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# Alternative Copper Fungicides for Blister Blight Management

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**ABSTRACT**

Copper fungicides such as copper hydroxide, copper oxychloride and copper oxide of 50% or 37.5% w/w metallic copper are currently recommended for Sri Lankan tea plantations to manage blister blight. The recommended forms of these fungicides are either wettable powder (WP) or wettable granule (WG) formulations. Even though these fungicides are effective in blister blight management, due to environmental and health concerns of Cu, there is a growing demand for fungicides with reduced Cu contents. In the present study, suspension concentrate (SC) formulation of two copper fungicides with reduced Cu contents, copper oxychloride-copper hydroxide mixture (20% w/w metallic Copper) (Airone) and tribasic copper sulphate (26.95% w/w metallic Copper) (Cuproxat), were tested for their field efficacy to manage blister blight of tea. Copper oxychloride- copper hydroxide mixture treatments at 0.12 - 0.16 % and tribasic copper sulphate treatment at 0.5% gave blister blight control comparable to 50 % w/w copper oxychloride treatment. The Maximum Residue Limit (MRL) for copper (40 ppm) in made tea set by European Union (EU) could be achieved 7-day after spraying when copper oxychloride-copper hydroxide mixture and tribasic copper sulphate sprayed at 0.12 - 0.14% and 0.5%, respectively. Cost of copper oxychloride-copper hydroxide mixture is comparable to that of the recommended 50% w/w copper oxychloride WP. However, cost of tribasic copper sulphate is considerably higher compared to the control. Copper oxychloride-copper hydroxide mixture and tribasic copper sulphate suspension concentrates are dust free formulations and readily dissolve in water. Therefore, the new fungicides would be potential alternatives to the recommended copper fungicides to ensure user and environmental safety.

**Key words**: Blister blight, Copper hydroxide, Copper oxychloride, Copper residues, tea

## INTRODUCTION

Blister blight caused by the fungus *Exobasidium vexans* is one the most problematic disease in tea. The disease first appeared in Sri Lanka in 1946 (Tubbs, 1947) and since then it remains challenging to manage. Wind-borne basidiospores initiate the infection on a susceptible leaf. After 6-10 days, translucent spots appear and they develop into white, powdery sporulating blisters in 18-21 days. The disease attacks young harvestable shoots resulting in direct crop loss. Blister blight infection of at nursery stage or young stages (tea in new clearing and recovering tea bushes after pruning) results in shoot dieback affecting the growth and extends the non-productive period of tea.

In the integrated disease management, application of synthetic fungicides is found to be the most effective method of blister blight management (Balasuriya, 2003). Tea Research Institute of Sri Lanka (TRISL) recommends systemic fungicides and contact fungicides for the management of blister blight. DeMethylation inhibitors (DMI) fungicides (FRAC code 3) such as Hexaconazole, Bitertanol, Propiconazole are used as systemic fungicides to manage blister blight in India and Sri Lanka (Baby 2002; Balasuriya, 2003). Copper fungicides have been traditionally used as contact/protective fungicides in blister blight management in Sri Lanka (Tubbs, 1947), China (Chen and Chen, 1982) and India (Baby and Preamkumar, 1999; Seenivasan and Muraleedharan, 2015).

Copper fungicides forming a layer on the leaf, protect the leaf from germinating spores of the fungus. When the fungus enters into the leaf, copper fungicides are of no use. Bordeaux Mixture (mixture of copper sulphate and lime) was first tested and found to be effective in managing blister blight (Annett and Chundra Kar 1910; Chen and Chen, 1982). However, this was very soon replaced by fixed copper oxychloride and copper oxide due to ease of use (Arulpragasam 1986). Copper dusting was abandoned due to several challenges such as lack of uniform distribution in the field, wash-off during the rain, difficulty in application during windy seasons and the requirement of early morning applications. For wet spray, different formulation of copper fungicides including wettable powders and different colloidal forms having varying levels of copper were tested against blister blight (Park and Webster 1956). Copper fungicides with 50% metallic copper were found to be superior and are still used in tea fields. Since discontinuation of Bordeaux mixture, copper sulphate-based formulation has not been used for the disease management in tea in Sri Lanka.

Copper fungicides belong to toxicity class II. Ecological impact on long term copper application has been reported (Zhou *et al.,* 2011). However, the global agricultural systems, both conventional and organic, still rely on copper-based fungicides for disease management against several economically important fungal and bacterial diseases in fruit crops, horticultural crops, vegetables *etc.* (Vitanovic, 2012). This is because copper fungicides are stable, easy to handle and relatively less toxic compared to other metallic elements (Lamichhane *et al*., 2018). However, copper usage reduction strategies have been adopted worldwide in order to reduce the negative ecological and health impacts. The approaches include optimizing time of application, reducing the rate and number of applications, adopting advanced spaying technologies and use of new modern formulations with reduced copper content and high efficacy (Lamichhane *et al*., 2018, La Torre *et al*., 2018). Due to the efficacy of copper against blister blight, tea industry is also in need of identifying an effective copper-based fungicide for better disease management.

Currently available copper fungicides for blister blight management are in the forms of copper hydroxide, copper oxychloride and copper oxide and they are available either as wettable powder (WP) or as wettable granules (WG) formulations of 50% or 37.5% w/w metallic copper. Though the major active ingredient is same among all these fungicides, their efficacy varies depending on the type of the formulation. In the current study, the efficacy of two liquid copper fungicides premix of copper oxychloride-copper hydroxide (Airone) and tribasic copper sulphate (Cuproxat) in managing blister blight of tea was assessed. These two fungicides were selected since they contain less copper content compared to the currently recommended copper-based formulations (50% w/w). For instance, Airone is a mixture of copper oxychloride and copper hydroxide having metallic copper of 20% and Cuproxat consists of tribasic copper sulphate 345 g/l having metallic copper of 26.95% (w/w).

## MATERIALS AND METHODS

### Field evaluation of fungicides against blister blight

The trial I for copper oxychloride-copper hydroxide mixture was conducted at Field No. 02 Upper Division, St. Coombs Estate, TRI, from July to September, 2014. The trial II was conducted at Field No 9, Lower Division, St. Coombs Estate, TRI, from June to August, 2015.

Both trials I and II for tribasic copper sulphate were conducted at Field No. 9, Lower Division, St. Coombs Estate, TRI (at two different locations in the field) from June to August and from September to December, 2015 respectively.

In both copper oxychloride-copper hydroxide mixture and tribasic copper sulphate trials the tea cultivar grown in the fields was TRI 2025 and the fields were in the $1^{st}$ year of pruning cycle. The basic experimental unit, a plot, had 40-50 tea bushes in Randomized Complete Block Design (RCBD). Each treatment had 3 replicates. The treatments were applied weekly after harvesting the leaves until the end of the experiment.

Both fungicides were tested in field conditions with varying concentrations in trials I and II as summarized in the Table 1 below.

Table 1. Treatment structure in copper oxychloride-copper hydroxide mixture and tribasic copper sulphate trials

| Copper oxychloride-copper hydroxide mixture treatments | | Tribasic copper sulphate treatments |
|---|---|---|
| Trial I | Trial II | Trial I & II |
| T1-0.08% Copper oxychloride-copper hydroxide | 0.12% Copper oxychloride-copper hydroxide | T1- 0.1% Tribasic copper sulphate |
| T2- 0.10 % Copper oxychloride-copper hydroxide | 0.14% Copper oxychloride-copper hydroxide | T2- 0.2 % Tribasic copper sulphate |
| T3-0.12 % Copper oxychloride-copper hydroxide | 0.16% Copper oxychloride-copper hydroxide | T3- 0.4 % Tribasic copper sulphate |
| T4-Untreated control | 0.2% Copper oxychloride (50% Cu W/W) | T4- 0.5% Tribasic copper sulphate |
| | Untreated Control | T5- 1.0 % Tribasic copper sulphate |
| | | T6- 0.2% Copper oxychloride 50%W/W |
| | | T7- Untreated Control |

**Blister blight severity assessment**

Blister blight severity was assessed as described in Sinniah *et al.* (2016). In brief, the treated and control plots were plucked separately at 7 to 8-day intervals. One hundred shoots consisted of the bud and three immediate leaves were selected randomly. Blister blight severity was assessed on the 3rd leaf from the bud using 0-6 score blister blight assessment key (Table 2). Disease severity index (DSI) was calculated using the formula

$$\text{DSI} = \frac{Sum\ of\ all\ numerical\ ratings\ x100}{Total\ number\ of\ obervations\ x\ maximum\ score}$$

Sum of all numerical ratings is described as $[(0xn_1) + (1xn_2) \ldots. + (6xn_7)]$ where $n_1$-$n_7$ are number of infected leaves at each score.

Area Under the Disease Progress Curve (AUDPC) was calculated from DSI using the following formula,

$$\text{AUDPC} = \sum_{n}^{n-1} ([(X_i + X_{i+1}) / 2]) [t_{i+1} - t_i]$$

where: $X_i$ is the disease intensity at the $i^{th}$ observation, $T_i$ the time (in days) at the $i^{th}$ observation, and n is total number of observations. AUDPC summarizes the disease intensity over the experimental period.

***Statistical analysis:***

The data were analyzed using two-way ANOVA to see the statistical differences between the treatments and blocks in R statistical software (R 3.3.0).

Table 2. Blister blight severity assessment key for tea considering host reactions or sequential stages of symptom development.

| Score | Description |
|---|---|
| **0** | No translucent spots / blisters /necrotic spots (No disease) |
| **1** | All translucent spots show hypersensitive reaction (HR) |
| **2** | Translucent spots cover **<2%** leaf area |
| **3** | Blisters and/or necrotic spots cover **<5%** of total leaf area. If only translucent spots appear, it covers **2-15%** leaf area |
| **4** | Blisters and/or necrotic spots cover **5-15%** of total leaf area. If only translucent spots appear, it covers **>15%** leaf area |
| **5** | Blister(s) and/or necrotic spots cover **15-30%** of leaf area |
| **6** | Blisters and/or necrotic spots cover **> 30%** leaf area and/or Twig/stem is infected/ die back/ leaf fall/stem breakage |

**Residue trials**

In order to determine the copper residue levels after application, 0.16% copper oxychloride-copper hydroxide mixture and 0.5% tribasic copper sulphate were selected for the analysis. The declining trials were conducted at St. Coombs, Talawakelle during wet season as per FAO guidelines (1986) with two replicated blocks during June-August 2016. Fresh tea leaf samples were taken at 0, 1, 3, 5, 7, 10, 14, 21 days after the application of the fungicides and subjected to miniature processing. Copper residue in the final product was analyzed at Soils and Plant Nutrition Division, TRI following dry ashing method I (Jones, 1972). As copper oxychloride-copper hydroxide mixture at 0.16% resulted Cu residue 40 ppm, higher than the Maximum Residue Limit (MRL) established by European Union for tea, residual effect of copper oxychloride-copper hydroxide mixture at 0.12 and 0.14% also determined on day 0 (before spraying) and 7 days after spraying.

**Cost- benefit analysis**

Cost-benefit analysis was performed taking intended market price of both copper oxychloride-copper hydroxide mixture and tribasic copper sulphate fungicides given by the suppliers and compared with recommended 0.2 % copper oxychloride application. The field cost of fungicide application was considered as same for all the tree fungicides.

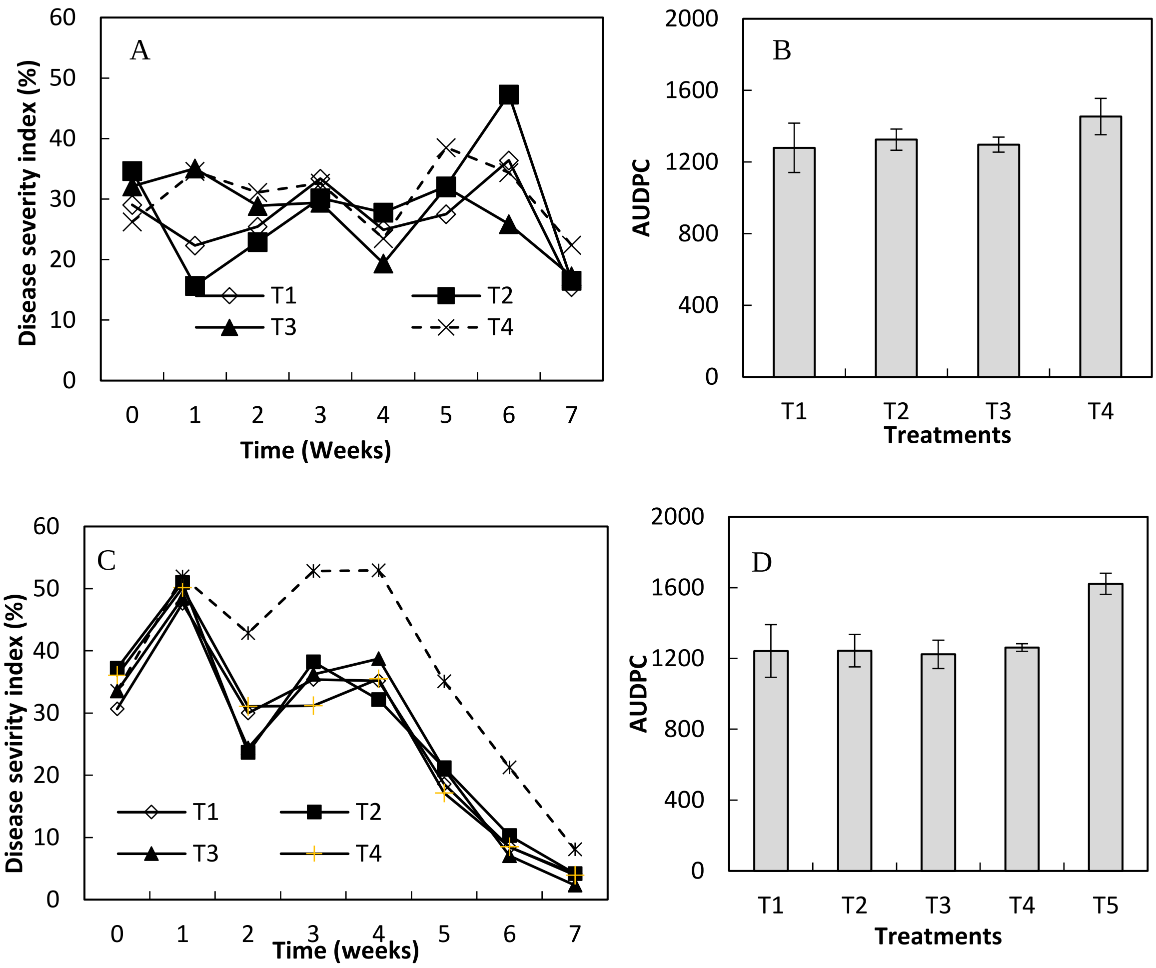


Figure 1. Disease progress curve (A) and a graph (B) showing AUDPC values in experimental plots treated with different concentration of copper oxychloride-copper hydroxide mixture (T1- 0.08%, T2- 0.10 % and T3- 0.12 % and T4-unsprayed control) in Trial I conducted during July to September, 2014. Disease progress curve (C) and a graph (D) showing AUDPC values in Trial II conducted during June to August, 2015. (T1- 0.12%, T2- 0.14 % and T3- 0.16 %, T4-0.2% copper oxychloride and T5-unsprayed control).

## RESULTS AND DISCUSSION

### Testing Copper oxychloride-Copper hydroxide mixture against blister blight

Figure 1 shows the changes in disease severity with time after application of copper oxychloride-copper hydroxide mixture treatments in Trial I and II. Slight reduction in AUDPC was observed in copper oxychloride-copper hydroxide mixture treatments when compared with the untreated control in Trial I. However, AUDPC values were not statistically significant among the treatments (P>0.05). Increased copper oxychloride-copper hydroxide mixture concentrations in trial II resulted disease severity and AUDPC values significantly lower (P<0.05) than the untreated control (Figure 1C, D).

When compared to the standard copper oxychloride treatment, copper oxychloride-copper hydroxide mixture treated plots showed slightly lower AUDPC values. However, the reduction in AUDPC values in copper oxychloride-copper hydroxide mixture treated plots was not statistically significant indicating that the disease control achieved by copper oxychloride-copper hydroxide mixture was comparable to the 50% w/w copper oxychloride. When copper oxychloride-copper hydroxide mixture treatments alone were compared, significant difference in AUDPC was not observed with increased concentration.

### Testing tribasic copper sulphate against blister blight

Figure 2 shows the changes in disease severity with time and AUDPC after application of treatments in trial I and II. In both trials, blister blight disease severity differed among treatments. The differences were statistically significant in trial II but not in trial I. In both trials, blister blight disease severity was higher in unsprayed control compared to the fungicide treatments. There was not significant difference among the copper fungicide treatments (T1 –T6). In the trial II, there was significant reduction in disease severity in terms of AUDPC in tribasic copper sulphate treatments at 0.5% and 1.0% when compared to unsprayed control (p<0.05).

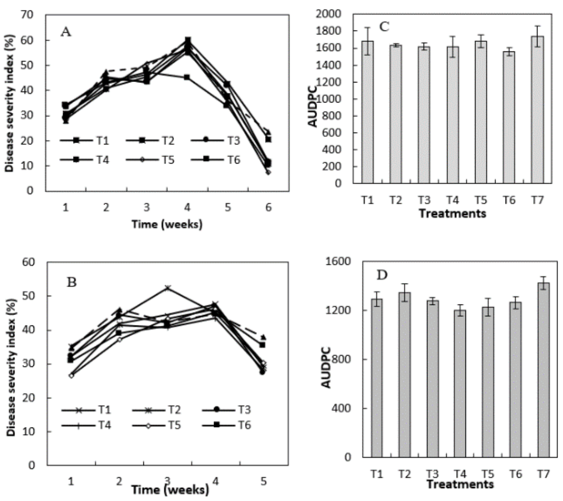


Figure 2. Disease progress curves in trial I (A) and trial II (B) and graphs showing AUDPC values in trial 1 (C) and trial II (D) in experimental plots treated with different concentration of tribasic copper sulphate (T1- 0.1%, T2- 0.2 % T3-0.4% T4-0.5%, T5-1.0%, T6-0.2 % 50% copper oxychloride and T7-unsprayed control.

## Copper residues in made tea

Figure 3 A and B show the levels of Cu residues in the final product processed in the miniature scale after spraying of copper oxychloride-copper hydroxide mixture and Tribasic copper sulphate, respectively. At the recommended pre-harvest interval for Cu fungicide i.e. 7 days after spraying, copper oxychloride-copper hydroxide mixture at 0.16% resulted higher amount (52.5 ppm) of residue than the MRL allowed by EU (40 ppm). When copper oxychloride-copper hydroxide concentration was reduced to 0.12-0.14%, the residue presence in made tea after 7 days was less than 40 ppm. Tribasic copper sulphate spraying at 0.5%, showed residue level 38 ppm 7 days after spraying.

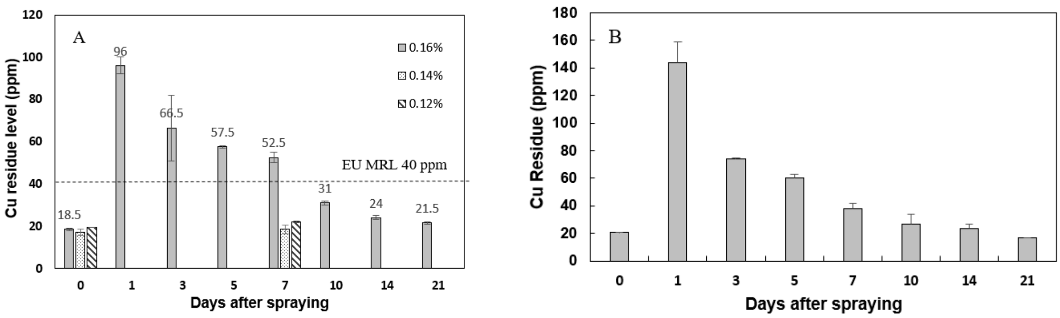


Figure 3. A) Levels of copper residue in the final product when copper oxychloride-copper hydroxide mixture is sprayed at 0.16%, 0.14% and 0.12%. B) Levels of copper residue in the final product when tribasic copper sulphate is sprayed at 0.5%. The broken line indicates the European Union (EU) Maximum Residue Limit (MRL) for Cu 40 ppm. Residue levels were analysed only on day 0 and day 7 for copper oxychloride-copper hydroxide concentration 0.12% and 0.14%.

## Cost benefit analysis

Table 3 shows the cost benefit analysis of copper oxychloride-copper hydroxide mixture and tribasic copper sulphate in comparison with TRI recommended 0.2% copper oxychloride application, keeping the cost of spraying and spraying frequency as constants. As per the analysis, cost of 50% copper oxychloride per hectare was Rs 4.12 lower when compared to copper oxychloride -copper hydroxide mixture. Further, the cost for spraying tribasic copper sulphate per hectare was 2.84 times higher than that of 50% copper oxychloride. The current TRI recommendation appears to be cost effective in comparison to tribasic copper sulphate and copper oxychloride-copper hydroxide mixture.

Table 3. Cost comparison considering only chemical cost (spraying frequency and cost remains same for both fungicides)

| | Copper oxychloride + copper hydroxide at 0.14% | Tribasic copper sulphate at 0.5% | Copper oxychloride at 0.2% |
|---|---|---|---|
| Volume required for a hectare (170 L water) | 238 ml | 850 ml | 280 g |
| Pack size | 1000 ml | 1000 ml | 1000 g |
| Price of 1 L or 1 kg in LKR | 1900 | 1500 | 1600 |
| Coverage with 1000 ml /g in hectare | 4.2 | 1.1 | 3.57 |
| Chemical cost per ha (approximately) | 452.38 | 1275.00 | 448.17 |

Copper oxychloride-copper hydroxide mixture and Tribasic copper sulphate are dust free suspension concentrate formulations and readily dissolve in water. These features are advantageous for handling of these fungicides. Further, both fungicides have lower metallic Cu content. Considering issues with reregistration of copper fungicides at Registrar of Pesticides (ROP) and unavailability of copper fungicide in local market, these new and safer formulations, have higher chances of obtaining product registration through the ROP office and be available at the market place for tea industry.

However, the problem faced during the experiment was measuring the fungicide in small volumes using pipettes. The thick suspension was sticking to the glass surface. Providing measuring cup with non-stick nature by the fungicide suppliers would assist to overcome this issue.

**Summary**

Copper oxychloride-copper hydroxide mixture treatments gave comparable blister blight control to current TRI recommendation 0.2% copper oxychloride treatment. Increasing concentrations of copper oxychloride-copper hydroxide mixture to 0.12 to 0.16% did not result in significant reduction in blister blight severity. Considering environmental and economic benefits, MRL and ease of handling 0.12-0.14% Copper oxychloride-copper hydroxide mixture is suitable for field application against blister blight. The lower dose is for normal monsoon conditions and higher dose is for sever monsoon conditions. Cost of premix of copper oxychloride-copper hydroxide application is more or less comparable to recommended 50% w/w copper oxychloride.

Tribasic copper sulphate treatments gave comparable blister blight control to 50% standard copper oxychloride treatment when results of Trial 2 are considered. The residue level present in made tea 7 days after spraying, satisfied the MRL of EU. However, cost of application of tribasic copper sulphate is significantly high compared to current TRI recommendation 50% w/w copper oxychloride.

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