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Colour measurement of ripening mango fruits as influenced by pre-harvest treatments using $L^* a^* b^*$ coordinates

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Abstract

Colour development is one of the important ripening processes for determining the quality of mango fruits. In this experiment, pre-harvest treated 'Alphonso' mango fruits were measured objectively for peel and pulp colour using Hunter ($L^* a^* b^*$) colour system. Azoxystrobin at 0.1% treatment resulted in brighter and superior reddish yellow colour development of peel of mango. Peel values of fruits under this treatment for L^* (63.38), a^* (17.66) b^* (61.03), C^* (62.13) and h^0 (70.48) were significantly higher than control and other treatments. Mango fruits under the same treatment achieved greater colour development for the pulp with L^* (64.16), a^* (28.43) b^* (66.34), C^* (75.18) and h^0 (68.54) that were in significance with rest of the treatments. Control fruits on the other hand showed poor values for each of colour coordinates indicating lack of proper colour development during ripening.

Keywords: Mango, L^* , a^* , b^* , C^* , h^0 , Azoxystrobin, ripening, colour

Introduction

Mango is one of the most popular fruits in India and many countries in the world. 'Alphonso', the choicest mango cultivar known for its appealing reddish-yellow colour, unique taste and flavour with subtle blend of acid and sugar stands first in the export market of our country. Mango colour development involves significant physiological changes during ripening. The fruit shows climacteric pattern of respiration during ripening (Lalel *et al.*, 2003; Brecht and Yahia, 2009) [10, 2] that coincides with the increased ethylene production (Mattoo and Modi, 1969) [14] boosting respiration and breakdown of peel carotenoids (yellowing) (Krishnamurthy and Subramanyam, 1970; Akamine and Goo, 1973) [8, 1]. Peel colour of mango fruits alters from dark green to yellow colour where chloroplasts in the peel are converted to chromoplasts comprising red and yellow pigments, (John *et al.*, 1970; Lizada, 1993) [5, 11] facilitated through the action of enzyme chlorophyllase, oxidative system and pH change or photo degradation (Gowda and Huddar, 2001; Wills *et al.*, 1982) [4, 18]. Alphonso mango pulp upon ripening was proposed to have 16 carotenoid fractions with β -carotene occupying 50 per cent of total carotenoids (Jungalwala and Cama, 1963; John *et al.*, 1970) [6, 5]. Mango carotenogenesis in the pulp is mediated by phosphatase enzyme (Mattoo *et al.*, 1968) [13]. Measurement of colour is one of the important aspects for determining the ripening quality of mango fruits. Hunter (L^* , a^* , b^*) is most commonly used colour system for measuring colour. It uses rectangular coordinates that is labelled with three dimensions of a colour as L^* , a^* , b^* . The L^* coordinate denotes lightness, the a^* coordinate represents redness ($+a^*$) or greenness ($-a^*$), and the b^* coordinate symbolizes yellowness ($+b^*$) or blueness ($-b^*$). In this system, an object for colour measure is assigned L , a , b value that defines the colour and its location in the three-dimensional colour space. The distance between two colours, each with their own colour space location, refers to a computable colour difference. Chroma or saturation (C^*) indicate purity of the hue and measures colour to a neutral grey of the same lightness (Shafiee *et al.*, 2010; Kevin, 2002) [17, 7]. (Hue (h^0) angle is represented in degrees, with 0° for $+a^*$ axis (red), till 90° for the $+b^*$ axis (yellow), 180° for $-a^*$ (green) and lastly 270° for $-b^*$ (blue) (Kevin, 2002) [7]. Mango fruits despite being attractive and delicious, suffers with many postharvest diseases and anthracnose caused by the fungus *Colletotrichum gloeosporioides* Penz being the deadliest of all. The disease is carried as latent infection from the field and becomes conspicuous upon ripening of fruits leading to rotting. The appearance of the fruit is thus affected resulting in low market value. Current investigation is the continuation of the previous experiment which included pre-harvest treatment of mango fruits with different fungicides, bioagents and botanicals for anthracnose management. In this experiment the resultant fruits from the latter experiment were studied at ripening for colour changes using Hunter $L^* a^* b^*$

colour coordinates to know the influence of pre-harvest treatments on mango colour development.

Materials and Methods

Twelve year old, uniformly grown, Alphonso mango trees aged 12 years, raised by approach grafting were imposed with fungicides like carbendazim (Bavistin 50 DF), tricyclazole (Beam 75 WP), Azoxystrobin (Amistar 250 SC), thiophanate methyl (Topsin M 70 WP) at 0.1% each and Zineb (Dithane Z-78) at 0.2%; botanicals like *Eupatorium odoratum* and *Nerium oleander* each at 5% and bioagents like *Trichoderma viride* and *Trichoderma harzianum* each at 0.5% three times before harvest during pre-flowering (Dec), peanut (Feb) and marble (March) stages of fruits during the years 2012-13 (year I) and 2013-14 (year II) respectively. Three replications per treatment and two trees per replication were assigned. Non-sprayed trees were considered as control. During harvest, 25 mango fruits with uniform maturity, size, free from diseases were selected per treatment, packed in CFB boxes and brought to the laboratory, Department of Post-Harvest Technology, Kittur Rani Channamma College of Horticulture, Arabhavi, India. The fruits were stored at ambient temperature and allowed for natural ripening to study the colour change.

Preparation of fungicides, bioagents and botanicals

Fungicides were obtained from Karnataka Agro Chemicals, Dharwad. Systemic fungicides like Azoxystrobin (Amistar, 250 SC), thiophanate methyl (Topsin-M, 70 WP), carbendazim (Bavistin, 50 DF), Tricyclazole (Beam, 75 WP) were prepared at 0.1 per cent by dissolving 1 g/ 1 ml of each fungicide was dissolved in 100 ml of water and volume made up to 1000 ml. While Zineb of 0.2% was prepared with 2 g of Zineb in 100 ml of water and then making up the volume to 1000 ml. Bioagents, *Trichoderma viride* and *Trichoderma harzianum* were obtained from the Department of Plant Pathology, University of Agricultural Sciences, Dharwad. Each were set at 0.5 per cent concentration by dissolving 5 g of respective culture in 100 ml of water and then making up the volume to 1000 ml. Leaves of botanicals *Eupatorium odoratum* (Siam weed) and *Nerium oleander* (Oleander) were collected, rinsed, extracted juice and the solution was then made up to 1000 ml volume with distilled water to achieve 5 per cent concentration. According to the concentration adopted each for fungicides, bioagents and botanicals, 6 litres of spray solution were prepared for the complete canopy coverage.

Instrumental analysis of peel and pulp colour (L^* , a^* , b^* , C^* , h^0)

The colour of samples was measured using a Lovebird colour meter (Lovebird RT300, Portable spectrophotometer, The Tintometer Limited, Salisbury, UK) fitted with 8mm diameter aperture. The instrument was calibrated using the black and white tiles provided. Colour was expressed in Lovebird units L^* (Lightness/darkness), a^* (redness/greenness), b^* (yellowness/blueness), C^* (chroma) and h^0 (hue angle). Ripened mango fruits were directly placed under the aperture of the colour meter and measured on three sides per fruit for both peel and pulp colour analysis and the values were averaged.

Statistical analysis

Statistical analysis Statistical analysis was performed for both the years separately and then pooled. All data were collected

and analysed by randomized block design (RBD). Significant differences among means at $P \leq 0.05$ were determined by post hoc tests using Duncan's multiple range test (Duncan, 1955) [3].

Results

Peel colour

The observations on peel colour of mango fruits as influenced by different pre-harvest treatments registered significant differences with respect to L^* , a^* , b^* , C^* and h^0 (Table 1, 2). The L^* values were significantly higher in the fruits of mango trees subjected to the treatment Azoxystrobin at 0.1% during year I (63.23), year II (63.52) and in pooled analysis (63.38). Conversely, in year I and year II, the control (37.52 and 42.07) documented significantly low L^* values for peel colour. However, the treatments *Nerium oleander* at 5% (39.48 and 43.06) and *Trichoderma harzianum* at 0.5% (40.78 and 43.65) were found to be on par with control. In pooled analysis, control with minimum peel L^* value (39.80) was found to vary non-significantly with *Nerium oleander* at 5% (41.27). Perusal of data on a^* values of mango peel colour indicated that the treatment Azoxystrobin at 0.1% was remarkably superior during year I, year II and even after pooled analysis of data (16.73, 18.59 and 17.66 respectively). In contrast, the fruits of control (2.61, 3.69, 3.15) contained minimum a^* values during both the years and even when pooled. In addition, the fruits of *Nerium oleander* at 5% (3.61, 4.43, 4.02 respectively), *Trichoderma harzianum* at 0.5% (4.36, 5.61, 4.99) and Tricyclazole at 0.1% (4.84, 5.95, 5.40) were at statistical parity with control during year I, year II and in combined analysis. Significantly higher b^* values of mango peel colour was established in Azoxystrobin at 0.1% (60.39, 61.66, 61.03) for two years and when average was analyzed. The least b^* values were found in the fruits of control (41.58, 42.58, 42.08). Control fruits did not differ statistically with fruits of *Nerium oleander* at 5% (42.48, 43.56, 43.02) and Tricyclazole at 0.1% (43.69, 45.44, 44.57) when observed during year I, year II and in pooled analysis. The study on C^* of mango peel colour connoted that the Azoxystrobin at 0.1% stood significantly than all the treatments during year I (61.31), year II (62.95) and in pooled analysis (62.13). Distinctively, significantly minimum C^* values were observed in control during the two years of investigation (44.60, 46.12) and even when combined (45.36). The treatments *Nerium oleander* at 5% (45.58, 47.26, 46.42) and *Trichoderma harzianum* at 0.5% (45.68, 47.21, 46.45) were statistically at parity with control at similar intervals. However, the treatment Tricyclazole at 0.1% (47.51) exhibited non-significance with the control during year I (48.11) and in pooled analysis (47.82). Hue angle (h^0) of mango peel colour was significantly minimum in the treatment Azoxystrobin at 0.1% during year I, year II and even after combined analysis (72.55, 68.40, 70.48). The maximum (h^0) value was observed in control (88.81, 87.59 and 88.20) and it was found to be on par with *Nerium oleander* at 5% (87.06, 84.99 and 86.02), *Trichoderma viride* at 0.5% (86.34, 84.65 and 85.50) and *Trichoderma harzianum* at 0.5% (86.16, 84.88 and 85.52) throughout the investigation.

Pulp colour

Pulp colour of mango fruits as influenced by different pre-harvest treatments showed significant differences with respect to L^* , a^* , b^* , C^* and h^0 (Table 3, 4). The L^* (Lightness) values were observed to be significantly higher in the treatment Azoxystrobin at 0.1% during year I (63.55), year II

(64.75) and in pooled analysis (64.16). Minimum and almost close values of L^* in year I was noticed in the treatments control (46.25), Tricyclazole at 0.1% (46.49), *Nerium oleander* at 5% (46.69) and *Trichoderma harzianum* at 0.5% (45.61) and they were on par with *Trichoderma viride* at 0.5% (48.34). During year II, the control fruits control (47.32) recorded the least L^* value and it was followed non-significantly by Tricyclazole at 0.1% (49.68). Statistical analysis of combined data revealed that the control (46.79) continued to document the least L^* value and it was on par with the treatments Tricyclazole at 0.1% (48.09), *Nerium oleander* at 5% (49.01) and *Trichoderma harzianum* at 0.5% (48.63). The inspection of data on a^* (Redness) values of mango pulp colour indicated that treatment Azoxystrobin at 0.1% was significantly higher during year I, year II and even after analysing the pooled data (27.04, 29.82 and 28.43 respectively). In year I, minimum a^* value was noticed in control (13.48) followed by *Trichoderma harzianum* at 0.5% (13.97), *Nerium oleander* at 5% (14.03) and *Trichoderma viride* at 0.5% (15.14). However, in year II, both control (13.22) and *Nerium oleander* at 5% (13.42) were found to record the least and intimate values of a^* and both were at parity with control (15.40) Tricyclazole at 0.1% (16.45). Pooled analysis registered almost similar trend as in year II with lower a^* value in control (13.36) closely followed by *Nerium oleander* at 5% (13.73) and *Trichoderma harzianum* at 0.5% (14.69). The highest significant b^* (Yellowness) values of mango pulp colour was seen in Azoxystrobin at 0.1% (65.42, 67.26 and 66.34) during the two years and when the average was analysed. The least b^* values in year I and year II were found in the fruits of control (48.29 and 50.01) and the treatment was statistically like *Nerium oleander* at 5% (48.95 and 50.85), *Trichoderma harzianum* at 0.5% (49.50 and 51.71) and Tricyclazole at 0.1% (49.43 and 51.50). But after analysis of pooled data, control (49.15) with minimum b^* value was had no significant difference with *Nerium oleander* at 5% (49.90), *Trichoderma harzianum* at 0.5% (50.61) and Tricyclazole at 0.1% (50.47). Mango pulp colour with respect to C^* (Chroma) presented significantly higher values in Azoxystrobin at 0.1% during the individual years and after pooling them (year I- 73.73, year II- 76.64 and pooled- 75.18). On the other hand, lower C^* values were observed in control (52.80) and this treatment was at parity with *Nerium oleander* at 5% (55.27), *Trichoderma harzianum* at 0.5% (55.69) and Tricyclazole at 0.1% (55.81) during the experimental year I. While in year II and pooled analysis, control (54.21 and 53.51) registered lower C^* value and it did not differ statistically with *Nerium oleander* at 5% (55.39 and 55.33) and *Trichoderma harzianum* at 0.5% (55.30 and 55.50). Hue angle (h^0) of pulp colour during year I was minimum in Azoxystrobin at 0.1% (70.32) followed by carbendazim at 0.1% (72.33). Both Azoxystrobin at 0.1% and carbendazim at 0.1% at this period registered significant difference over rest of the treatments. However, in year II and in pooled analysis Azoxystrobin at 0.1% (66.76, 68.54) excelled with significantly minimum h^0 value. In opposition, the maximum and close values of h^0 were shared by the treatments *Trichoderma harzianum* at 0.5% (87.62) and control (87.23) and they were found at parity with *Nerium oleander* at 5% (84.59) and *Trichoderma viride* at 0.5% (84.51). Similarly, in year II and combined data analysis, control with maximum h^0 value (85.18 and 86.21) was

followed by *Nerium oleander* at 5% (82.96 and 83.78), *Trichoderma viride* at 0.5% (82.47 and 83.49) and *Trichoderma harzianum* at 0.5% (82.49 and 85.06).

Discussion

Quality of 'Alphonso' mango is characterized by unique aroma and flavour, perfect sugar-acid blend influencing taste and the presence of appealing colour at edible ripe stage. Anthracnose disease is one of the major constraints for the 'Alphonso' mango growers as it affects the appearance of fruits thereby lowering their quality and market value. In the present investigation, the peel colour of the mango fruits showed superiority with Azoxystrobin at 0.1% in respect of L^* (lightness) (63.38), a^* (redness) (17.66), b^* (yellowness) (61.03), C^* (chroma) (62.13) and h^0 (hue angle) (70.48) values (Table 1, 2). During ripening, dark green chloroplasts in the peel of mango fruits converts into yellow red chromoplasts (John *et al.*, 1970; Lakshminarayana, 1980) [15]. Unripe mango peel encompasses well-arranged grana and osmiophilic globules in the chloroplast cells. This granal membrane loses integrity through ripening and osmiophilic globules appear signifying the conversion of chloroplasts to chromoplast with red or yellow pigments (Parikh *et al.*, 1990) [15]. Thus, significant reduction of latent infection in the field and its manifestation in the storage by pre-harvest application of fungicide Azoxystrobin at 0.1% as seen in the previous experiment (Manasa *et al.*, 2018) [12] appears to have facilitated the proper conversion of peel colour indicating the normal process of ripening in mango fruits. Diseased tissue tends to have higher respiration rates resulting in enhanced ethylene production thus impairing the ripening behaviour (Schiffmann-Nadel *et al.*, 1985) [16]. On the other hand, control fruits that suffered maximum disease stress recorded significantly minimum peel colour values of L^* (lightness) (39.80), a^* (redness) (3.15), b^* (yellowness) (42.08), C^* (chroma) (45.36) and h^0 (hue angle) (88.20). For similar reasons, the treatments *Nerium oleander* at 5%, *Trichoderma viride* at 0.5% and *Trichoderma harzianum* 0.5% followed the control fruits with respect to peel colour. Correspondingly, pulp colour of the fruits showed similar trend where the pre-harvest sprays of Azoxystrobin at 0.1% recorded significantly maximum pulp colour parameters with respect to L^* (lightness) (64.16), a^* (redness) (28.43), b^* (yellowness) (66.34), C^* (chroma) (75.18) and h^0 (hue angle) (70.48) (Table 3, 4). This result can be correlated with the significant increment in the β carotene content (data not shown) obtained under the same treatment in this work. Among the 16 fractions of carotenoids present in the ripe pulp of Alphonso mango, β -carotene occupies 50 per cent of total carotenoids (Jungalwala and Cama, 1963) [6]. Mango carotenogenesis mechanisms involve dephosphorylation with increase in the geraniol and mevalonic acid content and a concurrent surge in carotene content. The enzyme phosphatase that catalyzes carotenogenesis in ripe mangoes (Mattoo *et al.*, 1968) [13] was probably effectively functional in pre-harvest treatment with azoxystrobin than other treatments employed in the study. On the other hand, pulp colour of control fruits was significantly lower with L^* (lightness) (46.79), a^* (redness) (13.36), b^* (yellowness) (49.15), C^* (chroma) (53.51) and h^0 (hue angle) (86.21) values and β -carotene (data not shown) content during storage.

Table 1: Influence of pre-harvest sprays of fungicides, botanicals and bioagents on instrumental analysis of colour of peel (L^* , a^* , b^*) of mango fruits cv. 'Alphonso'

Treatments	Peel colour								
	L^* (Lightness)			a^* (Redness)			b^* (Yellowness)		
	Year I	Year II	Pooled	Year I	Year II	Pooled	Year I	Year II	Pooled
Control	37.52g	42.07f	39.80g	2.61f	3.69f	3.15f	41.58h	42.58g	42.08h
Carbendazim at 0.1%	58.06b	59.26b	58.66b	13.63b	14.80b	14.22b	55.61b	57.02b	56.32b
Tricyclazole at 0.1%	44.12de	49.17d	46.64d	4.84ef	5.95ef	5.40ef	43.69gh	45.44g	44.57gh
Azoxystrobin at 0.1%	63.23a	63.52a	63.38a	16.73a	18.59a	17.66a	60.39a	61.66a	61.03a
Thiophanate methyl at 0.1%	52.49c	54.54c	53.51c	11.95bc	13.46bc	12.71bc	54.42bc	54.60bc	54.52bc
Zineb at 0.1%	46.50d	50.96d	48.74d	9.70c	10.74cd	10.22cd	51.70cd	53.37cd	52.54cd
<i>Eupatorium odoratum</i> at 5%	44.14de	51.50cd	47.82d	9.48cd	10.55d	10.02d	50.28de	50.14de	50.21de
<i>Nerium oleander</i> at 5%	39.48fg	43.06ef	41.27fg	3.61f	4.43f	4.02f	42.48gh	43.56g	43.02gh
<i>Trichoderma viride</i> at 0.5%	41.54ef	45.51e	43.53e	6.89de	7.52e	7.20e	47.46ef	49.14ef	48.30ef
<i>Trichoderma harzianum</i> at 0.5%	40.78efg	43.65ef	42.22ef	4.36ef	5.61ef	4.99ef	45.02fg	45.89fg	45.46fg

Note: Values within the column with the same letter are not significantly different by Duncan Multiple Range Test at $P \leq 0.05$

Table 2: Influence of pre-harvest sprays of fungicides, botanicals and bioagents on instrumental analysis of colour of peel (C^* , h°) of mango fruits cv. 'Alphonso'

Treatments	Peel colour					
	C^* (Chroma)			h° (Hue angle)		
	Year I	Year II	Pooled	Year I	Year II	Pooled
Control	44.60g	46.12f	45.36g	88.81a	87.59a	88.20a
Carbendazim at 0.1%	57.39b	59.18b	58.29b	74.15e	73.01d	73.58e
Tricyclazole at 0.1%	47.51ef	48.11ef	47.82fg	84.28bc	83.35bc	83.82bc
Azoxystrobin at 0.1%	61.31a	62.95a	62.13a	72.55e	68.40e	70.48f
Thiophanate methyl at 0.1%	54.21c	56.29bc	55.25c	77.54d	76.04d	76.79d
Zineb at 0.1%	51.66cd	54.66d	53.16cd	82.20c	80.82c	81.51c
<i>Eupatorium odoratum</i> at 5%	49.10de	52.75d	50.93de	82.34c	80.76c	81.56c
<i>Nerium oleander</i> at 5%	45.58fg	47.26ef	46.42fg	87.06ab	84.99ab	86.02ab
<i>Trichoderma viride</i> at 0.5%	48.48e	49.33e	48.91ef	86.34ab	84.65ab	85.50ab
<i>Trichoderma harzianum</i> at 0.5%	45.68fg	47.21ef	46.45fg	86.16ab	84.88ab	85.52ab

Note: Values within the column with the same letter are not significantly different by Duncan Multiple Range Test at $P \leq 0.05$

Table 3: Influence of pre-harvest sprays of fungicides, botanicals and bioagents on instrumental analysis of colour of pulp (L^* , a^* , b^*) of mango fruits cv. 'Alphonso'

Treatments	Pulp colour								
	L^* (Lightness)			a^* (Redness)			b^* (Yellowness)		
	Year I	Year II	Pooled	Year I	Year II	Pooled	Year I	Year II	Pooled
Control	46.25f	47.32g	46.79f	13.48f	13.22f	13.36e	48.29f	50.01g	49.15g
Carbendazim at 0.1%	59.04b	60.75b	59.90b	23.55b	25.66b	24.61b	61.58b	63.76b	62.67b
Tricyclazole at 0.1%	46.49f	49.68fg	48.09ef	16.44de	16.45ef	16.44d	49.43ef	51.50fg	50.47fg
Azoxystrobin at 0.1%	63.55a	64.75a	64.16a	27.04a	29.82a	28.43a	65.42a	67.26a	66.34a
Thiophanate methyl at 0.1%	56.79bc	59.03bc	57.91b	21.30bc	23.39bc	22.34bc	57.61c	60.25c	58.94c
Zineb at 0.1%	54.43cd	56.10cd	55.27c	19.33cd	21.80c	20.57c	56.58cd	57.08cd	56.83cd
<i>Eupatorium odoratum</i> at 5%	52.13de	53.58de	52.86cd	19.50c	21.17cd	20.34c	54.83d	55.82de	55.33de
<i>Nerium oleander</i> at 5%	46.69f	51.33ef	49.01ef	14.03ef	13.42f	13.73e	48.95ef	50.85fg	49.90g
<i>Trichoderma viride</i> at 0.5%	48.34ef	52.51ef	50.43de	15.14ef	18.09de	16.62d	51.58e	53.75ef	52.67ef
<i>Trichoderma harzianum</i> at 0.5%	45.61f	51.65ef	48.63ef	13.97ef	15.40ef	14.69de	49.50ef	51.71fg	50.61fg

Note: Values within the column with the same letter are not significantly different by Duncan Multiple Range Test at $P \leq 0.05$

Table 4: Influence of pre-harvest sprays of fungicides, botanicals and bioagents on instrumental analysis of colour of pulp (C^* , h°) of mango fruits cv. 'Alphonso'

Treatments	Pulp colour					
	C^* (Chroma)			h° (Hue angle)		
	Year I	Year II	Pooled	Year I	Year II	Pooled
Control	52.80f	54.21g	53.51g	87.23a	85.18a	86.21a
Carbendazim at 0.1%	68.47b	72.72b	70.59b	72.33f	71.68f	72.01e
Tricyclazole at 0.1%	55.81ef	57.32ef	56.57ef	82.63bcd	81.60bcd	82.12bc
Azoxystrobin at 0.1%	73.73a	76.64a	75.18a	70.32f	66.76g	68.54f
Thiophanate methyl at 0.1%	65.54bc	67.74c	66.64c	76.47e	76.62e	76.55d
Zineb at 0.1%	63.48cd	65.49cd	64.49cd	79.59de	79.36cde	79.48cd
<i>Eupatorium odoratum</i> at 5%	61.72d	63.47d	62.60d	80.55cd	78.51de	79.53cd
<i>Nerium oleander</i> at 5%	55.27f	55.39fg	55.33fg	84.59ab	82.96ab	83.78ab
<i>Trichoderma viride</i> at 0.5%	58.65e	59.39e	59.02e	84.51abc	82.47abc	83.49ab
<i>Trichoderma harzianum</i> at 0.5%	55.69ef	55.306fg	55.50fg	87.62a	82.49abc	85.06ab

Note: Values within the column with the same letter are not significantly different by Duncan Multiple Range Test at $P \leq 0.05$

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