the reason that higher concentration of STR negatively affected seed germination of rice in our study. TC group antibiotics (TC, CTC, OTC etc.) have been reported to pose negligible or no effects on seed germination in several previous literatures which used TCs at 1-100 mg/L concentrations (Hillis *et al*., 2011; Yang *et al*., 2010), though however, the germination was affected at higher doses.

Compared to germination percentage, the mean germination over time could be a better indicator to test the effect of antibiotics on seed germination. STC was found to delay germination as compared to TC and STR but insignificant delaying was also reported on treatment with TC and STR. Among the doses, 2RD was found to negatively affect MGT as compared to RD, 0.5RD and control in all treatments. It supports previous observations that antibiotics, which are active against gram negative microorganisms, delay the seed germination process (Goss, 1962). By comparing the germination and MGT data, it can be concluded that antibiotics do not decrease the germination percentage but delay the process thus causing a slowing down the biochemical events during germination. Also, the seeds for which germination was delayed are likely to face competitive pressure for further growth in terms of root and shoot development.

After seed germination the correct development of shoots and roots is critical for plant establishment and survival. Some antibiotics may have lesser toxic effects on seed germination, but pose direct negative effects on emerging roots and shoots (An *et al.*, 2009). In our study shoot development were found significantly affected by antibiotic treatments. Earlier it was reported that TC at 0.01 mg/L stimulated the shoot growth of lettuce, carrot, cucumber and tomato (Pan and Chu, 2016). The authors also reported a declined trend for shoot elongation on increasing the concentration of TC. In our study, however, a decrease in shoot and root growth as well as their biomass were observed at RD and at 2RD of individual antibiotic as well as in the combination, though insignificant stimulation was noticed at 0.5 RD. Toxic effects on root elongation at high TC dosages have been reported with by other authors in other edible crops (Pan and Chu 2016; Liu *et al.*; 2009).The significant effect of antibiotics on total length of seedlings and total dry weight of seedlings is reflected as Vigour indices confirming that vigour index can be a reliable indicator of the toxicity assessment of antibiotics.

No remarkable difference in the shape of OJIP rise with treatment indicated that the antibiotics moreor less equally affected the fluorescence rise at all levels. In most cases a good value of φP_0 (>0.7) was observed that the chemicals did not cause severe inhibition of photosynthetic performance as seen in other stressors (Shasmita *et al.,* 2020; Strasser *et al.,* 2004; Stirbet and Govindjee, 2011). Nevertheless there was a rising trend of V_J, especially with STC treatment indicating that the photosynthetic performance of plants was affected with treatment (Qiu *et al.,* 2004). Similar change was also reported with respect to ψ_0 and φE_0 indicating that not only the primary photochemistry but and the electron movements from PS II is affected by the treatments, especially at higher dose (2RD). A good level of heat dissipation was observed at higher doses of antibiotic treatment showing that the plants released the excess absorbed energy as a protection mechanism against light stress. This is because of the fact that the proportion of closed photosystems increased with antibiotic doses, which was not significant, this resulted in lowering of P fluorescence as seen from the transients. Consequently the absorption area per reaction centre also increased. No significant change in the magnitude of V_K is an indicator of lack of the effect of treatment on donor side of PS II (Strasser, 1997; Lazar, 2003).

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