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RESEARCH ARTICLE

DETERMINATION OF RESIDUE LEVELS OF NATURAL PYRETHRIN (PYAGRO 4% EC) AND PRE-HARVEST INTERVAL (PHI) ON TEA IN KENYA

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ABSTRACT

Kenya is a one of the major tea-producers of tea in the world .The tea is grown in the highland areas with adequate rainfall and low temperatures. The main tea-growing area is in the Kenyan Highlands, west and east of the Rift Valley, at altitudes between 1800M m. a. s. l. and 2400M m. a. s. l. Tea is a major foreign exchange earner, and the main source for 17 to 20 percent of Kenya's total export revenue. Pests are responsible for yield losses up to 30% in tea world wide. In Kenya pests especially mite pests cause appreciable losses especially during dry period. The most common pests include mite pests, weevils, aphids and mosquito bugs (*Helopeltis sp.).* A number of pesticides have been tested for their efficacy in controlling these pests. Any chemical used to control pests in tea should not contaminate the final product the made tea. Any residues in the product should comply with international standards on the Maximum residue Limits (MRLs). The current study was carried out in order to establish residue levels of Pyrethrins in fresh tea leaves, black tea and brewed tea after foliar application. The study monitored the decline of pesticide residues under normal harvest time intervals and also evaluated the effect of tea manufacturing process on the Pyrethrin residue levels in black tea. Tea samples were collected by picking two leaves and a bud at various pre-harvest intervals after application of the pesticide Pyagro 4%EC at maximum proposed application rates. Results show that the levels of the pesticide residues decreased with increase in the pre-harvest interval in days. The results reveal that residues found in samples collected on the first day after application contain the highest residue levels and those collected fourteen days after application contained the lowest residue levels. The processing and brewing of tea appear to lower the residues of Pyrethrins (Pyagro) significantly. The residue levels from the study are lower than the maximum residue levels (MRLs) allowed within the European Union. Therefore, if Pyagro 4% EC pesticide is used according to the established pattern for control of tea pests it will pose no risk to the consumers of tea.

INTRODUCTION

Kenya is one of the major tea-producers in the world only third to India and Shri-Lanka. It has more than 110,000 hectares of land devoted to tea. In Kenya, tea is grown in the highland areas with adequate rainfall and low temperatures. The main tea-growing area is in the Kenyan Highlands, west and east of the Rift Valley at altitudes between 1800M m.a.s.l. and 2400M m a s l. Tea is a major foreign exchange earner, and the main source for 17 to 20 percent of Kenya's total export revenue. The study of pesticide residues in food is a major component in pesticide development. Each crop or food product for which a pesticide is registered must be analyzed for residues and a tolerance established (Elliot *et al.,*(1972), McEwen *et al*, 1979 EPA, 1986). In the recent years pesticide use has in different crops increased tremendously in East African countries. These are the countries that engage in high valued cash crop production such as floriculture, coffee, tea, cocoa, and cotton. When pesticides are applied to food crops they degrade through chemical and biological processes at a rate determined by the nature of the chemical and plant surface or soil in which the pesticide is placed (Al-Agha *et al.,*2005). This fact is recognized in establishment of tolerances and acceptable daily intake (McEwen *et al,* 1979). The main concern in the study of pesticide residues in food is to ensure safety of the food supply (Kenneth and Hassal (1982), McEwen and Stephenson (1979). Acceptable pesticide daily intake (ADI) has been established for a number of pesticides (McEwen *et al,* 1979). Reports have shown that normal methods of food preparation significantly reduce pesticide residues (McEwen *et al.,* 1979), Meinen and Bergman 1991, Moye,1981, Stefan, 1997). Tea as a product undergoes various preparations from the fresh leaf to the black brewed tea for consumption. The current study establishes the maximum residue likely to be present in raw agricultural commodity i.e. fresh tea leaves, processed tea, and brewed tea. Tea worldwide is susceptible to a number of insect and mite pests, therefore during outbreak conditions application of

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pesticides such as Pyagro 4%EC for control of the pests such as tea thrips, tea aphids, tea mosquito bug (*Helopeltis*) and tea weevils is envisaged. Pyagro 4% EC is a natural Pyrethrin formulation containing 4% EC Pyrethrins. Pyagro 4% EC is a broad spectrum insecticide formulated at pyrethrum Bureau, Kenya for control of mites and other insect pests in a wide range of crops (Casida and Quistad 1995; Samantha and Dan Morris,1995; Wangai and Kitazi 2001). The current study was aimed at determining the residue levels of Pyagro 4% EC, in tea crop when applied at recommended rates. This study is also necessary in order to recommend correct use of pesticides on tea. Pre- Harvest Interval is also important in order to avoid residues in made tea and to adhere to the International Standards.

METHODLOGY

In the field experiments Pyagro 4EC pesticide was used in replicated plots. Sampling of leaves for residue determination was done 0, 7 and 14 days after application so as to establish the pre-harvest interval. This was to ensure that pesticide residue on the crop becomes within acceptable and safe limits for human use (Al-Agha *et al.,* 2005). The plant residues were isolated by liquid-liquid extraction (LLE). Clean up was done using florisil column chromatography (Tekel *et al.,* 2001). Residue data for samples of fresh, black and made tea were determined using Gas Chromatography (GC), fitted with an electron capture detector with a packed column.

Black tea processing

Black tea processing was performed in a miniature-scale tea processing facility at the Tea Research Foundation of Kenya, Kericho. The process is designed to simulate as closely as possible the commercial black tea processing procedure that is standard in Kenya (Stefan, 1997). The tea processing includes withering which results in moisture loss. Biochemical changes also occur within the leaf matrix. Enzymes begin to gradually ferment the leaf material to add the complexity of Flavor and quality of tea. This is followed by leaf maceration a step in which the cell structure of the leaf matrix is physically destroyed to allow fermentation to occur by a process of crush, tear and curl (CTC). This process allows air to circulate into the tea matrix, where oxygen works with enzymes from the plant cells to ferment the entirerity of the matrix. Fermentation or oxidation is a process by which tea quality is achieved. The macerate leaf is placed in several trays whereby humidified ambient air is blown between the trays in order to supply oxygen to the fermentation reaction and also dissipate heat that is generated by the exothermic reaction. Tea drying terminates the fermentation process. The tea is considered dried at moisture content of approximately 2.5 to 4%.

Calibration curves

Known concentrations of the pesticides were analyzed to generate a five point calibration curve of the type stated below:

The Power curve fit: $y = bx^m$ (McKenzie Laboratories, 1995).

Where y = the detector response, peak height
b = y intercept
x = nanograms injected
m = the slope of the line

The standard concentrations were given in terms of nanograms injected for example if 2 µl of 0.1 ppm (0.1ng/ µL) was injected; this is equivalent to 0.2 ng. The power curve was chosen because it gives all the concentrations as positive values including those of peak heights lower than the y-intercept of the calibration line, which would otherwise be given as negative concentration if a linear curve of the form $y=ax + b$ is used. Secondly, the rate at which the concentration changes is not constant because the factors responsible for the change are continually changing. These factors include plant growth, sunlight intensity and amount of rainfall. In cases where data do not follow a linear trend, an exponential or power curve fit is used. (Frank *et al*., 1969)

Calculation method

Sample concentration

The sample peak heights from chromatograms were used in the standard equation obtained to calculate the nanograms found for each sample. The Pyrethrins in all calculations were listed as ppm Pyagro. The ppm of the pesticides was determined from the nanograms found from the calibration equation using the following steps:

$$\text{g-final weight} = \frac{\text{g-initial sample wt} \times \text{mL-aliquot}}{\text{mL-extraction solvent}}$$

$$(\text{ng/mg})\ \text{ppm} = \frac{A \times B \times C}{D \times E}$$

A = ng- found
B = final volume, µL
C = dilution factor
D = µL- injected
E = final weight, mg

After obtaining the concentration of each sample, the mean concentration of each triplicate was determined.

Fortification recoveries

Analytical procedures are validated by fortification of control samples, results within the standard range of 70 % to 120% show acceptable accuracy (Stefan, 1997). Percentage recoveries of fortified control samples were calculated using the equation

$$\%\ \text{recovery} = \frac{\text{ppm found in fortified sample - ppm in control}}{\text{ppm fortification level}} \times 100$$

Treated sample residues were corrected upward using the percentage recoveries for each set of samples.

Statistical analysis

The data was analyzed using M-stat statistical package. The level of significance is at 0.05 (Frank *et al.,*(1969)

RESULTS

The residue levels recorded are based on the total peak heights of all the Pyrethrins peaks detected, therefore decrease in the number of peaks and peak heights imply decline in the pesticide residues. The decrease is caused by growth dilution (spread of the pesticide to new tissues as the plant grows, leading to lower concentrations as the number of tissues increase), photo degradation (i.e. Pyrethrins are decomposed by UV light) and rainfall which could wash away the pesticide from the leaf surface.

Residue decay curves

The residue decay curves given in figures 1, 2 and 3 show a decline in the pesticide concentration in the samples collected 0, 7 and 14 days after pesticide application. Pesticides decay gradually from the time of application and also as they undergo tea preparation procedures. Figure 1 shows decay in the fresh leaves, this decay is mainly attributed to growth dilution, photo degradation and rainfall. Figure 2 show residues in black tea samples which is significantly lower than the residues of the respective fresh tea samples. The difference is as a result of thermal decomposition during the manufacture of black tea. Figure 3 shows residues in brewed tea which are further reduced by high temperatures during brewing. The decay curves for the fresh tea leaf samples go below zero because of the big range between the values for zero day samples (1.077 ppm) and the 14-day value (0.001). Similarly the curve for the black tea sample due the range between the values for zero day samples (0.160 ppm) and the 14-day value (0.0003 ppm)

a) Fresh leaf samples

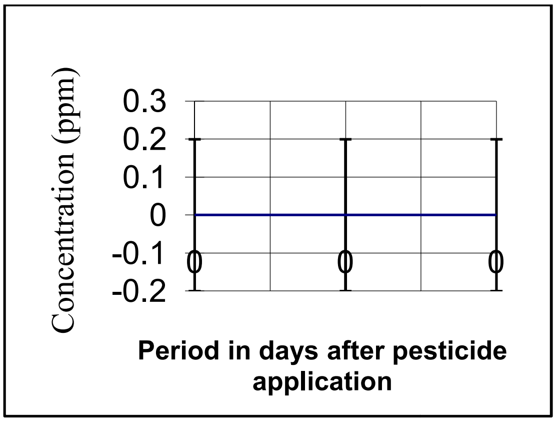

Fig 1: Pyagro fresh leaf samples decay curve

b) Black tea samples

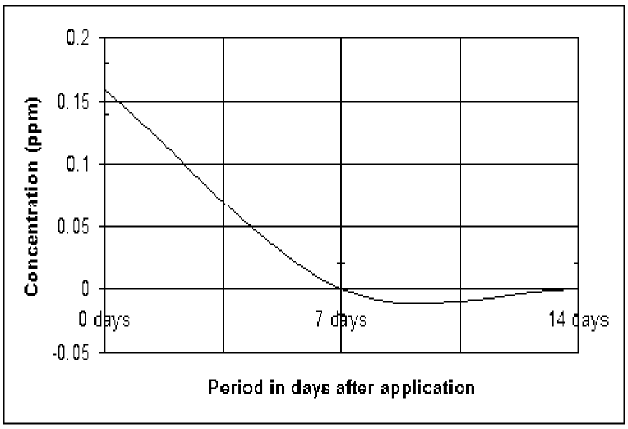

Fig 2: Pyagro Black tea samples decay curve

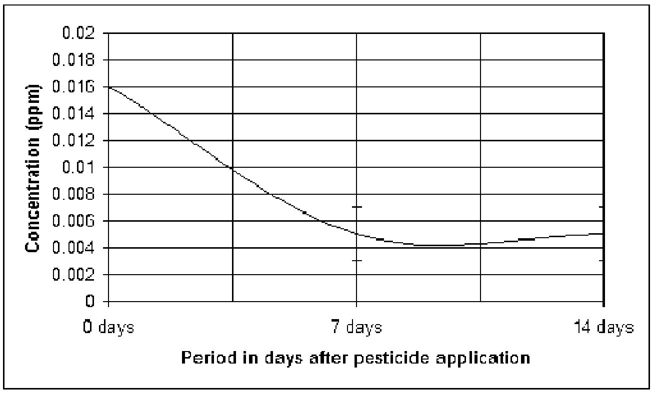

Fig 3: Pyagro Brewed tea samples decay curve

Effect of processing on pesticide residue levels

Pesticides are generally lost before the manufacture of black tea, by evaporation, rainfall, photo degradation and through growth dilution. Further degradation takes place due to thermal decomposition during manufacture of black tea, as the tea is exposed to high temperature. More so, during preparation of brewed tea, pesticide residues contained in black tea are further degraded by high temperature. The reduction of the pesticide residues due to processing are shown in figures 4 and 5. Pesticide residues may leach into the brewed tea or remain in the spent tea depending on their solubility in water. Pyagro residues pass into the brewed tea though in small amounts as shown in figure 4 and 5.

c) Brewed tea samples

Figure 5 shows a decrease in Pyrethrums residues in the different tea matrices, which were collected on the first day after spraying tea with Pyagro. There is a clear drop from 1.077 ppm in the fresh leaf sample to 0.16 ppm in the black tea, a decrease of 85.1%. This decrease could be as a result of exposure to high temperatures of about 120°C during manufacture of black tea. The graph shows a decrease of 90% from 0.16 ppm in black tea to 0.016 ppm in the brewed tea, this suggests that Pyrethrins are soluble in water and leach into the tea infusion. The residue level in the brewed tea is much lower than the residues found in the black tea; this could be attributed to exposure to high temperatures during brewing. The high temperatures cause decomposition of the residues as Pyagro is said to degrade at temperatures above 70^0C (Wangai and Kitazi, 2001; Yamamoto *et al.* (1971).The residue levels found in the 7- day samples are lower than the residue levels found in the 0- day samples. Figure 9 shows a steady decrease from 0.03 ppm in the fresh leaf sample to 0.007 ppm in the black tea, a 76.7% decrease. This decrease could be due to exposure to high temperatures of about 120°C during the manufacture of black tea. Brewed tea contains 0.005 ppm which is lower than the concentration in the black tea by 28.6%. The decrease could be due to exposure to high temperatures during brewing of tea. The high temperatures may have caused decomposition of the residues and subsequent evaporation. (Wangai and Kitazi, 2001).

The residues found in the tea matrices collected on the 14^{th} day after pesticide application were very low thus the difference between the residues in the three matrices may be insignificantly different. The residues are much lower than the residues detected in the 7th day samples. The residues decrease from 0.0007 ppm in the fresh leaf tea matrix to 0.0003 ppm in black tea. This decline could be due to thermal decomposition and subsequent evaporation during manufacture of black tea. The residue found in the brewed tea was found to be 0.0005 ppm, which is higher than the residues in the black tea, this could be due to the low concentrations hence the margin of experimental error could make the values overlap.The graph for the residue values obtained from the 14-day samples may not give the actual effect of processing on residue level therefore it is not presented.

a) 0-day Samples

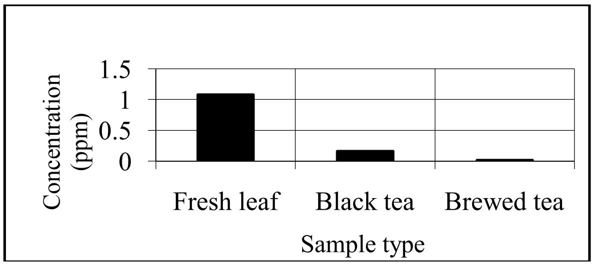

Fig 4: Pyagro 0-day; Effect of processing on residue levels

b) 7-day samples

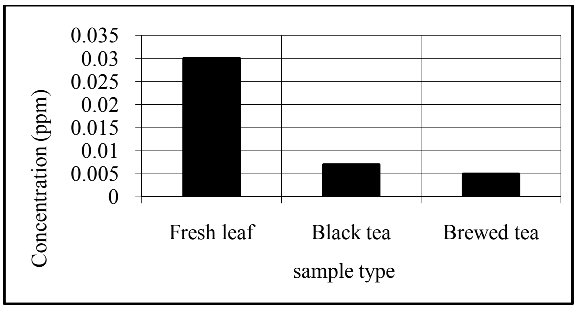

Fig 5: Pyagro 7-day; Effect of processing on residue levels

Conclusion

The residue data shown in the residue decay curves (Figures 1, 2 and 3) and the bar graphs (Figures 4 and 5) show that application of Pyagro 4% EC in fresh leaves result in residues in the tea product and that tea preparation procedures lead to reduction of pesticide residues in the tea product. The residues found in the tea after application of the pesticide at maximum proposed rates of 5kg/ha indicate that the correct time of harvesting (pre-harvest interval) after pesticide application is seven days. It was also established that tea processing and preparation play an important role in reducing the pesticide residue levels. The application parameters considered during the field trial may be proposed for use in Kericho, Kenya. Based on the results presented above; treatment of mature tea bushes with Pyagro 4%EC according to the intended use pattern tested in Kenya resulted in very low residue levels of Pyrethrin (Pyagro 4% EC) residues in brewed tea. Generally it will give low levels of Pyrethrin residues in fresh and black tea.

The pesticide residues found in this study lie below the acceptable Maximum Residue Limits (MRLs) of 0.1ppm in dried (black) tea established within the European Union (E.U).

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