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The Effect of Pyraclostrobin Application on Yield of Tea (*Camelia sinensis* (L.) O. Kuntze) under Wet Season

Arief Rahman[1], Eka Tarwaca Susila Putra[2], Sriyanto Waluyo[2]
[1]Postgraduate Student, Department of Agronomy, Faculty of Agriculture Universitas Gadjah Mada
Jln. Flora no. 1, Bulaksumur, Sleman, Yogyakarta 55281, Indonesia
[2]Department of Agronomy, Faculty of Agriculture Universitas Gadjah Mada
Jln. Flora no. 1, Bulaksumur, Sleman, Yogyakarta 55281, Indonesia
***Corresponding email: arrahman.arief@gmail.com**

ABSTRACT
This field experiment aimed to study the effect of pyraclostrobin on tea production and to determine optimum concentration in order to maintain high yield during the period of wet season. The experiment was arranged in Randomized Completely Block Design (RCBD) using two factors (tea clones and pyraclostrobin doses) with three blocks as replications. The pyraclostrobin doses, consisted of 4 levels (0, 50, 100, and 150 $g.ha^{-1}$), each of which were be tested on three tea clones (TRI 2025, Gambung 9 and PGL 15). Data were evaluated for chlorophyll content, photosynthetic rate, number of pecco, number of dormant shoot (banjhi), number of total shoot, pecco fresh weight, banjhi fresh weight, total shoot fresh weight, and shoot dry weight. Data were analysed by Analysis of Variance (ANOVA) and LSD test at $\alpha = 5\%$. The results informed that pyraclostrobin was able to improve chlorophyll content on TRI 2025 clone, and improved photosynthetic rate on Gambung 9 clone. However, there was no significant difference among plants treated with pyraclostrobin on a yield components, i.e., number of pecco, number of banjhi, pecco fresh weight, banjhi fresh weight, total shoot fresh weight, and total shoot dry weight.

Keywords: Concentration, pyraclostrobin, shoot tea, wet season, yield

INTRODUCTION

Tea (*Camellia sinensis* (L.) O. Kuntze) is one of estate crop commodities which is categorized a top export commodity of Indonesia. This commodity plays a strategic role for agricultural development as well as for economy development of Indonesia. Based on data from Indonesian Central Bureau of Statistics (2017), exported tea from local market has been considered a main foreign exchange earnings as it contributes to USD 114,232 million (± Rp 1 trillion) per year. From health aspect, tea has high beneficial nutrients and minerals, such as polyphenol, vitamins, and fluoride (Khan and Mukhtar, 2011; Vanessa and Williamson, 2004).

In Indonesia, tea is commonly cultivated in high altitude so that surrounding environmental condition will fit to its original habitat. Ministry of Agriculture of Indonesia (2014) stated that total area grown for tea plantation was 124,573 ha. From those area, 80,792 ha (more than 60%) was a smallholder tea plantation that was managed by a farmer family. The great potency has recently not been optimized yet due to low productivity of tea plantation in Indonesia. Up to now, tea production in Indonesia was approximately 142.4 tons of fresh leaves per year that gradually decreased comparing to production in late years, mainly from 2009 to 2014 (Indonesian Central Bureau of Statistics, 2015).

Indonesia is a tropical country, having two seasons in a year. During dry season, low rainfall causes growth of tea shoot retarded or leaf size reduced, the air and leaf temperature increased, (Kartawijaya, 1992) and young shoots died, which then contribute to low production (Rahardjo *et al*., 1991). In contrary, during wet season which is followed by high rainfall may cause poor light intensity and inhibit shoot development. High humidity also occurs and potentially causes several diseases. Therefore, one of alternatives to face rainfall variation is by applying certain substrate that is able to maintain crop productivity, besides clone selection and adaptation of newly selected clones that are susceptible to extreme climate.

Pyraclostrobin is one of substrates having potential benefits for restoring plant physiology and surpressing blister leaf blight that is a major disease on tea. Krieger *et al*. (2001) informed that pyraclostrobin is a chemical substance that is classified into strobilurin fungicides. Strobilurins, including pyraclostrobin, work by inhibiting spore germination. The active compounds in strobilurins inhibit mitochondrial respiration by binding to the quinol or ubiquinone oxidation site in the cytochrome-bc1 complex. Binding this site halts important cellular biochemical process, such as electron transfer in the inner membrane of the mitochondria, which prevents adenosine triphosphate (ATP) from forming and so prevents further energy transfer in the fungal spore. Beside as fungicide, pyraclostrobin is also able to work as plant growth regulator that may positively affect physiological process, including increasing water and nutrient absorption rate, increasing nitrate reductase activity, and initiating biosynthesis of auxin (IAA) so as to improve crop productivity (Venancio *et al*., 2003). In addition, other researchers claimed that fungicide containing active derivative compounds of strobirulin may contribute to the increase of leaf photosynthetic activity and the improvement of nitrogen content and quality in wheat grain (Beck *et al*., 2002 cit. Ribeiro *et al*., 2014).

The previous studies informed that tea clones such as TRI 2025, Gambung 9 and PGL 15 have high yield potential. Especially, PGL 15 has high-shoot yield potential and resistance to various levels of rainfall (Krisyando *et al*.,2012; Wijoseno *et al*., 2012; Yuliana *et al*., 2013). This present research was therefore conducted purposively to study the effect of pyraclostrobin on tea production and to determine optimal concentration of pyraclostrobin so as to maintain high production during wet season.

MATERIALS AND METHODS

The experiment was conducted in Production Unit of Pagilaran, PT Pagilaran, Keteleng village, Blado District, Batang Region, Central Java for six months, from January to June 2016. The experiment was arranged in Randomized Completely Block Design (RCBD) using two factors (tea clones and pyraclostrobin doses) with three blocks as replications. The treatments tested on three tea clones were pyraclostrobin doses, consisted of 4 levels, 0, 50 100 dan 150 $g.ha^{-1}$. The commercial pyraclostrobin used was Cabrio 250 EC (contained pyraclostrobin 250 $g.L^{-1}$). Three clones selected were PGL 15, Gambung 9, and TRI 2025. Experiment used the tea plantations overlays by two years pruning age (TP 2) and the conditions were homogeneous. Each overlay was mapped as an experimental plot with an area 720 m^2 (30 m × 24 m). Each experiment plot was further divided into three blocks that served as replication. Each block was further divided into four patch treatments in the same area. Each patch treatment used land area of 23.04 m^2 (4.8 m × 4.8 m). In each patch treatment was sprayed pyraclostrobin with different concentration in accordance with the treatment being tested. Cabrio was applied by spraying it once per plucking cycle with the period of one plucking cycle was 30 days. Spraying was done on the day after tea plucking. Cabrio doses were applied according to treatments arranged on each tea shrub in triplicate. The observation was conducted for chlorophyll content, photosynthetic rate, number of pecco, number of dormant shoot (banjhi), number of total shoot, pecco fresh weight, banjhi fresh weight, total shoot fresh weight, and shoot dry weight.

The observation of chlorophyll content was done by grinding 1 g of leaf in a mortar and pestle and mixing it in 20 mL of acetone 80%. Fine extract was sieved by using filter paper in a beaker glass. The solution was pipetted and put into cuvette up to limit line. Before measuring it with Spectrophotometer (Spectronic 21-D, Milton Roy, Germany), the calibration was done by placing the pure acetone in cuvette as a standard. The first measurement was set at a wavelength of 645 nm and the absorbance was also set to zero value. Afterwards, absorbance value was recorded as the primary data during measurement. The second measurement was set at a wavelength of 663 nm and the first method was repeated. The chlorophyll content was calculated by using the following formula: total chlorophyll content = 0.0202 x A645 + 0.00802 x A663. A645 was absorbance recorded at 645 nm and A663 was absorbance recorded at 663 nm. The measurement of photosynthetic rate was done using Photosynthetic Analyzer (LI CORR 6,400, USA).

The collected data were subjected by Analysis of Variance (ANOVA) according to RCBD and then followed by LSD test at 5% alpha level if there was significant difference among treatments. The statistical analysis was done by SAS program.

RESULTS AND DISCUSSION

Chlorophylls are key pigment molecules that play a major role in photochemical reaction at the reaction center of photosynthesis. The main function of chlorophyll in photosynthetic apparatus includes light absorption, excitation energy transfer to photosystem, and ion charge separation on photosynthetic membrane (Scheer, 2006). In fact, the presence of chlorophyll molecule in photosynthetic apparatus is capable of producing energy efficiently by minimizing the lost energy. Chlorophyll-a works directly in the reaction of conversion of radiation energy into chemical energy and then absorbs and collects the energy into molecule reaction center. Meanwhile, chlorophyll-b works as a radiation energy absorber that is furthermore transferred to chlorophyll-a.

Table 1 showed that there was a significant effect of pyraclostrobin on TRI 2025 clone. At dose of 50 g pyraclostrobin ha^{-1}, chlorophyll content was higher than at dose of 100 and 150 g pyraclostrobin ha^{-1}. Efendi *et al*., (2014) noted that the application of pyraclostrobin were capable of increasing chlorophyll content. The increase of chlorophyll content may stimulate photosynthesis that will contribute to accumulation of photoassimilate in storage organ (called source). Pyraclostrobin is also substrate that contains nitrogen as one of nutrients that is highly needed in chlorophyll synthesis.

Chlorophyll is synthesized in leaf and readily absorbs sunlight at a different amount of interspecies. Chlorophyll synthesis is highly affected by several factors, such as light, sugar or carbohydrate, water, temperature, genetics, and nutrients (N, Mg, Fe, Cu, Zn, S, and O) (Hendriyani and Setiari, 2009). Chlorophyll is an important chloroplast component and its content is positively correlated to photosynthetic rate (Li *et al*., 2006).

As shown in Table 2, PGL 15 and TRI 2025 clone was not significantly affected by pyraclostrobin spray. It was suspected due to the presence of shading leaves that may lower photosynthetic rate as an increase of leaf area expansion. As layers of leaves were added, shading occured within the canopy. At this point, light interception became less in lower leaves so that it decelerated photosynthetic capacity. The photosynthetic capacity was highly affected by the

Table 1. Chlorophyll content under various pyraclostrobin doses and clones in wet season

Pyraclostrobin Dose($g.ha^{-1}$)	Chlorophyll Content ($mg.g^{-1}$ leaf fresh weight)		
	PGL 15	TRI 2025	Gambung 9
0	1.03 a	0.66 a	0.76 a
50	0.93 a	0.78 b	0.85 a
100	0.89 a	0.67 a	0.83 a
150	0.90 a	0.67 a	0.82 a
Mean	0.94	0.70	0.70
CV	11.09	6.21	14.65

Note: Mean values within columns followed by the similar letter were not significantly different by LSD test at 5% alpha level.

Table 2. Photosynthetic rate under various pyraclostrobin doses and clones in wet season

Pyraclostrobin Dose($g.ha^{-1}$)	Photosynthetic Rate ($\mu mol\ CO_2.m^{-2}.s^{-1}$)		
	PGL 15	TRI 2025	Gambung 9
0	154.19 a	153.65 a	151.06 b
50	157.63 a	154.16 a	157.11 a
100	146.84 a	143.49 a	154.11 ab
150	152.35 a	152.68 a	154.87 ab
Mean	152.75	151.00	154.29
CV	2.72	4.04	1.27

Note: Mean values within columns followed by the similar letter were not significantly different by LSD test at 5% alpha level.

Table 3. Number of pecco, number of banjhi, and number of total shoot under various pyraclostrobin doses and clones in wet season

Pyraclostrobin Dose (g.ha^{-1})	Number of pecco (pecco.m^{-2}.plucking^{-1})			Number of banjhi (bud.m^{-2}.plucking^{-1})			Number of total shoot (bud.m^{-2}.plucking^{-1})		
	PGL 15	TRI 2025	Gambung 9	PGL 15	TRI 2025	Gambung 9	PGL 15	TRI 2025	Gambung 9
0	14.25 a	15.50 a	15.25 a	72.75 a	36.92 a	45.00 a	87.00 a	52.42 a	60.25 a
50	11.00 a	12.00 a	14.67 a	92.92 a	32.17 a	40.25 a	103.92 a	44.17 a	54.92 a
100	9.50 a	13.25 a	19.17 a	89.83 a	34.92 a	43.08 a	99.33 a	48.17 a	62.25 a
150	12.08 a	14.17 a	15.08 a	65.92 a	38.92 a	46.00 a	78.00 a	53.08 a	61.08 a
Mean	11.71	13.73	16.04	80.35	35.73	43.58	92.06	49.46	59.63
CV	9.38	28.79	23.20	28.42	16.74	28.57	25.32	9.69	20.54

Note: Mean values within columns followed by the similar letter were not significantly different by LSD test at 5% alpha level. 3) Data were transformed into (log x + 1) prior to ANOVA.

Table 4. Weight of pecco, banjhi, and total shoot under various pyraclostrobin doses and clones in wet season

Pyraclostrobin Dose (g.ha^{-1})	Weight per Shoot (g)								
	Pecco			*Banjhi*			Total		
	PGL 15	TRI 2025	Gambung 9	PGL 15	TRI 2025	Gambung 9	PGL 15	TRI 2025	Gambung 9
0	1.95 a	1.16 a	1.67 a	2.06 a	1.49 a	1.98 a	2.00 a	1.33 a	1.75 a
50	2.00 a	1.12 a	2.19 a	1.89 a	1.35 a	2.10 a	1.94 a	1.24 a	2.10 a
100	1.75 a	1.20 a	1.75 a	1.82 a	1.36 a	1.83 a	1.78 a	1.28 a	1.74 a
150	1.61 a	1.12 a	1.93 a	2.12 a	1.30 a	2.01 a	1.86 a	1.21 a	1.95a
Mean	1.95	1.16	1.67	2.06	1.49	1.98	2.00	1.33	1.75
CV	23.22	14.03	21.59	9.23	10.34	7.99	15.22	7.30	13.26

Note: Mean values within columns followed by the similar letter were not significantly different by LSD test at 5% alpha level.

amount of light received and absorbed in leaf surface.

On Gambung 9 clone, pyraclostrobin spray affected photosynthetic rate positively (Table 2). 50 g dose of pyraclostrobin ha^{-1} gave better result than that of control, followed by of 100 and 150 g of pyraclostrobin ha^{-1}. It was suspected due to the the content of active compound in pyraclostrobin which was able to improve metabolism activity in photosynthesis, including in optimizing the enzyme activity in photosynthesis.

The number of shoots on plucking table was a criteria of tea productivity and capacity (Spillane, 1992) so that more or less the yielding shoot may reflect the tea productivity. Table 3 shows that the number of pecco and banjhi shoot on three clones were not significantly different under various pyraclostrobin doses. During five months treatment, an increase on the number of shoots was not observed yet. In other words, the significant effect of pyraclostrobin spray may take longer time (more than five months). Koehle *et al*. (2003) informed that the response of nitrate absorption increment on wheat was obviously seen 7 days after treatment and its biomass increase was observed after two weeks. It was indicated that the response of tea clones on the increase of N absorption and distribution coming from pyraclostrobin may be seen in months after application and potentially increase the plant growth. Kaido *et al*. (2013) and Nasir (2002) stated that each treatment may give various response from any input from environment and nutrient intake meaning that the maximum yield could be obtained if there was optimal combination from water and nutrient as well as management practice which was responded by plant genetics.

The number of total shoot was the summation between number of pecco and number of banjhi. According to Table 3, it is seen that the number of total shoot was not significantly different among treatments. It was indicated that pyraclostrobin application had not increased the number of shoot for all tested clones yet. It was suspected that environmental factors, such as rainfall and temperature affected the growth and yield of tea. Putri *et al*. (2015) informed that rainfall and temperature highly affected the number of shoots. It was proven by the higher number of pecco at low rainfall rather than that at high rainfall.

The weight per shoot was obtained by the capability of source in distributing photo-assimilate and bud

Table 5. Shoot fresh weight under various pyraclostrobin doses and clones in wet season

Pyra-clostrobin Dose (g.ha^{-1})	Shoot Fresh Weight (g.m^{-2}.plucking^{-1})								
	Pecco			*Banjhi*			Total		
	PGL 15	TRI 2025	Gambung 9	PGL 15	TRI 2025	Gambung 9	PGL 15	TRI 2025	Gambung 9
0	30.53 a	15.13 a	22.61 a	161.83 a	54.71 a	92.30 a	274.82 a	108.95 a	82.23 a
50	20.87 a	13.68 a	26.66 a	175.70 a	45.15 a	93.71 a	269.78 a	91.76 a	83.00 a
100	16.21 a	15.65 a	33.13 a	159.10 a	47.83 a	92.55 a	240.96 a	93.47 a	103.35 a
150	23.18 a	15.03 a	26.86 a	140.54 a	51.57 a	103.66 a	241.33 a	98.51 a	96.06 a
Mean	22.70	14.87	27.32	159.29	49.81	95.56	256.72	98.17	91.16
CV	44.69	18.68	34.57	34.08	17.07	31.59	30.24	15.23	15.31

Note: Mean values within columns followed by the similar letter were not significantly different by LSD test at 5% alpha level.

Table 6. Total shoot dry weight under various pyraclostrobin doses and clones in wet season

Pyraclostrobin Dose(g.ha^{-1})	Total Shoot Dry Weight (g.m^{-2}.Plucking^{-1})		
	PGL 15	TRI 2025	Gambung 9
0	63.23 a	24.27 a	34.84 a
50	55.93 a	20.04 a	35.14 a
100	55.35 a	20.81 a	38.96 a
150	49.34 a	21.82 a	39.28 a
Mean	55.96	21.73	37.06
CV	23.09	13.68	14.72

Note: Mean values within columns followed by the similar letter were not significantly different by LSD test at 5% alpha level.

initiation to sink (De Costa *et al*., 2009). Table 4 presented that the pecco weight treated with pyraclostrobin was not significantly different compared to control. The environmental factors during experiment might contribute to this result. Besides, it was suspected that the presence of accumulative impact from environmental factors several years ago. In addition, pyraclostrobin containing nitrogen might not yield significant effect on plant weight since it might contribute to biosynthesis of amino acid and synthesis of other secondary metabolites. This assumption was in accordance with Chamuah (1988) *cit*. Mondal (2014) who stated that high nitrogen requirement on tea was not only for growth and yield, but also for biosynthesis of amino acids and secondary metabolites.

There were young and old shoots on banjhi. In contrast to pecco, despite more young shoots on banjhi, photosynthate translocation from maintained leaves to the shoot was very low. Even though the weight per shoot on banjhi was higher than that on pecco, banjhi had lower dry weight so that it took longer time to pluck for the upcoming cycle and had higher sensitivity to adapt with climate change than pecco (Visher, 1963 cit. Muningsih, 2014).

Pecco fresh weight of plants treated with pyraclostrobin was similar to those of control plants (Table 5). The pecco fresh weight was affected by the number of pecco and weight per pecco. In this present study, both variables showed insignificant result among all treatments tested. It also occurred on banjhi fresh weight that was not significantly different among all treatments.

Total shoot fresh weight was the accumulation of fresh weight of pecco, banjhi, leaves, and old shoot twig. Table 5 shows that the significant difference among all treatments on total shoot fresh weight was absent. It was suspected that high rainfall resulted in the insufficient amount of light radiation so it inhibited photosynthesis at the end.

As shown in Table 6, the effect of pyraclostrobin doses did not significantly affect the total shoot dry weight. The higher total shoot dry weight potentially led to the higher yield. Muningsih *et al*. (2014) and Yuliana *et al*. (2013) stated that as long as crop canopy did not shade each other, the production per area unit might be greater if there was more shrubs per area unit. If the shading over crops was present, the different planting space might not give significant difference on yield. The crop yield was also determined by soil fertility, sunlight intensity, and optimal rainfall.

De Costa *et al*. (2007) and Mohotti and Lawlor (2002) declared that under mutual shading condition, tea had been naturally equipped with adaptable photosynthesis apparatus according to its function and its maximum capacity. Mutual shading was able to affect the photosynthetic rate directly through the leaf temperature regulation. This present study showed that the photosynthesis function would be accustomed to maximum capacity under mutual shading condition and however, it would affect the yield adversely.

CONCLUSIONS

Pyraclostrobin was able to improve chlorophyll content on TRI 2025 clone and to accelerate photosynthetic rate on Gambung 9 clone on 50 $g.ha^{-1}$ dose apliccation pyraclostrobin. The application of pyraclostrobin could not increase yield on all clones yet or had no significant different among plants treated with pyraclostrobin on yield components, such as number of pecco, number of banjhi, pecco fresh weight, banjhi fresh weight, total shoot fresh weight, and total shoot dry weight.

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