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# Insecticide residues in tuber crops and its effect on soil microbes

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## Abstract

Sucking pests are one of the most important problems in tuber crop cultivation. For the management of sucking pests' whitefly, mealybug, scale *etc*., systemic insecticides like imidacloprid and dimethoate are recommended for drenching and spraying. In the present study, these insecticides were tested for their impact on soil micro-organisms and for their presence in cassava.Beneficial soil microbes *Bacillus cereus*, *Beauveria bassiana* and *Trichoderma* spp. were isolated from rhizosphere soil of tuber crops and their growth in insecticide applied media were compared. Residue analysis of the plant samples (cassava leaves and tubers) was conducted after the application of insecticides at recommended doses to the plants. Observations were taken after 1, 7, 14 and 30 days of application. From the LC-MS and GC-MS study, it was found that tuber (edible part) is safe from imidacloprid residue even 24 h after the application. But, 0.051 ppm dimethoate residue was noticed in cassava tubers after 30 days of drenching with the insecticide.

**Keywords:** Insecticide residue, Tuber crops, Soil microbes, Dimethoate, Imidacloprid

## Introduction

The term pesticide encompasses a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others. Among them are organochlorine insecticides (OCs), which have been used successfully to control a number of diseases such as malaria and typhoid and were banned or restricted in most technologically advanced countries after the 1960s (Pathak et al., 2022). The introduction of other synthetic insecticides, organophosphate insecticides (OP) in the 1960s, carbamates in the 1970s and pyrethroids in the 1980s, contributed significantly to pest control and agricultural production. Ideally, a pesticide must be lethal to the target pests but not to non-target species, including humans. Unfortunately, this is not the case, which is why the controversy over the use and misuse of pesticides has come to the surface. The widespread use of these chemicals, true to the saying, 'if little is good, a lot more will be better' is having devastating effects on humans and other life forms (Aktar et al., 2009).

Soil microorganisms are the most abundant biota in soil and are responsible for nutrient and organic matter cycling, soil fertility, soil remediation, plant health and primary production of the ecosystem. Beneficial microorganisms include those that form symbiotic associations with plant roots (rhizobia, mycorrhizal fungi, actinomycetes, diazotrophic bacteria), promote nutrient mineralization and availability, produce plant growth hormones, and are antagonists of plant pests, parasites, or diseases (biocontrol agents). Many of these organisms already occur naturally in the soil, although in some situations it may be beneficial to increase their populations either through inoculation or through

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the use of various agricultural management techniques that increase their abundance and activity. Although microbes are ubiquitous on Earth, their distribution across different habitats is not uniform. Microbial populations are usually found in nutrient-rich niches with a constant supply of easily usable nutrients, such as the rhizosphere. The rhizosphere has an enormous pool of soil microorganisms and is considered a hotspot for microbial colonization and activity. It is the largest ecosystem on earth with enormous energy flow (Barriuso et al., 2008).

Bacteria are the most abundant microbes in the rhizosphere and have a significant impact on the plants growing there. Up to 15% of the total root surface can be covered by different bacterial strains (Van Loon, 2007). The most common bacterial genera reported in the rhizosphere are *Bacillus* and *Pseudomonas*. Different *Bacillus* strains represent the most important Gram-positive inhabitants of the rhizosphere (up to 95% of all Gram-positive soil bacteria) (Barriuso et al., 2008). *Bacillus* can form endospores and produce antimicrobial substances that inhibit other competitors. Representatives of the genus *Bacillus* are increasingly used in agriculture to promote plant growth and to protect against plant pathogens (Qiaoet al., 2017). Antifungal agents are widely used for biological control of both plant fungal diseases and insect pests. Various non-pathogenic (saprophytic) strains of *Trichoderma* spp. have been used to reduce damage (root rot, wilt, desiccation, and bald patches) caused by other pathogenic fungi (e.g., *Pythium, Sclerotium, Verticillium*) (Cook, 1994). According to Mascarin et al. (2019), fungal entomopathogens like *Beauveria* are very good options against (biocontrol agents) arthropod pests. Also, many researchers (Barreto et al., 2004; Imoulan and Elmeziane, 2014; Amnuaykanjanasin et al., 2013) underlined the role of *B. bassiana* as potent entomopathogens.

Over the last 50 years, there has been an increasing use of pesticides in the environment. The ideal pesticide should be toxic only to the target organisms, be biodegradable, and not leach into groundwater. In the European Union, approval systems for new pesticides are governed by common guidelines (Lynch, 1995), which state that effects on microbial processes should be measured by testing a sensitive soil that represents a worst-case scenario. Such effects of pesticides in the environment have classically been studied using functional parameters such as microbial activities in the soil (Greaves, 1982), which are important to hold onto soil nutrients also (Savonen, 1997). Insecticide residues in agricultural products are of great concern due to their potential impact on human health, the environment and food safety. Several types of insecticides are used in agriculture, including organophosphates, pyrethroids, neonicotinoids, and more. Each has different chemical properties and modes of action, which can influence their persistence in the environment and the likelihood of residues on crops. Many countries have established regulations and MRLs for insecticide residues in food. These limits ensure that the level of residues on agricultural products is within safe and acceptable levels for human consumption. Violation of these limits may result in product recalls or restrictions on the sale of products. Research has led to the development and improvement of analytical methods for the detection and quantification of insecticide residues in foods. High-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) are commonly used techniques.

The management of insecticide residues for soil health and safe agricultural produces is a multifaceted issue involving public health, environmental protection, and sustainable agriculture. Ongoing research and the adoption of best practices are essential to ensure food safety, protect the environment, and safeguard public health.

## Materials and Methods

### Materials

Rhizosphere soil was collected from tuber crop fieldsof Thrissur, Idukki and Thiruvananthapuram districts of Kerala, to know the impact of insecticides on important beneficial soil microbes. For culturing bacteria, nutrient agar and for fungi, Rose Bengal Agar (initial screening)/ *Potato Dextrose Agar* (sub-culturing) media were used. Insecticides imidacloprid 17.8% SL and dimethoate 30% EC are used in the study, as these are the most common ones used against sucking pests of tuber crops. For residue analysis LC-MS and GC-MS were employed.

### Methods

Soil collected from rhizosphere of tuber crops and uncultivated soil were taken in earthen pots with 30 cm × 30 cm size. Rhizosphere soils were treated with different concentrations of imidacloprid (0.3, 0.5 and 1.0 ml $l^{-1}$) and made three replications each. Similarly, for dimethoate also three concentrations (1.5, 2.0 and 2.5 ml $l^{-1}$) were used. The soils were sieved to get rid of pebbles and other larger particles. One grams of the fine soils were weighed using weighing balance. Serial dilution was done with 1gm of above samples mixed with 9ml of distilled water ($10^{-1}$). Mixed well and made serial dilutions up to $10^{-6}$.

#### i. Enumeration of microbial population

Bacteria: One ml of samples from $10^{-6}$ dilutions were poured into sterile Petri plates using pipette. Nearly 15 ml of molten nutrient agar was poured over the sample and mixed gently. After the media were solidified, the plates were incubated for 24-48 h. Three replicas were maintained.

Fungi: One ml of samples from $10^{-4}$ dilutions were poured into sterile Petri plates using pipette. Nearly 15 ml of molten Rose Bengal Agar was poured over the sample and mixed gently. After the media were solidified, the plates were incubated for 48-72 h. Three replicas were maintained.

Subculture of promising microorganisms: After the incubation, the growth of the colonies of microorganisms were observed. The colonies were differentiated based on their morphology. The same procedures were repeated for bacteria and fungi. The dissimilar colonies were then inoculated to different test tubes for the purpose of sub culturing (nutrient agar medium for bacteria and potato dextrose agar medium for fungi).

To study the effect of insecticides on bacteria, 60, 100 and 200 $\mu$l imidacloprid (@ 0.3, 0.5, 1.0 $mlL^{-1}$) was added to 200 ml of nutrient agar taken in 500 ml conical flasks. Also, 300, 400 and 500 $\mu$l dimethoate (@ 1.5, 2.0 and 2.5 $mlL^{-1}$) was added to 200 ml of nutrient agar. Agar plates without adding insecticides were taken as control. Streaking was done with selected bacteria having promising growth. Growth was observed after 24 h. Bacterial identification was by using NCBI blast, after DNA isolation, PCR and Sanger sequencing. To study the effect of insecticides on fungi, 60, 100 and 200 $\mu$l imidacloprid (@ 0.3, 0.5, and 1.0 ml $l^{-1}$) was added to 200 ml of PDA taken in 500 ml conical flasks. Again, 300, 400 and 500 $\mu$l dimethoate (@ 1.5, 2.0, and 2.5 ml $l^{-1}$) was added to 200 ml of PDA taken in 500 ml conical flasks. PDA plate without adding insecticides were taken as control. Plating was done with selected promising fungi and observed the growth after 48 h. Fungi were using NCBI BLAST after Sanger sequencing.

**ii. Residue analysis in cassava plant parts**

Insecticides, imidacloprid 17.8% SL and dimethoate 30% EC were taken at different doses. Imidacloprid was taken at doses 0.3 ml $l^{-1}$, 0.5 ml $l^{-1}$, 1.0 ml $l^{-1}$, whereas dimethoate was used at 1.5 ml $l^{-1}$, 2 ml $l^{-1}$, 2.5 ml $l^{-1}$. These insecticides were treated by both spraying and drenching in 5-month-old cassava plants. The plant samples (cassava leaves and tubers) were collected after 1, 7, 14 and 30 days. Residues in the plant parts were detected using LC-MS and GC-MS.

## Results and Discussion

**Effect of insecticides on microbial growth**

After adding insecticides imidacloprid and dimethoate at recommended doses in nutrient agar medium, growth of *Bacillus cereus* was compared. The same procedure was followed for the growth of *Trichoderma* sp. and *Beauveria bassiana* in PDA plates. After inoculation and incubation of the bacteria into Petri plates containing insecticides, it was observed that *Bacillus* growth was comparable in control and imidacloprid added plate, whereas very poor growth/ no apparent growth was observed in dimethoate added one (Fig. 1). Similarly, from the PDA plates it was observed that *Trichoderma* growth was not found in plates containing dimethoate at different concentrations. But growth was detected in plates containing imidacloprid, even though it was lesser compared to control (60 per cent of control's growth after 48 h)(Fig. 2). The trend was similar in case of *Beauveria* also.

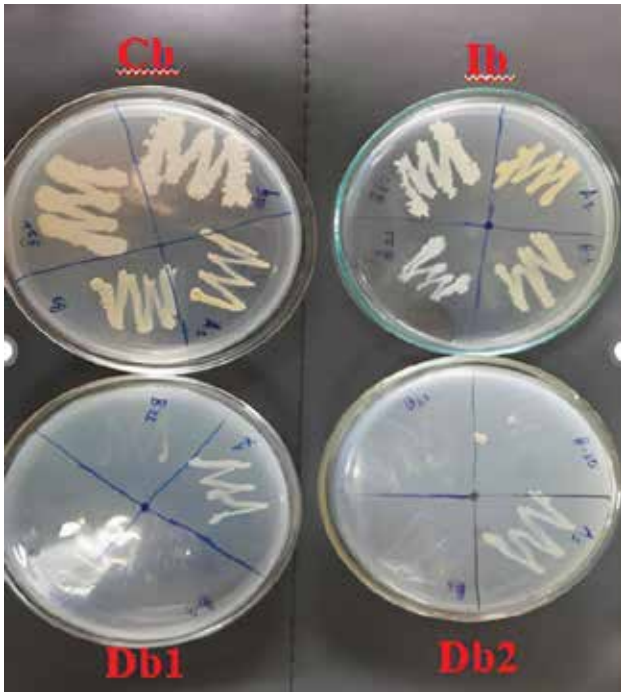

Fig. 1. *Bacillus* growth in nutrient agar plate (Ib-imidacloprid added medium,Cb-Control, Db 1 & Db 2- dimethoate added medium)

Heavy treatment of soil with pesticides can cause populations of beneficial soil microorganisms to decline. According to soil scientist Dr. Elaine Ingham 'Soil deteriorates when we lose both bacteria and fungi'. The excessive use of chemical pesticides has similar effects on soil organisms as the excessive use of antibiotics in humans. Indiscriminate use of chemicals may work for a few years, but after a while there are no longer enough beneficial soil organisms to hold onto the nutrients (Savonen, 1997).

*Bacillus* are ubiquitous in nature (Parker and Duerden, 1990) and a number of members of the genus *Bacillus* are natural agents for biological control of invertebrate pests and are the bases of many biological commercial insecticides (Molina et al., 2010). Studies by Emmert and Handelsman (1999), states that interaction of *B. cereus* with the host plant revealed some promising avenues for improving biocontrol. *Trichoderma* can not only prevent diseases, but also promote plant growth, improve nutrient utilization efficiency, increase plant resistance, and improve environmental pollution caused

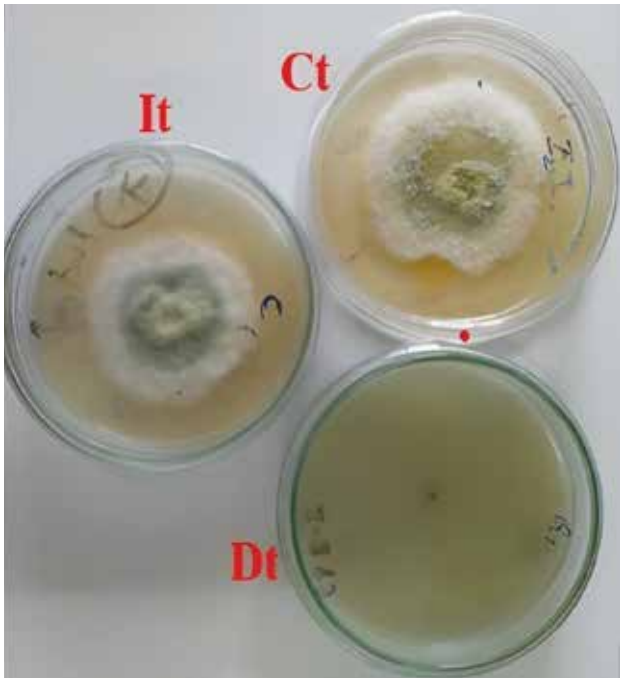


Fig. 2. *Trichoderma* growth in PDA plate (It- imidacloprid added medium, Ct- Control, Dt- dimethoate added medium)

by agrochemicals. Barreto et al. (2004), Imoulan and Elmeziane (2014) and Amnuaykanjanasin et al., (2013) emphasized the role of *B. bassiana* as potent entomopathogens. Based on the study conducted by Dara (2017), *B. bassiana* is compatible with many chemical fungicides. For the control of nymphs and adults of whitefly (*B. tabaci*), among entomopathogenic fungi, *B. bassiana* found to be one of the best options (Harish et al., 2019). Based on the literature available about the potential of soil microbes, *B. cereus, Trichoderma* and *B. bassiana* were used for the study of effect of insecticides on them.

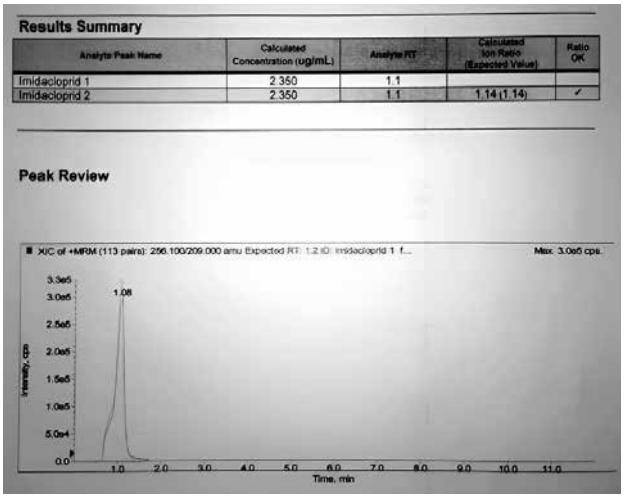


Fig. 3. LC-MS study result of imidacloprid residue in cassava plant 24 h after treatment

Many studies were conducted to realize the persistence of imidacloprid. The works of Juraske et al., (2009), Donnarumma et al., (2011) and Mohapatra et al., (2012) revealed not much of residual toxicity above MRL both in plants and soil for the insecticide. Butin case of dimethoate, like in the present study, found to cause many adverse effects in non-target organisms (Van Scoy et al., 2016). The study conducted by Getenga et al., (2000) shows that dimethoate is highly persistent in soil.

**Residue of insecticides in cassava**

Imidacloprid residue was detected only in the case of spraying, not in drenching and that too in leaves only. The quantity observed was 2.35 ppm after 24 hours of treatment. Residue was not detected in LC-MS study, for IT1D, IT1S and IL1D (Table 1 & Fig. 3). GC-MS study for dimethoate showed, at a spraying dose of 2.5 $mlL^{-1}$, 4.63 ppm of residue was present in leaves after 24 h of treatment. In the same dose, 0.051 ppm of dimethoate residue was noticed in cassava tubers 30 days after drenching (Table 2 & Fig. 4).

Table 1. Imidacloprid residue at various parts of cassava plants one day after treatment (LC-MS)

| Sl. No. | Sample code | Identification code* | Pesticide detected | Residue (ppm) |
|---|---|---|---|---|
| 1 | RF/0231/03/19 | IT1D | Nil | Nil |
| 2 | RF/0232/03/19 | IT1S | Nil | Nil |
| 3 | RF/0233/03/19 | IL1D | Nil | Nil |
| 4 | RF/0234/03/19 | IL1S | Imidaclo-prid | 2.35 |

*(I-imidacloprid, T-tuber, L-leaf, D-drenching, S-spraying)

According to the study, dimethoate is highly persistent in cassava tubers (@ 2.5 $mlL^{-1}$, even after 30 days of drenching. In the present study 0.051 ppm dimethoate could be detected using GC-MS study in tubers (Table 2 & Fig. 4). According to European Commission standards (ECS), 0.01 ppm is the safe limit for dimethoate in food crops (European Commission Standards on food crops,

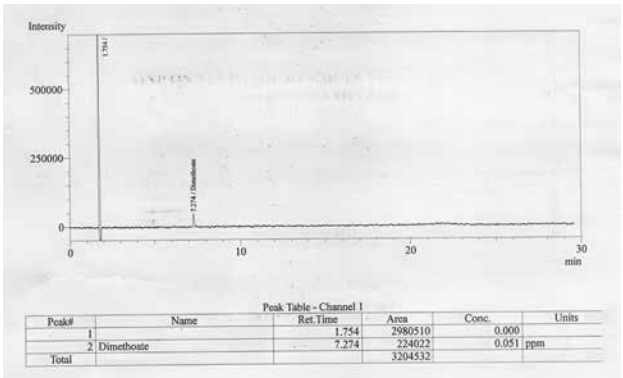


Fig. 4. GC-MS study result of dimethoate residue in cassava tuber 30 days after treatment

Table 2. Dimethoate residue at various parts of cassava plants until 30 days after treatment (GC-MS)

| Sl.No. | Sample code | Identification code | Pesticide detected | Residue (ppm) |
|---|---|---|---|---|
| 1 | RF/0235/03/19 | DT1D | Nil | <LOQ* |
| 2 | RF/0236/03/19 | DT1S | Nil | <LOQ |
| 3 | RF/0237/03/19 | DL1D | Nil | <LOQ |
| 4 | RF/0238/03/19 | DL1S | Dimethoate | 4.63 |
| 5 | RF/0255/03/19 | DT14D | Nil | <LOQ |
| 6 | RF/0256/03/19 | DT14S | Nil | <LOQ |
| 7 | RF/0257/03/19 | DL14D | Nil | <LOQ |
| 8 | RF/0258/03/19 | DL14S | Nil | <LOQ |
| 9 | RF/0259/03/19 | Control (Tuber) | Nil | <LOQ |
| 10 | RF/0260/03/19 | Control (Leaf) | Nil | <LOQ |
| 11 | RF/0274/03/19 | DT30D | Dimethoate | 0.051 |
| 12 | RF/0275/03/19 | DT30S | Nil | <LOQ |
| 13 | RF/0276/03/19 | DL30D | Nil | <LOQ |
| 14 | RF/0277/03/19 | DL30S | Nil | <LOQ |

*LOQ (Limit of Quantification) = 0.05 ppm
(D- dimethoate, T-tuber, L-leaf, D-drenching, S-spraying)

2022). In case of Imidacloprid, residue above MRL could not be detected after one week both in leaves and tubers but detected after 24 h (Table 1 & Fig. 3). The residual problem of dimethoate was observed in drenching not in spraying. So, we can say even if someone use the pesticide, try not to go for drenching and can opt for spraying in shoots.

Likewise, dimethoate toxicity and safety of imidacloprid was explained by Rehberg et al., (2022) in his study. According to El-Sheikh et al., (2022), a study conducted in Egypt shows that dimethoate is above the MRL in many fruits and vegetables collected from farmers' markets and may cause many potential health risks to humans.

## Conclusion

Imidacloprid is found to be safe against beneficial soil microbes compared to organophosphorus insecticide, dimethoate. Also, imidacloprid is comparatively innocuous to use in cassava at recommended dose, whereas, dimethoate may only be used in spraying, but not for drenching in cassava and other tuber crops.

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