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Enhanced plant growth of cowpea supplemented with *Trichoderma* under biotic stress of *Sclerotium rolfsii*

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Abstract
In the present context of agriculture, which depends on hazardous chemicals to meet the growing need of presently increasing population and as a result, the conditions of soil as well as the environment is diminishing day by day. The only alternative of these problems is to utilize the natural resources i.e. biocontrol agents to combat with various types of biotic as well as abiotic factors. In the present study, *Trichoderma viride* was used as a biocontrol agent against *Sclerotium rolfsii* in cowpea plant. The germination percentage, plant height, number of stem, number of leaves, phenol (OD) and fresh weight was found maximum in cowpea plants which were soil and seed treated with biocontrol agent. Disease Incidence was also found minimum in the plants treated with maximum amount of biocontrol agent. These biocontrol agents are proving a better substitute to harmful agrochemicals and should be used as well as explored in a large fashion for better production of crops in a sustainable manner, which alongside insurance against pests and diseases are reliable for small farmers.

Keywords: Biocontrol agents, disease, pest, *Sclerotium rolfsii*, *Trichoderma viridae*

Introduction
Agriculture, in now a days are challenged by various types of biotic as well as abiotic factors. The main challenge is to feed the world in low availability of natural resources, which is demolished with effects of hazardous chemicals and ultimately the problem of food production, have become quite common threats for the farming community. The prime mother of farming i.e. soil, due to continuous applications of hazardous chemicals are at the alarming stress, which is a serious matter of concern for the global agriculture. Biotic stress also exerts a serious impact on crop productivity throughout the world. Among the biotic limitations against plants, most destructive soil borne fungus is *Sclerotium rolfsii* Sacc., which is predominantly distributed in tropical and subtropical countries. It is common where high temperature exists during the rainy season. According to Weber (1931) [18] and Garret (1956) [6] the fungus survived in the soil for many years by producing sclerotial bodies. Symptoms like pre-emergence as well post-emergence death of seedlings has been reported by Agrawal and Kotashane (1971) [1]. The effect of some synthetic fungicides on the environment and men, when used for plant disease management, has been proved deleterious. Since the realization of this fact, scientists have launched the search for alternatives that would be environment and men friendly. Promises has been found in some antagonists which are known to compete with pathogenic fungi to affect their spread (Okereke *et al*., 2007) [12].
The rhizosphere of plants contains several microorganisms, which are having major role in ecosystem (Kai *et al*., 2016) [9]. Plants discharges about half of their photosynthetic carbon into the surrounding of roots (Hutsch *et al*., 2002) [8], which attract various microorganisms (Perry *et al*., 2007) [14]. These microorganisms are of great importance for their contribution to plant nutrition, hormonal control and disease suppression (Bastida *et al*., 2009) [3]. Among these microorganisms, *Trichoderma* spp. have more ability to colonize the root zone of the plants. The antagonistic effect of rhizospheric *Trichoderma* spp., are found on some fungal plant pathogens and its use as a biocontrol agent has been established also (Kumar *et al*., 2012) [10]. *Trichoderma*, as a soil borne mycoparasite have been found very effective in controlling several soil borne phytopathogens (Dolatabadi *et al*., 2012) [5].

These soil borne plant pathogens can be biologically controlled by seed, soil and seedling application with the antagonists. The aim of this investigation was to study the antagonistic effect of *Trichoderma* spp. in cowpea against *Sclerotium rolfsii* in different forms and assess its ability in suppressing the populations of *Sclerotium rolfsii* in cowpea.

Materials and Methods

A pot experiment was conducted to study the effect of *Trichoderma viride* in the growth of cowpea plants under the biotic stress of *Sclerotium rolfsii*. The treatments were formed with the bioagent as T1 (seed treatment), T2 (soil application), T3 (seed + soil application), T4 (Hexaconazole 5% EC) and T5 (control). Plastic pots of 10×10 cm were filled-up with the sterilized soil mixed with the sclerotia of *Sclerotium rolfsii* at the rate of 100 sclerotia per 100 g of soil (Yaqub and Shahjad, 2005) [19] and was replicated four times. Cowpea seeds were surface sterilized with 70% ethanol for 1 min followed by 1% sodium hypochlorite for 5 min and after each step seeds were washed with sterilized distilled water. Five seeds were sown in each pot with four replications at 1cm depth and kept in controlled conditions for germination of the seeds. Parameters of cowpea plant like plant height, no. of stems, no. of leaves and phenol content that utilizes Folin-Ciocalteu (F-C) reagent were observed after 30 days of sowing. Another pot experiment was conducted to analyse the effect of bioagent on the soil supressiveness activity. Around 250 g of garden soil was taken in each pot. The biomass of the pathogen was mixed well with sterilized soil. *Trichoderma* cell mass was uniformly mixed with the pathogen pre-mixed soil at different cell mass concentrations (w/w) viz., 0% (Ta), 2% (Tb), 4% (Tc), 6% (Td), 8% (Te) and 10% (Tf). Ten seeds were sown in each pot with four replications by following the above steps of seed sterilization. Observations were taken 15 and 30 days after sowing (DAS) for the damping off of cowpea seeds caused by the pathogen. Both the experiments were replicated twice. The IBM SPSS Statistics software package version 20 was used for the analysis.

Results and Discussion

The efficiency of *Trichoderma viride* was assessed against the soil borne pathogen *Sclerotium rolfsii*. The assessment of various plant parameters was done 30 DAS and disease incidence were recorded 15 and 30 DAS.

Seedling germination and Plant parameters:

The experiment clearly indicated that the bioagent inoculated in different forms (T1 to T4) significantly affected the plant growth, if compared with non-inoculated one (T5) in both the experiments. All the sets of treatments were found significantly superior and effective in increasing germination of cowpea as compared to the control set in twice replicated experiment. However, the rate of enhancement varied with different treatments. More than 70% seed germination occurred in bioagent inoculated seeds as well as soil treated i.e. in treatments 1 to 4 but reduction in germination per cent was observed in untreated one (T5) (Table 1). The bioagent strains initiated the germination of cowpea seed on 3rd day after sowing connecting to active participation in germination system whereas in control set initiation of germination occurred on 6th day after sowing. All the treatments gave better results in terms of plant height as compared to control. Plant height was found maximum in T3 followed by T2, T1, T4, and T5 in both the experiments. Also, the number of stems were found maximum in the plants treated with T3 followed by T2, T1, T4, and T5, and the similar trends were observed in case of number of leaves as well as for their fresh weight (g) of the plants in both the experiments (Table 1, Fig. 1 and Fig. 2). Antagonism of *Trichoderma* species against several pathogens were reported by Reddy *et al*. (2013) [15]; Sundaramoorthy & Balabaskar (2013) [17] and Hanan & Mohamed (2014) [7]. *Trichoderma* spp. influences seed germination and seedling vigor of cowpea by several mechanisms (Zheng and Shetty, 2000[20]; Clear and Valic, 2005[4]). Inspite of *Trichoderma* spp. *Pseudomonas* spp. also shows positive relation with seed germination and plant vigor by using alone or in combination with *Trichoderma* spp. against root diseases (Shanmugaiah *et al*., 2009) [16]. Azarmi *et al*. in 2011[2] used *Trichoderma* species (*Trichoderma harzianum* isolate T969 and *T. harzianum* isolate T447) and found that, seed germination rate was not affected by *Trichoderma* application, but leaf number, shoot height, diameter, fresh and dry weight and root fresh and dry weight in tomato seedlings were interestingly increased as compared to the control. These previous findings are in support with the present study.

Phenol estimation

Plants grown under biotic stress of *S. rolfsii* were used for this experiment. Plants inoculated with T3 produced greater amount of total phenol in terms of Optical Density (OD), which was followed by T2, T1, T4, and T5 in both the experiments (Table 1 and Fig. 2). The correlation between total Phenol (OD) and other parameters like plant height (cm), number of stems, number of leaves and fresh weight (g) of plants in different treatments for both the experiments were found to be positive for each experiment (Table 2). The mechanical strength of plant cell wall is enhanced by the phenolic compounds, which produces toxins. (Pandey *et al.*, 2005) [13]. An organized protection reaction framework is enacted in host plants and defense related proteins or pathogenesis related proteins (PR proteins) are created during the host-pathogen interaction. Various morphological changes including cytoplasmic disruption and loss of protoplasmic content are exhibited by the hyphae of the pathogens which are bounded by phenolic compounds. The present study showed higher accumulation of phenolic in the plant augmented with bioagent. These phenolic compounds also have an antagonistic role against various pests, as these chemicals occur constitutively and functions as preformed inhibitors which constitute an active defense response against pest and diseases (Nandi *et al.*, 2013). [11].

Soil supressiveness

In the control, where soil was mixed only with pathogen (*S. rolfsii*) and not with antagonists (Ta), 80% of damping off of cowpea seedlings in experiment 1 and 74.50% in experiment 2 respectively was observed 15 DAS and after 30 days of sowing, 100% damping off took place in both the experiments. Disease incidence (DI) in percentage, after 15 as well as 30 days was found maximum in Ta followed by Tb, Tc, Td, Te and Tf in both the experiments (Table 3 and Fig. 3). The DI was found in increasing trend if compared between 15 DAS and 30 DAS in both the experiments. The maximum and minimum difference was found in Ta and Tf respectively (Fig. 4). The percent reduction over the control was observed maximum in Tf followed by Te, Td, Tc and Tb after 15 and 30 DAS in both the experiments (Table 3 and Fig. 5). The plants were found healthy in terms of their fresh weight which was found maximum in Tf followed by Te, Td, Tc and Tb

when noted 30 DAS in both the experiments (Table 3 and Fig. 5). From the *in vitro* findings, it can be suggested that the antagonists such as *Trichoderma viride* can be used as a bio-control agent against *Sclerotium rolfsii*. It also affirming that the microorganisms naturally remain in the soil are having more or less similar potential antagonistic effect on the various crop disease caused by different pathogens. These can be utilized as a potential bio-control agent to diminish the disease as well as pest frequency and to build crop efficiency. Therefore, further work should be taken up to explore the possibility of the use of the antagonists under study in field condition for the biological control of the diseases caused by *Sclerotium rolfsii*.

Table 1: Plant parameters in different treatments

Treatments	Experiment 1						Experiment 2					
	Germination %	Plant height (cm)	No. of stems	No. of leaves	Phenol (OD)	Fresh weight (g)	Germination %	Plant height (cm)	No. of stems	No. of leaves	Phenol (OD)	Fresh weight (g)
T1	82.5(65.3)	22.33	7.5	14.25	0.4058	1.230	85.0(67.2)	21.68	7.25	14.50	0.4018	1.193
T2	87.5(69.3)	22.35	8.25	15.75	0.3923	1.575	92.5(74.1)	22.50	8.5	15.75	0.4128	1.535
T3	95.0(77.1)	25.50	10	19.25	0.4355	1.625	100.0(90.0)	25.65	10.75	18.25	0.4613	1.820
T4	72.5(58.4)	19.20	7	14.25	0.2970	0.915	75.0(60.0)	18.50	7.25	14.50	0.3118	0.898
T5	67.5(55.2)	16.05	6.75	11.25	0.2813	0.735	65.0(53.7)	16.50	6.25	12.00	0.2618	0.635
C.D.	0.785	1.147	1.145	1.456	0.054	0.198	0.760	0.820	0.962	1.546	0.056	0.130
SE(m)	0.258	0.377	0.376	0.479	0.018	0.065	0.250	0.269	0.316	0.508	0.018	0.043

values in the bracket are the angular transformed values
values are the mean of four replications

Table 2: Correlation among various plant parameters

	Experiment 1					Experiment 2				
	A	B	C	D	E	A	B	C	D	E
A	1.000					1.000				
B	0.891	1.000				0.922	1.000			
C	0.936	0.953	1.000			0.947	0.973	1.000		
D	0.933	0.878	0.881	1.000		0.985	0.938	0.952	1.000	
E	0.957	0.830	0.816	0.923	1.000	0.989	0.858	0.906	0.969	1.000

A: Plant height (cm), B: No. of stems, C: No. of leaves, D: Fresh Weight (g), E: Phenol (OD)

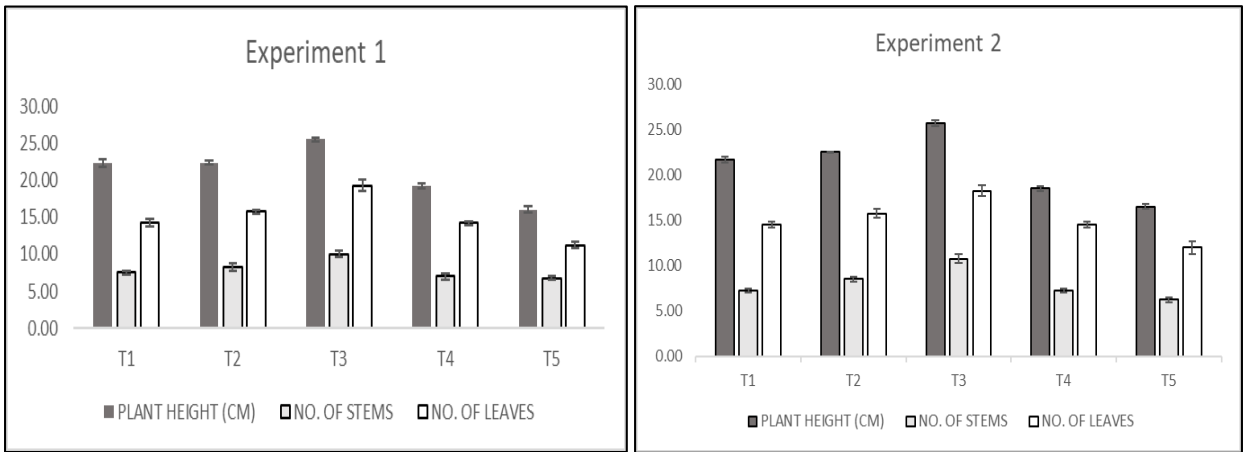

Fig 1: Various plant parameters under different treatments, 30 days after sowing

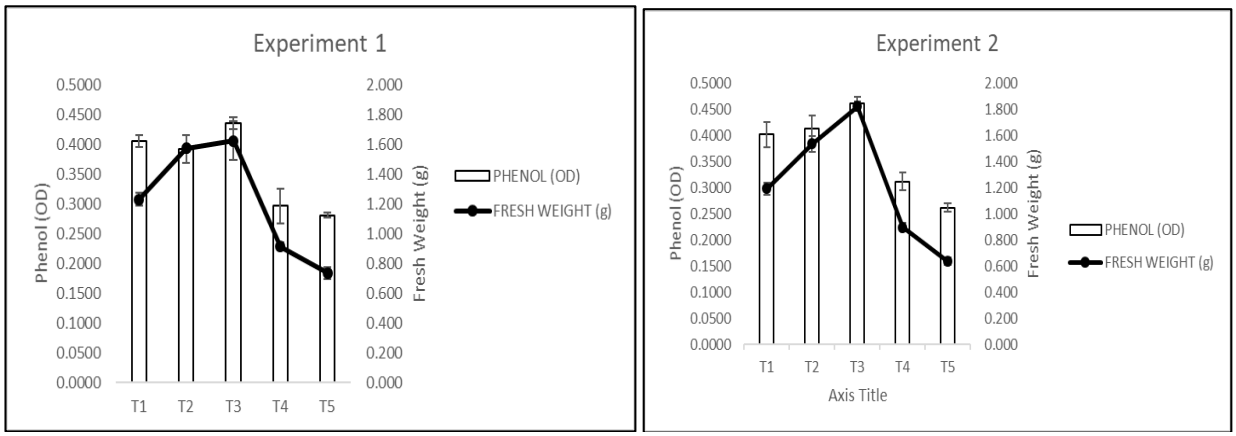

Fig 2: Total Phenol (O.D.) and Fresh weight of plants under various treatments

Table 3: Disease Incidence and fresh weight against different treatments

	Experiment 1					Experiment 2				
Treatments	**PDI after 15 days**	**Percent reduction over control**	**PDI after 30 days**	**Percent reduction over control**	**Fresh weight (g)**	**PDI after 15 days**	**Percent reduction over control**	**PDI after 30 days**	**Percent reduction over control**	**Fresh weight (g)**
Ta	80.00 (63.43)	-	100.00 (90.00)	-	-	74.50 (59.67)	-	100.00 (90.00)	-	-
Tb	59.75 (50.62)	25.31	64.75 (53.58)	35.25	0.82	64.50 (53.43)	13.42	72.00 (58.05)	28.00	0.78
Tc	44.25 (41.70)	44.69	51.50 (45.86)	48.50	1.12	42.75 (40.83)	42.62	62.25 (52.09)	37.75	0.96
Td	31.25 (33.99)	60.94	39.50 (38.94)	60.50	1.29	28.50 (32.27)	61.74	40.00 (39.23)	60.00	1.17
Te	19.25 (26.02)	75.94	30.00 (33.21)	70.00	1.38	20.75 (27.10)	72.15	27.50 (31.63)	72.50	1.40
Tf	11.75 (20.05)	85.31	15.75 (23.38)	84.25	1.52	12.75 (20.92)	82.89	16.00 (23.58)	84.00	1.50
C.D.	1.743		1.483		0.036	1.488		1.264		0.043
SE(m)	0.783		0.495		0.012	0.497		0.422		0.014

values in the bracket are the angular transformed values
values are the mean of four replications and five for plant fresh weight

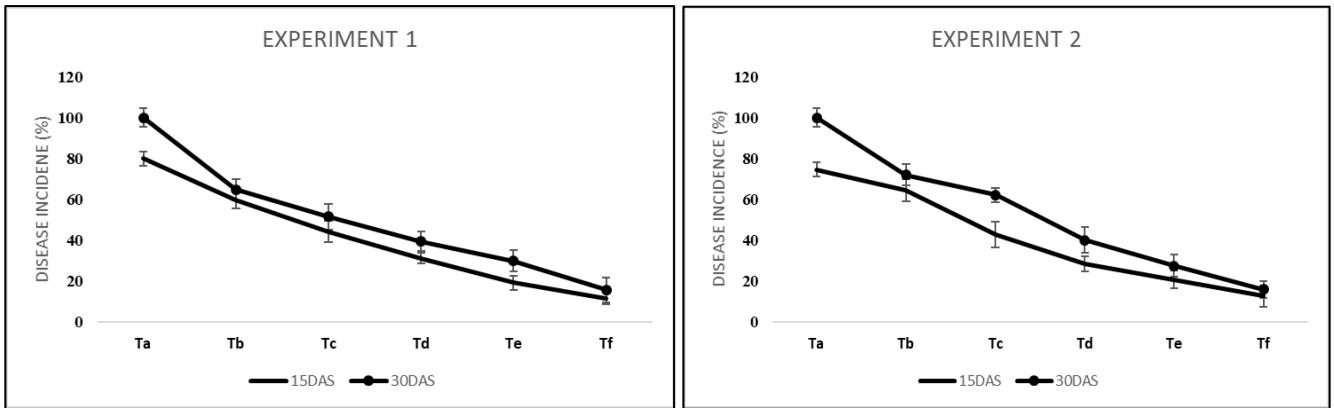

Fig 3: Disease Incidence in different treatments

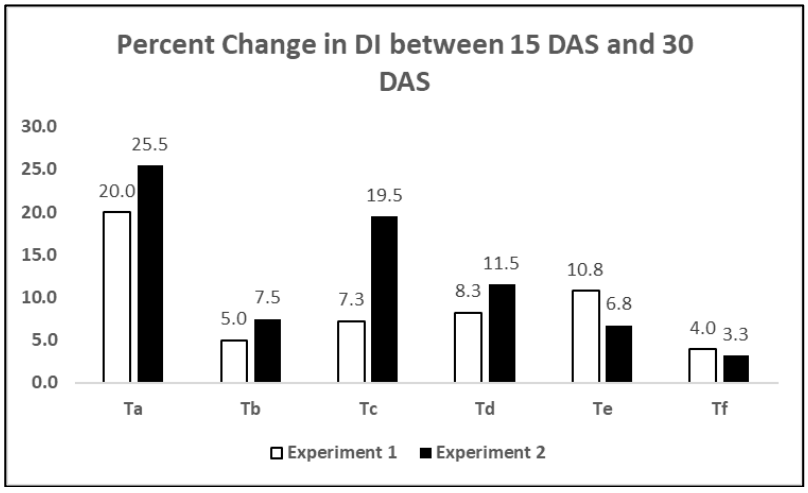

Fig 4: Different in Percent Disease Incidence on two dates

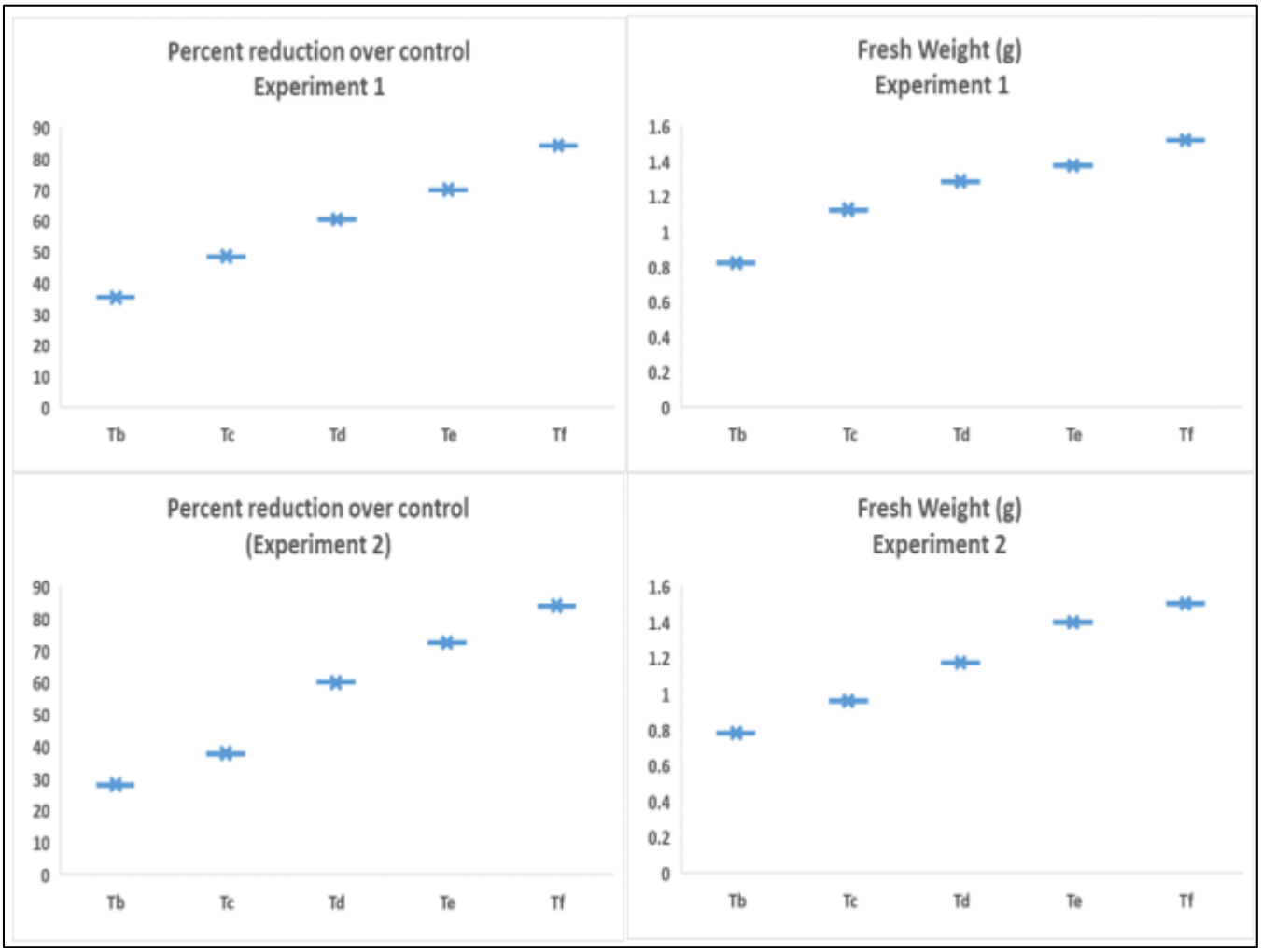

Fig 5: Percent reduction against control and fresh weight (g)

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