Study the presence of tetramethylthiuram disulfide residue in three selected microgreen species

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ABSTRACT
Microgreens are tender leafy vegetables which are popular among consumers due to their pleasing colors, textures, flavors, and high nutritive values. Microgreens are generally consumed fresh and in relatively large portion sizes. Tender cotyledons of carrot, beetroot, lettuce, amaranthus, cabbage etc. are popular as microgreens among local consumers at present. All most all exotic vegetable seeds found in domestic markets are treated with fungicide (Thiram: Tetramethylthiuram disulfide) thus pose a health risk. Therefore, this study was conducted to identify the presence of fungicide residue in 3 microgreen varieties Amaranthus (*Amaranthus viridis*; var Red Thampala), Carrot (*Daucus carota* subsp. sativus; var New Kurodaand and Mungbean (*Vigna radiata*; var MI 5). Seeds were sawn at 125 g/m^2 rate on trays with a coir dust medium and 30-36 LUX light was supplied continuously. Temperature, pH and RH of the growth medium were maintained at 28–30 °C, 5.5–6.0 and 90–95% while 70–78 % RH and 30–34 °C temperature were maintained as environmental conditions. Height at harvesting of 10 – 14 days old carrot and amaranthus microgreens were 7.5 cm while that of mungbean was 14 cm. Chlorophyll content and fungicide residue were analyzed in harvested microgreens and microbial growth of growth medium was checked. Amaranthuss and carrot had 1.1 CCI and mungbean had 2.4 CCI values as average chlorophyll contents at harvesting. According to FTIR analysis thiram presented only in carrot microgreens and further quantifications are on progress. Mean results of standard plate count of growth medium were 10 CFU/ml in amaranthus and 11 CFU/ml in carrot and mungbean.

Keywords: microgreens, fungicide residue, FTIR.

1 INTRODUCTION

Microgreens are tender immature greens which are produced by seeds of vegetables and herbs with two fully grown cotyledon leaves either with or without the development of a rudimentary pair of first true leaves (Xiao *et al*.,2012) which can be harvested within a period of 7-14 days after germination. (Treadwell *et al*., 2010). These tender greens are used to enhance the color, texture and flavor of salads, soups and sandwiches as a new culinary trend and microgreens have proven to be have numerous health benefits due to the presence of bio active compounds. Studies on microgreens highlight that baby spinach (*Spinacia oleracea* L.) had higher levels of phytonutrients than mature leaves (Lester *et al*., 2010). Oh *et al* reported that lettuce (*Lactuca sativa*) seedlings had highest antioxidant capacity compared to mature leaves.

Even though microgreens are very popular at global scale, there is no much production and consumption in local markets in Sri Lanka due to various reasons. A major difficulty in growing microgreens is the control of fungal infection at germination and early growth (Sharma *et al.*, 2003).

Mostly “Thiram” is used (Tetramethylthiuram disulfide) as a fungicide to protect the crops in the field and to protect the crops harvested during storage and transport. It is also used as a seed protectant to protect fruit and vegetable crops from fungal diseases (Vaneet *et al.,* 2003).

Since microgreens are consumed as fresh in salads or in slightly cooked form, the presence of fungicide residues pose a health threat. However, so far there is no research has been carried out to trace the fungicide travel from seeds to sprouts. Thus, objective of this study was to detect the presence of fungicide residues in the microgreens using Fourier transform infrared spectrometry (FTIR) (Cassella *et al.*,2000) and in the growth medium by fungal analysis in the growth medium when germinating the fungicide treated seeds of Amaranthus (*Amaranthus spinosus* var. Red Thampala), Carrot (*Daucus carota* var. New Kuroda) and Mung bean (*Vigna radiata* var. MI 5).

2 METHODOLOGY

a) Selection of microgreens

Three suitable crop species were selected to proceed the experiment where carrot and amaranths were selected from the category of common microgreens especially as seeds are easily available in the market and higher demand as microgreens when compared to other microgreens and mung bean was selected as a new micro green but a commonly available seed type in Sri Lanka, to analyze its potential as a microgreen. One variety was selected from each including “New curoda” from carrot, “Red thampala” from amaranths and “MI 5” from mung bean.

b) Treat seeds with Thiram and germinate

Already treated seeds of carrot and amaranthus were purchased from the market and mung bean seeds were treated with thiram powder at the same rate of other two types by manual mixing and keeping overnight (10 g of 80 % pure thiram per 50 kg of seeds). Plastic trays (0.077 m^2) were filled with 2.5 cm height coir dust layer as the growth medium and medium was tested for pH and EC. Seeds were sawn separately and evenly in plastic trays with coir dust medium at the rate of 80 g/m^2. Water was sprayed using a spray bottle and trays were kept in a rack with artificial light supply. Light intensity, RH and temperature of the micro environment of seedlings were measured and monitored throughout the research period.

c) Monitoring microgreens

Number of days to germinate and pest and disease incidents were observed while seedling height and chlorophyll content were measured once in three days. Mung bean microgreens were harvested 10 days after germination (just before the emerging of first two true leaves) while amaranths and carrot were harvested 14 days after germination. Seedlings were harvested without roots and initial wet weights were

recorded and oven dried at 105 ˚C for 24 hours to remove moisture. Dry weights of the samples were recorded to calculate moisture content and dry matter content.

d) FTIR analysis

Fourier transform infrared (FTIR) spectra were recorded by a Bio-Rad Tracer interface using the direct deposition technique in combination with the FTS-45 FTIR spectrometer (Bio-Rad Co., Hercules, CA) of dried powdered plant samples after crushed using mortar and pestle. Fungicide residue also analyzed in FTIR.

e) Fungal analysis

In order to evaluate the effect of fungicide residues in the medium, solutions were plated in photatodextrose agar medium and plate count method was conducted to microbial analysis. Growth medium (1g) of each planting trays were measured and dilution series were prepared to plate 0.01ml of solution in Potato Dextrose Agar (PDA) medium plates. Plates were incubated at 25 ˚C for 5 days. All dilutions were done in triplicates and colony count were obtained in each plate to analyze data.

$$\boldsymbol{CFU/ml = \frac{No.of\ colonies \times Dilution\ factor}{plated\ volume}}$$

3 RESULTS AND DISCUSSIONS

a) Monitoring growth conditions

It is recommended to have a pH of 5.5 - 6.5 and a low electrical conductivity less than 500 µS/cm in growing media for micro greens (Abad, Noguera, & Bures, 2001). Even though peat and peat based media are commonly used in micro greens production, coconut coir can be used as an alternative (Prasad, 1997). pH and EC values of used coir dust medium were 5.83 and 421 µS/cm respectively. RH of the micro environment varied between 90 – 95% while soil temperature varied from 28 – 30 ^{0}C and air temperature fluctuated between 30 – 34 ^{0}C throughout the experimental period. Light intensity value was maintained at 30 – 36 LUX. It took 5 days for carrot, 4 days for amaranth and 3 days for mung beans to germinate after sowing.

b) **Harvesting**

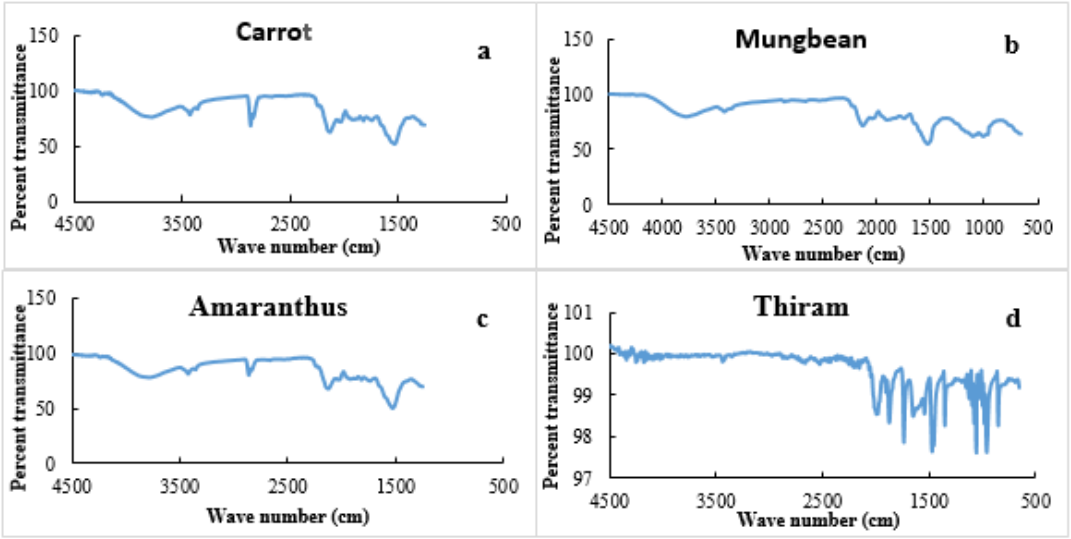
Mung bean was harvested 10 days after germination at the height of 14.2 cm while carrot and amaranthus were harvested after 14 days at the heights of 7 – 8 cm. Average chlorophyll contents at harvesting were 2.4 CCI in mung bean and 1.1 CCI in other two species. Even though mung bean was harvested as 10 days old, there were some immature pubescence present which could lower the consumer acceptability.

c) FTIR analysis

Thiram is a dithiocarbamate fungicide and has reported adverse effects on hepatic system (Dalvi and Deoras, 1986), reproductive system (Bjorge *et al*., 1996; Mishra *et al.,* 1998), developmental process (Korhonen *et al.*, 1982) and residue effects of thiram in diet combined with nitrite has the potential of forming carcinogenic nitrosamine (IARC, 1991). According to European Union considerations, the maximum residue limits for thiram is 7 mg/kg expressed as carbon disulfide and acceptable daily intake is 0.01 mg/kg body weight, toxicity class III WHO (Active ingredient).

The variation of FTIR spectra of different plant material are shown in (Fig.1). The surface functional groups (SFGs) of Thiram and plant material are derived by the organic components in seedlings such as proteins, fat, cellulose and hemicellulose were identified. Three major bands of FTIR spectra were identified at wavenumbers 3200–3500 cm^{-1} and 2820–2980 cm^{-1} corresponding to –OH stretching of alcohol/carboxylic and $–CH_2$ stretching of polar groups respectively. The peak at 885–750 cm^{-1} is representative of out of plane isolated and substituted C–H in the aromatic structure and also of carbonates present. The diminution of the alcohol/carboxylic –OH stretching can be attributed to dehydration whereas the disappearance of $–CH_2$ aliphatic bands indicated a decrease in polar functional groups. According to the peaks observed in the FTIR specturms, trace amount of thiram could be present in the seedlings. However, further analysis will be done in the future using HPLC methods with the extractions of plant materials.

Figure 1: FTIR spectra of microgreens of carrot (a), mung bean (b), amaranthus (c) and thiram powder (d)



d) Fungal analysis

Table 1: Mean Colony forming units of the cultured samples from growth medium

Type of Microgreens	Mean colony forming units of the 10^{-3} diluted soil sample (cfu ml^{-1})
Amaranthus	10
Carrot	11
Mungbean	11

According to the results of microbial counts, countable amount of colony forming units were present in the 10^{-3} soil dilution of all three samples (Table 1). Thereby confirming that even though all three seed types were fungicide treated initially, microbial infections had occurred in the growth medium. There were no significantly different of the colony forming units present between the carrot, mung and amaranthus. A method of soaking seeds in aqueous suspension of thiram have shown eradicate infection by several fungi. However, thiram cannot completely controlled all the fungi growth. The reason might be due to that the concentration of thiram was inadequate to prevent microbial infections or cannot control of some fungi pathogens. The fungicide concentration should be increased further to suppress the microbial growth in the growth medium and molecular studies should be done to further analysis of microbes (Madue *et al*., 1996).

4 CONCLUSIONS

According to the results of FTIR analysis, it was clear that thiram residues were present in all three microgreens and used thiram concentration could not suppress fungal growth totally in the growth medium. Therefore, another experiment is needed to be performed to quantify the thiram residue levels and conclude whether using pre-fungicide treated seeds in microgreen production is acceptable or not. And there is a huge potential of using mung bean as a microgreen by harvesting before the appearing of pubescence. There is a need of conducting another study to decide the best age of harvesting as microgreens for mung bean and other potential legumes of which seeds are freely available in Sri Lanka.

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