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Physicochemical properties and microbial diversity of triazole treated soil of *Solanum lycopersicum* L.

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Abstract
Studying the effect of hexaconazole and paclobutrozole application on physical, chemical and biological properties of soil an experiment was established in 2020 at the Indian Biotrack Research Institute of Thanjavur in Tamil Nadu, India. The aim of this study was to compare the impact of triazoles treated and untreated soil physicochemical properties and biological properties. The treatment of triazoles was prepared from hexaconazole (25mg), paclobutrozole (25mg), combined hexaconazole and paclobutrozole (25+25mg) and without triazole pot for standard. Changes in soil physicochemical properties and microbial community such as were investigated. Some of the physicochemical parameters pH, Electrical conductivity, Organic Carbon, Organic Matter, Available Nitrogen, Available Phosphorus, Available Potassium, Available Zinc, Available Copper, Available Iron, Available Manganese, Cat ion Exchange Capacity, Calcium, Magnesium and Sodium were influencing the growth of plant and microbial population.

Keywords: *Solanum lycopersicum* rhizospere soil, triazole, physicochemical, bacteria, fungi

Introduction
The increasing needs for agricultural production and for ensuring suitable crop safety require the maintaining and improving of soil fertility. Natural materials, industrial wastes, fertilizers, pesticides, mycocides, bacteriocides and by-products can be used for improving the physical and chemical properties and the fertility and biological activity of soils (Angin *et al.,* 2013). Soil properties can be influenced by long-term agricultural management practices as described in pedological literature (Vopravil *et al.*, 2021) [19]. Triazoles, that exhibits high efficacy in plant protection against fungal diseases. It's too frequent use may however, pose risk to soil ecosystems, leading to changes in their biological diversity. (Małgorzata *et al.*, 2021) [7].

Myclobutanil (CAS 88671-89-0) is a broad-spectrum systemic foliar-applied fungicide of the substituted triazole chemical compounds. Exposure may occur at workplaces where it is used or produced and the general population may be exposed via ingestion of contaminated food. The predominate toxicological effects reported in laboratory animals include liver toxicity (degeneration, necrosis, and hypertrophy), testicular atrophy after long-term exposures, and developmental effects (fetal death and skeletal variation). It exhibits high to very high persistence in soil and in aquatic environments it is expected to adsorb to suspended solids and sediment.

A part of the fungicides used in foliar treatment penetrates into the soil. It was changes in the bioavailability of (essential) elements in soil, fructification, the amount of green biomass and the production of phenolic compounds related solely to the presence of triazoles (penconazole and cyproconazole) in soil, injected as a single compound or their mixture. The triazoles presence has substantially affected the bioavailability of Fe, Cu and Zn in soil (Michal *et al.*, 2021) [8]. Hexaconazole and tebuconazole have been registered to control various fungal diseases such as powdery mildew, sheath blight, early and late blight of potato, scab, leaf spot rust, bunt etc. in India. Azoles fungicides are widely used due to their broad spectrum antifungal activities cost effectiveness, systemic action and their long lasting stability in different domains of environment such as soil, water etc., (Pankaj *et al.*, 2020) [10]. One of the most lucrative options is to look into the rhizosphere. Rhizosphere may be defined as the narrow zone of soil that surrounds and get influenced by the roots of the plants. It is rich in nutrients compared to the bulk soil and hence exhibit intense biological and chemical activities. A wide range of macro and microorganisms including bacteria, fungi, virus, protozoa, algae, nematodes and microarthropods co-exist in rhizosphere and show a variety of interactions between themselves as well as with the plant. (Prashar *et al.*, 2014).

Many contact and systemic fungicides are used to control plant pathogens to get disease free crop. Farmers use these chemicals to protect their crop like plantation crop, fiber crop, vegetable and fruit crops etc., from plant pathogenic fungi and their toxins which destroy their entire crop and finally economic loss occurred. But these fungicides also

affect soil microorganisms like decomposers and mycorrhizal fungi etc. Fungicides also show hazardous affect on human health. Soil and water pollution occur due to use of fungicides. Many fungi also play an important role in soil in increasing soil fertility. Some fungi like *Trichoderma, Penicillium, Aspergillus* etc., (Rani *et al*., 2017) [16]. The dissipation of tebuconazole and carbendazim was affected by concentration applied when the two fungicides were applied individually. However, the degradation of both fungicides accelerated at low concentrations and slowed down at moderate to high concentrations. Whether applied individually or in combination, low doses of both fungicides did not impart negative effects (Wang *et al*., 2016) [21].
Effect of triazole based fungicide, propiconazole on soil physicochemical properties, stimulatory impact of propiconazole residue on soil microbial communities (Praveen *et al.,* 2019) [15].

Materials and Methods

Collection of Triazoles

Hexaconazole and Paclobutrozole were purchased from Prakash Agrotech, RR Nagar, Thanjavur.

Pot experiment (Piscitelli *et al.*, 2020) [13]

Seeds of *Solanum lycopersicum* L., were soaked for 15 minutes in tap water and sterilized with sodium hypochloride (5%) for 15 min. Then, they were washed three times with sterile water and sown in glass beakers with sterile soil. Seeds were exposed to sunlight to germinate at the greenhouse. After 30 days the seedlings were transferred into open air to grow in four different experimental pots containing 2 kg of the selected soil. The pot culture experiments was carried out by Indian Biotrack Research Institute, Thanjavur, Tamil Nadu, India.

Treatment combination (Michal *et al.*, 2021) [8]

- USRV 1 - Control
- USRV 2 - Hexaconazole (25mg/100ml)
- USRV 3 - Paclobutrozle (25mg/100ml)
- USRV 4 - Hexaconazole 25mg + Paclobutrozole 25mg (50mg/100ml)

Solanum lycopersicum were planted in the pot experiment and foliarly-treated monthly with the same total triazoles per plant.

Physicochemical Parameters

Soil pH, Electrical Conductivity were determined as described by Mishra (1968) [9]. The organic carbon and organic matter of the soil were estimated by rapid titration methods of Walkly and Black (1934) [20] as described by Piper (1944) [12].
The Available nitrogen, phosphourus, potassium, zinc, copper, iron, manganese was estimated by the Microkjeldhal distillation method (Jackson 1958), Cat ion Exchange Capacity (Kai *et al.*, 2011) [6]. Calcium, Magnesium and Sodium (APHA, 1995) [2].

Isolation of bacteria from soil (Clegg *et al.*, 1998)

The standard serial dilution technique was used for the isolation of bacteria from soil samples. One gram of soil sample was mixed with l0 ml of sterile water and serially diluted (10^{-1} to 10^{-4}). From the serially diluted soil sample, 100 µl was mixed with warm nutrient agar medium and poured into Petri plates. Natamycin (Sigma-Aldrich, USA) at 20 µg/ml was amended with a molten nutrient agar medium at 50 C to prevent fungal growth. After 48 h, the plates had a lawn of mixed bacterial colonies. The individual colonies were picked using sterile toothpicks and streaked onto fresh nutrient agar plates to get pure cultures. The pure culture was stored

Isolation of fungi from soil (Warcup, 1950)

The pooled and collected sample is taken as the represented soil sample. The population dynamics of mycoflora was studied by soil dilution technique with PDA medium (pH 6.5).

Table 1: Analysis of physicochemical parameters from rhizosphere soil of triazole treated *Solanum lycopersicum* cultivated pot

Name of the parameters	Different places			
	USRV 1	USRV 2	USRV 3	USRV 4
pH	6.7	6.6	7.1	6.4
Electrical conductivity (dsm^{-1})	0.36	0.32	0.32	0.41
Organic Carbon (%)	0.46	0.45	0.44	0.38
Organic Matter (%)	0.32	0.45	1.28	0.28
Available Nitrogen (mg/kg)	1.98	1.10	10.8	0.93
Available Phosphorus (mg/kg)	2.06	1.02	8.00	0.35
Available Potassium(mg/kg)	7.03	6.52	8.03	2.8
Available Zinc (ppm)	1.45	1.62	1.04	0.89
Available Copper (ppm)	0.52	0.31	0.88	0.19
Available Iron (ppm)	4.62	3.3	3.28	5.35
Available Manganese (ppm)	1.84	1.51	1.05	0.74
Cat ion Exchange Capacity (C. Mole $Proton^{+}$/kg)	26.6	21.2	18.3	34.5
Calcium (mg/kg)	14.3	9.21	8.43	6.3
Magnesium (mg/kg)	10.5	7.15	7.05	5.2
Sodium (mg/kg)	4.29	3.01	2.29	1.23

Table 2: Isolation and Identification of bacteria from trizole treated rhizosphere soil of *Solanum lycopersicum* pot

Name of the bacteria	CFU/ml				Total no. of species
	USRV 1	USRV 2	USRV 3	USRV 4	
Aeromonas hydrophila	4	-	-	-	4
Bacillus substilis	18	9	11	7	45

Bacillus cereus	17	13	7	6	43
Bacillus firmus	-	8	13	-	21
E.coli	25	6	8	8	47
Pseudomonas fluorescens	8	-	3	4	15
Pseudomonas stutzeri	-	3	10	-	13
Total number of colonies	72	39	52	25	188

Table 3: Isolation and Identification of fungi from trizole treated rhizosphere soil of *Solanum lycopersi cum* pot

Name of the fungi	**CFU/ml**				**Total no. of species**
	USRV 1	**USRV 2**	**USRV 3**	**USRV 4**	
Aspergillus flavus	11	6	14	21	52
A. niger	19	18	23	7	67
A. sydowii	5	4	7	-	16
A. terreus	8	9	6	1	24
A.fumigatus	13	7	8	1	29
Fusarium solani	3	5	2	1	11
Penicillium chrysogenum	15	8	7	6	36
Penicillum citrinum	11	-	5	-	16
Trichoderma viride	-	7	1	1	9
Total number of colonies	85	64	73	38	260

USRV- U. Suresh, R. Velayutham

Result and Discussion

Analysis of physicochemical parameters such as, pH, electrical conductivity, organic carbon, organic matter, available nitrogen, available phosphorus, available potassium, Available zinc, available copper, available iron, available manganese, cat ion exchange capacity, calcium, magnesium and sodium from the different concentration of triazole treated *Solanum lycopersicum* rhizosphere soil.

An organic carbon (0.46%), available manganese (1.84ppm), calcium (14.3 mg/kg), magnesium (10.5 mg/kg) and sodium (4.29 mg/kg) was recorded from the sample of USRV 1 (Control). available zinc (1.62 ppm) was high in USRV 2 (Hexaconazole) highly recorded. The pH (7.1), organic matter (1.28%), available nitrogen (10.8 mg/kg), available phosphorus (8.00 mg/kg), available copper (0.88ppm) and available potassium (8.03 mg/kg) was highly observed in the sample of USRV 3 (Paclobutrozle) compared with other samples. An electrical conductivity (0.41 dsm^{-1}), available iron (5.35 ppm) and cation exchange capacity (34.5 C. Mole $Proton^{+}$/kg) was high in the sample of USRV 4 (Hexaconazole and Paclobutrozole combined) recorded respectively (Table-1).

Piscitelli *et al.*, (2020) was reported the physicochemical analyses revealed a moderately alkaline pH (8.0, in H2O). The electrical conductivity was 0.48 dS/m, the OC was 17.2 g/ kg while CEC value was 17.1 (cmol (+)/kg).

Małgorzata *et al.*, (2021) [7] was reported the tebuconazole was introduced into the soil (sandy loam with pH 7.0) in the following doses in mg kg^{-1} DM (dry matter) of soil: 0.00 (C), 0.02 (O), and 10.0 (T).

The isolation of bacteria from the different soil sample of triazole treated *Solanum lycopersicum* rhizosphere soil by the serial dilution factor of 10^{-4}, 10^{-5} and 10^{-6}. The 72 bacterial isolates from the USRV 1 sample, namely *Aeromonas hydrophila*, *Bacillus substilis*, *B.cereus*, *Eschericia. coli* and *Pseudomonas fluorescens* recorded respectively. 39 bacterial communities observed from the USRV 2 such as *Bacillus substilis*, *B. cereus*, *B. firmus*, *Eschericia. coli* and *Pseudomonas stutzeri* were recorded. 52 isolates identified from the USRV 3 soil sample, isolates names are followed, they are *Bacillus substilis*, *B.cereus*, *B. firmus Eschericia. coli*, *Pseudomonas fluorescens* and *P. stutzeri.* Bacterial isolates (25) identified from the sample of USRV 4 such as *Bacillus substilis*, *B. cereus*, *Eschericia.coli* and *Pseudomonas stutzeri* recorded respectively (Table – 2).

Zuluaga *et al.*, (2020) [23] was stated a total of 101 bacterial strains were found at population densities greater than 1 x 104 cells g-1 fresh weight of soil or unwashed *Solanum* roots.

Pérez-Rodriguez *et al.*, (2020) [11] was studied, a total of 90 bacteria were isolated from tomato roots and rhizosphere (40 and 50, respectively). Then, they were screened for N_2 fixation ability and from them, 36 isolated were able to grow in N-free media (50% from rhizosphere and 50% from roots).

Małgorzata *et al.*, (2021) [7] was reported at evaluating the effect of tebuconazole on population numbers, diversity, and structure of bacterial communities. It caused changes in the population numbers of diversity of bacteria as well as in the biochemical activity of soil. The application of tebuconazole to the soil in a dose of 10.0 mg kg^{-1} soil significantly stimulated the proliferation of organotrophic bacteria and Their population number increased by 62.4% compared to the control sample.

Mycodiversity of triazole treated four different rhizosphere soil sample of *Solanum lycopersicum* having number of fungus were identified, USRV 1 (72), USRV 2 (39), USRV 3 (52) and USRV 4 (25) by dilution facter of 10^{-3}, 10^{-4} and 10^{-5}. The USRV 1 was having a following communities, such as *Aspergillus flavus*, *A.niger*, *A.sydowii*, *A.terreus*, *A.fumigatus*, *Fusarium solani*, *Penicillium chrysogenum* and *P. citrinum* recorded respectively. USRV 2 having a following fungi such as, *Aspergillus flavus*, *A.niger*, *A.sydowii*, *A.terreus*, *A.fumigatus*, *Fusarium solani*, *Penicillium chrysogenum* and *Trichoderma viride* were recorded. USRV 3 soil sample containing following fungi namly as *Aspergillus flavus*, *A.niger*, *A.sydowii*, *A.terreus*, *A.fumigatus*, *Fusarium solani*, *Penicillium chrysogenum* and *P. citrinum* and *Trichoderma viride* were observed. *Aspergillus flavus*, *A.niger*, *A.terreus*, *A.fumigatus*, *Fusarium solani*, *Penicillium chrysogenum* and *Trichoderma viride* were recorded respectively in the sample of USRV 4 (Table – 3).

Shinkafi and Gobir, (2018) [17] was reported that the isolation and identification of Rhizosphere Mycoflora of tomato (*Lycopersicum esculentum*). Rhizosphere soil samples used and sample inoculation was carried was carried out using potato dextrose agar media. A total number of five fungi were isolated *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus oryzae, Rhizopus oryzae* and *Rhizopus stolinifer*.

One hundred fifty-six (156) experienced soils were collected from agricultural farms in Kenya and cultured on Sabouraud DextroseAagar. +e study isolated 48 yielded *Aspergillus fumigatus* and *A. flavus*. Out of the isolates, 3 had MIC of 32 and 1 had MIC of 16 against itraconazole, and 1 isolate had MIC of 32 against posaconazole. CYP51A gene was sequenced, and TR34/L98H mutation was identified. Triazole resistance existing in Kenya calls for rational use of azole-based fungicides in agriculture over concerns of emerging antifungal resistance in clinical practice (Edson *et al.,* 2018).

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