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Contribution of leaf growth on the disappearance of fungicides used on tea under south Indian agroclimatic conditions

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Abstract: The sprayed chemicals on tea leaves disappear over a period of time by the influence of rainfall elution, evaporation, growth dilution, and photodegradation. Influence of plant growth on the four fungicides (hexaconazole, propiconazole, tridemorph, and c) was studied to know the constructive loss of fungicides. The study shows that residues of fungicides sprayed on tea shoots got diluted by the growing process. The expansion of a leaf took 8 to 11 d and more than 50% of the fungicide residues were cleaned out during this leaf expansion period. Under south Indian agroclimatic condition, the fungicides are sprayed at an interval of 10 d, so it is safe that the tea is harvested on the 10th day of the application of fungicides.

Key words: Tea shoots, Growth, Dilution, Fungicides, Residue loss
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INTRODUCTION

Tea, one of the oldest and most popular beverages in the world, is obtained from the tender leaves of the plant *Camellia sinensis* (L). O. Kuntze. Tea plants are attacked by more than 300 insect and mite pests. The main pests of tea are mites, leafhoppers, plant bugs, aphids, thrips, leaf eating and leaf folding caterpillars and beetles (Muraleedharan, 1991). The tea plant is susceptible to a number of fungal pathogens, of which the fungi *Exobasidium vexans* causing blister blight and *Pestalotiopsis* spp. causing grey blight are of major economic importance in south India. In the absence of any control measures direct crop loss due to blister blight could be as high as 35% (Radhakrishnan and Baby, 2004). Fungicides are chemicals used in the control of fungal diseases. Premkumar and Baby (2005) have published the latest recommendations on the control of blister blight and grey blight in tea, using chemicals such as carbendazim, hexaconazole, propiconazole, and tridemorph. On tea, pesticides and fungicides are recommended for application after harvesting the tea shoots at an interval of 10~14 d throughout the disease prevalent season and they are generally cleaned out by evaporation, growth dilution of tea plant, photodegradation, and rainfall. These systemic fungicides are absorbed by the plant without harming it but inhibiting fungi.

Growth dilution plays an important role in the degradation of pesticide on plant surface, especially with tea plant that shows rapid growth rate (Chen and Wan, 1997). The pesticides sprayed on tea shoots are diluted by the growing process of the shoots. In general, the shoots with one bud and two or three leaves are plucked. Hence, when the pesticide is sprayed on the unfolded buds, its concentration will be diluted with the extension of the shoots. Studies by Garland *et al*.(1999; 2004) showed that decrease in the levels of propiconazole and tebuconazole residues in peppermint crops and boronia is affected by growth dilution. Angioni *et al*.(2003) reported that the increasing weight of the peach fruit during growth leads to the decrease in pesticide levels. Groenewoud *et al*.(1995) attributed the decrease of propiconazole residue levels in boronia leaves to dilution effects as a result of growth. However, no previous work has been found on the important mechanism of growth dilution on tea leaf growth. In the present paper, the residues of certain fungicides used on tea during the leaf growth period of tea were assessed.

MATERIALS AND METHODS

The experimental sites were located in the Anamallai hills in Valparai, India at 1 150 m above mean sea level (MSL). The fungicides were sprayed at the recommended doses, i.e., hexaconazole (Contaf 5 EC) at 200 ml/ha, propiconazole (Tilt 25 EC) at 125 ml/ha, tridemorph (Calixin 80 EC) at 100 ml/ha, and carbendazim (Bavistin 50% wettable powder) using a knapsack sprayer. The structures of these fungicides are shown in Fig.1. Dilution of the fungicide due to leaf expansion was determined by spraying the fungicide on the shoots comprising three leaves and a bud. For each chemical, one bush was selected and the untreated bushes in between were treated as control. The fungicide residues were assessed on one leaf and a bud, two leaves and a bud, and three leaves and a bud (Chen and Wan, 1997).

The fungicides were applied on the tea bushes, and tea shoots comprising three leaves and a bud were collected after 24 h of fungicide application, leaving behind two leaves and a bud and one leaf and a bud that were collected for the analysis of fungicide residues after expansion. When a shoot with the two leaves and a bud grew into a shoot with three leaves and a bud, the fungicides fallen on the two leaves and a bud spread onto three leaves and a bud so that the total quantity of residues was diluted on the larger shoot. The residues were then determined in the shoot with two leaves and a bud and the one with three leaves and a bud. This study was conducted during June 2006 and May 2007. The average rainfall (mm) was 538.4 mm and the minimum and maximum temperatures were 16.9 and 23.9 °C, respectively, in June 2006, and 916.4 mm and 18.2 and 24.0 °C in May 2007. Unfolding and expansion of the third leaf in the crop shoot comprising two leaves and a bud is called leaf expansion. The number of days taken for leaf expansion would vary with months, which was reported to be 8 to 9 d during May and 10 to 11 d in June (Murthy and Sharma, 1989; Sharma, 1979).

Hexaconazole and propiconazole residues in green tea shoots were extracted by the procedure described by Karthika and Sachin (2008) and Karthika and Muraleedharan (2009), respectively. The four common steps involved in the determination of fungicide residues in green leaves were extraction with suitable solvent, partition, elution, and analysis as follows:

Step 1: 10 g of control green leaves samples were placed in a 250-ml conical flask. The extracting mixtures of solvents were 100 ml *n*-hexane:water at 1:1 (v/v) for hexaconazole, 200 ml methanol:water at 1:1 (v/v) for propiconazole, 100 ml 0.02 mol/L hydrochloric acid:methanol at 80:20 (v/v) for carbendazim, and 100 ml of acetone for tridemorph. The mixtures were shaken on a mechanical shaker for 2 h.

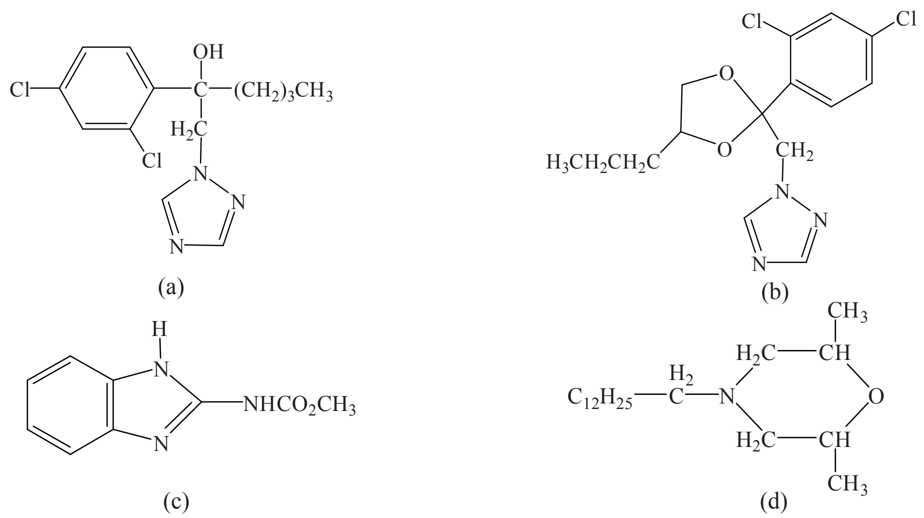

Fig.1 Structures of the four fungicides
(a) Hexaconazole; (b) Propiconazole; (c) Carbendazim; (d) Tridemorph

Supernatant was filtered through Whatmann No. 1 filter paper and transferred to a 500-ml separatory funnel.

Step 2: The filtrate was partitioned by adding 100 ml of saturated sodium chloride (NaCl) to the separatory funnel. For hexaconazole, the aqueous layer was discarded and the organic phase was once again partitioned with 50 ml of saturated NaCl. For propiconazole and tridemorph 200 ml of dichloromethane (DCM) (in three aliquots: 100, 50, and 50 ml) and for carbendazim 100 ml of DCM (in two aliquots: 50 and 50 ml) were added to the funnel and shaken vigorously.

Step 3: The organic phase was passed through a layer of anhydrous sodium sulphate (Na_2SO_4) into a 500-ml round bottom flask to remove moisture. The extract was concentrated to dry using rotary vacuum evaporator at a temperature of 35 °C. The residue was dissolved in specific solvent, 10 ml hexane for hexaconazole and 10 ml DCM for propiconazole, carbendazim, and tridemorph. Purification of the extract was performed by column chromatography using 10 g of preactivated adsorbent florisil (60~100 mesh). Activation of florisil was carried out by keeping it in an oven for 2 h at 400 °C, and the material was cooled, weighed, and transferred to the column. For carbendazim, 15 g of ready-to-use Extrelut N-20 was used as adsorbent material. In case of carbendazim, the residue dissolved in DCM was loaded into the column and adsorbed in Extrelut N-20 for 20 min before elution. Then 250 ml of methanol:DCM (1:1, v/v) was used for eluting carbendazim. Hexaconazole was eluted with 200 ml of 10% (v/v) ethyl acetate in DCM, 250 ml DCM:hexane at 3:1 (v/v) for eluting propiconazole, and 200 ml of acetone for eluting tridemorph.

Step 4: The eluate was evaporated to dry at 35 °C using rotary vacuum evaporator. For the determination of hexaconazole residue, the concentrate was dissolved in 5 ml of acetone, and for the detection of propiconazole, tridemorph, and carbendazim residues, the concentrates were dissolved in 10 ml of high performance liquid chromatography (HPLC) grade methanol prior to chromatographic analysis. HPLC analysis was performed with an Agilent 1100 series. The HPLC chromatographic column consisted of a Zorbax Rx C18 capillary column. The mobile phase used for propiconazole residue analysis was HPLC acetonitrile:(HPLC water+1 mmol ammonium acetate) (80:20, v/v). Detection was carried out at 220 nm wavelength. The flow rate of the mobile phase was 1.5 ml/min and the thermostat temperature during the performance was 25 °C. The mobile phase used for analyses of tridemorph and carbendazim residues was HPLC acetonitrile:HPLC water (80:20, v/v). The flow rate of the mobile phase was 1.0 ml/min and the thermostat temperature during the performance was 25 °C. The detector wavelength for tridemorph was 225 nm while 280 nm for carbendazim. The injection volume of the sample to chromatographic analysis was 10 μl.

Gas chromatographic analysis of hexaconazole was carried out on a Hewlett Packard GC (5890 series II) equipped with DB-5 column coupled with nitrogen phosphorus detector (NPD) and HP 3396 series III integrator. The GC was operated at the oven temperature of 225 °C, injector temperature 220 °C, detector temperature 250 °C, and carrier flow (nitrogen) 5 ml/min for the residue analysis of hexaconazole.

Method performance was assessed by evaluating quality parameters such as recovery percentage, repeatability, reproducibility, linearity, and limits of detection and quantification. Linearity was achieved over the range of 0.10~1.0 mg/kg with a correlation coefficient of 0.999. The instrumental limit of detection (LOD) for the fungicides was 0.10 mg/kg and the limit of quantitation (LOQ) in green tea shoots was 0.3 mg/kg. Recovery of spiked fungicides in control green tea shoots at the levels of 0.3, 1.0, 2.0, 4.0, and 5.0 mg/kg was greater than 90%.

RESULTS AND DISCUSSION

Table 1 presents growth dilution of various fungicides. More than 60% of hexaconazole was cleaned out when a bud and two leaves expanded into a bud and three leaves. The loss was 57% when a shoot with one leaf and a bud expanded into the one with two leaves and a bud. The amount of propiconazole lost when a bud and two leaves expanded into a bud and three leaves was almost 55%. About 59% of tridemorph residue was lost when a bud and two leaves expanded into a bud and three leaves. When a bud and two leaves expanded into a bud and three leaves, the residue of carbendazim on the latter was below 0.1 mg/kg, the lowest detection.

Table 1 Effect of growth dilution on residues*

Chemical	After spray		After expansion	
	Type of shoot	Residue (mg/kg)	Type of shoot	Residue (mg/kg)
Hexaconazole	A bud+three leaves	1.54±0.02		
	A bud+two leaves	0.79±0.03	A bud+three leaves	0.31±0.01
	A bud+one leaf	0.28±0.01	A bud+two leaves	0.12±0.02
Propiconazole	A bud+three leaves	1.18±0.03		
	A bud+two leaves	0.53±0.04	A bud+three leaves	0.24±0.01
	A bud+one leaf	0.16±0.02	A bud+two leaves	<0.1
Tridemorph	A bud+three leaves	0.82±0.01		
	A bud+two leaves	0.37±0.01	A bud+three leaves	0.15±0.02
	A bud+one leaf	0.10±0.03	A bud+two leaves	<0.1
Carbendazim	A bud+three leaves	0.64±0.02		
	A bud+two leafves	0.20±0.01	A bud+three leaves	<0.1
	A bud+one leaf	0.10±0.01	A bud+two leaves	<0.1

* Values as mean±*SD* (*n*=4)

These results show that the residues of fungicides sprayed on tea shoots got diluted by the growing process of the tea plant. The expansion of a leaf took 8 to 11 d and more than 50% of the residues of the fungicides were lost during this leaf expansion period of shoots from two leaves and a bud into three leaves and a bud, which are normally removed during harvest. Since the fungicides are sprayed at an interval of 10 to 14 d depending on the shoot growth and disease incidence, it can be stated that the major part of fungicide residues was cleaned out due to the growth of the tea shoot. Similarly, the residue levels on a bud and one leaf were lost by over 50% when it expanded into a bud and two leaves and in the case of propiconazole, carbendazim, and tridemorph, the residues after leaf expansion were below their lowest detection limit 0.1 mg/kg.

The growth of the tea shoot, however, did not result in the disappearance of the fungicides suddenly but rather gradually. Considerable levels of residues remained on a bud and three leaves after expansion. The slow disappearance of the residues was attributed to the systemic nature of the chosen fungicides (Schermerhorn *et al*., 2005). Once the fungicides are applied, they are absorbed by the plant leaves and translocated upwards in the plant, and persist in the fresh green leaves for a longer period of time. Angioni *et al*.(2003) reported that increasing weight of the peach during growth was the main factor leading to an apparent decrease in pesticide levels on a weight-by-weight basis. Chen and Wan (1997) reported growth dilution as one of the important parameters that contributed to the loss of chemicals sprayed on tea shoots. This work is a preliminary study to evaluate the percentage of residue loss during the said mechanism of growth dilution. In south India, the tea shoots are harvested between 7 to 10 d after fungicide application, and this study shows that during this period, the sprayed chemical is cleaned out significantly due primarily to the expansion of tea shoots; therefore, it is safe to harvest the tea leaves.

CONCLUSION

The above work brings insight on the important degradative mechanism of a sprayed chemical on tea shoots, viz., growth dilution. The study illustrates that almost 50% of the residues were cleaned up due to the extension of shoots from the growth stage of two leaves and a bud to the one of three leaves and a bud. The fungicides employed are all systemic in nature and are known to persist in the sprayed environment for a long period, which may pose a threat for the presence of their residues in the final product. Our study reveals that a major contribution to the loss of residues is influenced by growth dilution. In south Indian tea plantations, the tea shoots are harvested on the 10th day after each fungicide application. The maximum residue limit prescribed by the European Union (EU) is 0.05 mg/kg for hexaconazole and 0.1 mg/kg for the rest of the fungicides. It can be seen that more than 50% of the fungicide residues were lost during the shoot expansion period that varies from 8 d in the slow growth season to 11 d in the fast growth season. Hence upon the day of harvest, i.e., the 10th day, the levels of residues on the tea shoots are definitely lower than the limits due mainly to growth dilution and other cumulative influence of disappearance processes such as rainfall elution, thermal degradation, and photodegradation.

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