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SENSITIVITY OF THE BACTERIA AGAINST THE LOW CONCENTRATION OF PESTICIDES AND HEAVY METALS

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Abstract– For this study ten frequently used pesticides were used Viz; Cypermethrin, Chloropyriphos, Acephate, Spinosad, Captan, Carbendazim, Metalaxyl, Mancozeb, Chlorothalonil, 2-4-D as per European Union standard concentration. Seven heavy metals were used viz; Copper (cu), Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Arsenic (As), Mercury (Hg) as per Indian standards. Total 21 isolates were isolated and screened for cypermethrin, 16 for chloropyriphos, 11 for acephate, 16 for spinosad, captan 22, carbendazim 16, metalaxyl 12, mancozeb 9, chlorothalonil 14 and for 2-4-D 20 at low concentration of these pesticides. Among the heavy metals total 30 isolates were isolated and screened for copper, for lead 14, cadmium 10, chromium 20, nickel 10, and for arsenic 12. These isolates were tested for sensitivity against low concentration of pesticides and heavy metals. The result of experiment showed that very few isolates CAR-8, CTG-10, DIM-5 showed sensitivity to carbendazim at 2.5 ppm, captan at 1ppm and 2-4-D at 2.5 ppm respectively and isolates NIV-7 and COM-2 showed sensitivity to heavy metals like nickel at 0.04ppm and copper at 0.01ppm concentration.

INTRODUCTION

Railway track farming are carried out from Charni road station till Borivali station along the Western line, from Byculla station till Dombivli station on central line and from Sewri station till Panvel station on harbour line of Mumbai in Maharashtra. Different types of vegetables like Spinach, Lady's finger and Radish are grown throughout the year along these railway tracks. The water source for such agricultural activity is sewage water where their discharge of the effluents is from various industries, water from drainage pipelines and from domestic source, which lead to the contamination of the soil, fruits and vegetables (Doshi and Zele, 2014). Generally heavy metals are not biodegradable they have long half-life with the potential for accumulation in different body organs leading to unwanted side effects (Sathawara *et al.,* 2004). They include Pb, Cd, Zn, Cu, Co, Ni, Ar, Hg and Cr (Sharma, 2006). The total dietary intake of pesticides residues that remain on agricultural commodities are known as carcinogens or toxins and therefore it is desirable to reduce these residues (Zawiyah *et al.*, 2007). The main objectives of the present work are to detect the pesticide residues and heavy metals in fruits and vegetables by using pesticides and heavy metals sensitive bacteria. For this purpose experiment of screening and testing sensitivity of the bacteria against the low concentration of different pesticides and heavy metals were carried out at Department of Plant Pathology and Agril. Microbiology MPKV, Rahuri.

MATERIALS AND METHOD

Isolation and Media used

Isolation of Bacteria were carried out by serial dilution, pour plate and food poison technique (Nene and Thapliyal, 1979). Media used for isolation was Nutrient agar medium (Anon, 1957).

Source of chemicals used

Chemicals used were obtained from Department of Agricultural Entomology and Department of Plant

pathology and Agricultural Microbiology M.P.K.V., Rahuri. Chemicals were used - Cymbush 25% EC, San chloro XtraTc, TaTaAsataf 75% SP, Spintor 45% SC, Captaf 50% WP, Bavistin 50% WP, Raxyl 35% WS, Indofil M-45and Kavach. Heavy metals - Copper Sulphate, Lead Nitrate, Cadmium Chloride, Chromium Trioxide, Nickel Sulphate, Arsenic Trioxide, and Mercury Chloride.

Method

Screening of isolates at low concentration of individual pesticides and heavy metals

After 4-5 days of incubation, plates were observed. Those colonies were not seen in the treated plates containing soil and water samples and low concentration of pesticides and heavy metals, but colonies seen in the control petri plates containing soil and water samples with no pesticides and heavy metals were selected. These isolates were further screened and purified.

Testing of isolates for their sensitivity to respective pesticides and heavy metals

Isolates were tested for their sensitivity against respective pesticides and heavy metals at low concentration by streaking plate technique. The sterilized nutrient agar medium of 500 ml of each pesticides of each concentration as per given in a treatment were prepared and poured in sterilized Petri plate just before solidification (40 °C temperature) under aseptic conditions. After solidification, the plates were streaked using isolates which were screened for particular pesticides at EU standard concentration. Same procedure was followed for streaking of plates for particular heavy metals at Indian standard concentration. Then plates were kept at 28±2 °C in Bacteriological incubator for 4-5 days.

RESULTS AND DISCUSSION

The result of experiment at 0.05, 1.25 and 2.5 ppm concentration of Cypermethrin 21 isolates were screened and tested, out of them no any isolate showed sensitivity to Cypermethrin at 0.05, 1.25 and 2.5 ppm by poison food technique. Same results were seen at 0.05, 1.25, 2.5 ppm concentrations of Chloropyriphos, at 0.01, 0.25 and 0.5ppm concentration of Acephate, at 0.05, 1.25 and 2.5 ppm concentration of Metalaxyl, at 0.07, 1.75 and 3.5 ppm concentration of Spinosad, at 0.01, 0.25 and 0.5 ppm concentration of Chlorothalonil and at 0.05, 1.25 and 2.5 ppm concentration of Mancozeb. At 0.1, 2.5 and 5ppm concentrations of Carbendazim 16 isolates were screened, out of them Isolate CAR-8 showed sensitivity to Carbendazim at 2.5 ppm (Plate 1) and at 0.1ppm and 5ppm all the 16 isolates showed the full growth on nutrient agar plate. When Carbendazim–Mancozeb fungicidal mixture applied at concentration of 2.34mg.kg^{-1} soil had a greater ($p<0.05$) inhibitory effect on nitrogen fixers, nitrifying bacteria and cellulolytic organisms (Fawole *et al.*, 2009). Few isolates of *Septoriapyricola* failed to form colonies with Carbendazim concentration 0.1 to 10 mg.L^{-1}(Pappas *et al.*, 2010). At 0.02, 0.5 and 1ppm concentration of Captan, 22 isolates were screened, out of them one isolateCTG-10 showed sensitivity at 1 ppm concentration of Captan (Plate 2). (Banerjee and Banerjee (1987) reported that in captan treated soil total count of fungi, bacteria and actinomycetes decreased significantly only at a relatively high fungicide concentration 1ppm (Di-ciocco and Rodriguez, 1997) reported that the 8 h growth in rotary shaker of *A.brasilense* was inhibited with 1mg. L^{-1} pure captan. At 0.05, 1.25 ppm concentration of 2-4-D 20

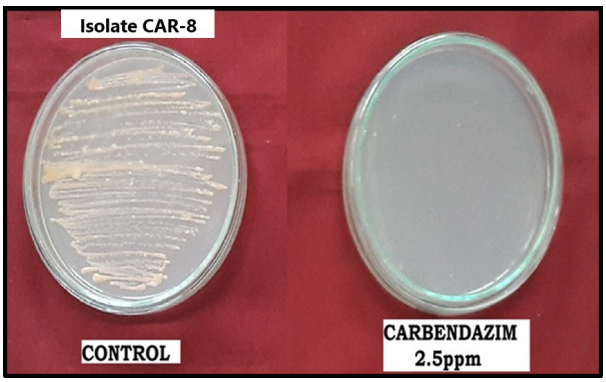

Plate 1. Sensitivity of Bacteria at 2.5 ppm concentration of Carbendazim

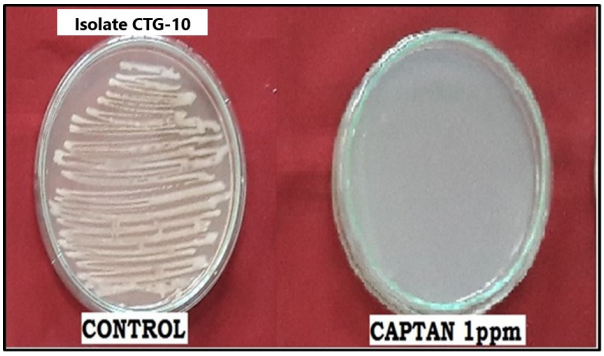

Plate 2. Sensitivity of Bacteria at 1 ppm concentration of Captan

Table 1. Permissible limits for Heavy metals in $mg.kg^{-1}$

Indian Standard Awasthi (2000)	Cu	Pb	Cd	Cr	Ni	As	Hg
	30.0	2.5	1.5	20	1.5	1.1	NL

NL=no limit

Table 2. Maximum Residue limits of Pesticide in Fruits and Vegetables

Pesticides	Europian Union Standard (2016) in $mg.kg^{-1}$
1.Cypermethrin	0.05
2.Chloropyriphos	0.05
3.Acephate	0.01
4.Spinosad	0.07
5.Captan	0.02
6. Carbendazim	0.1
7.Metalaxyl	0.05
8.Mancozeb	0.05
9.Chlorothalnil	0.01
10.2-4-D	0.05

Treatment

1. Treatment + soil samples
2. Control= Soil samples
3. Treatment + Water samples
4. Control= Water Samples

Pesticides		Treatment		
1.	Cypermethrin	0.05	1.25	2.5
2.	Chloropyriphos	0.05	1.25	2.5
3.	Acephate	0.01	0.25	0.5
4.	Spinosad	0.07	1.75	3.5
5.	Captan	0.02	0.5	1.0
6.	Carbendazim	0.1	2.5	5.0
7.	Metalaxyl	0.05	1.25	2.5
8.	Mancozeb	0.05	1.25	2.5
9.	Chlorothalonil	0.01	0.25	0.5
10.	2-4-D	0.05	1.25	2.5
Heavy metals		Treatment		
1.	Copper (Cu)	30.0	0.01	
2.	Lead (Pb)	2.5	0.5	
3.	Cadmium (Cd)	1.5	0.07	
4.	Chromium(Cr)	2.0	1.00	
5.	Nickel(Ni)	1.5	0.04	
6.	Arsenic(As)	1.1	0.05	
7.	Mercury(Hg)	NL	NL	

isolates were screened, out of them none of the isolates showed sensitivity to 2-4-D at 0.05, 1.25. At 2.5 ppm concentration isolate DIM-5 showed sensitivity to 2-4-D. (Plate 3) (Worth *et al.*, 1948) reported that growth of the aerobic bacteria was prevented by 0.0002 % 2-4-D. Herbicide 2-4-D, atranex and agroxone inhibited the occurrence of *Rhizobium phaseoli* and *Azatobacter vinelandii* and their population further decreased with increase in herbicide concentration (Meena *et al.*, 2019).

In the heavy metals experiment at 1.5 and 0.04 ppm concentration of Nickel 10 isolates were screened, out of them none of the isolates showed sensitivity to Nickel at 1.5ppm, except NIV-7 showed sensitivity at 0.04ppm concentration by serial dilution, pour plate and poison food technique (Plate 4). Toxic effect of nickel, cadmium and EDTA on growth of the plant growth promoting *Rhizobacterium, Pseudomonas brassicacearum.* The inhibition was much greater at low concentration of Ca^{2+} (25 umol/l) and (Mg^{2+} 100 umol/l) (EC 50 Cd^{2+} 85$\pm$0.5 umol/l and EC Ni^{2+}, 62 ± 1.8 umol/l) (Krujatz

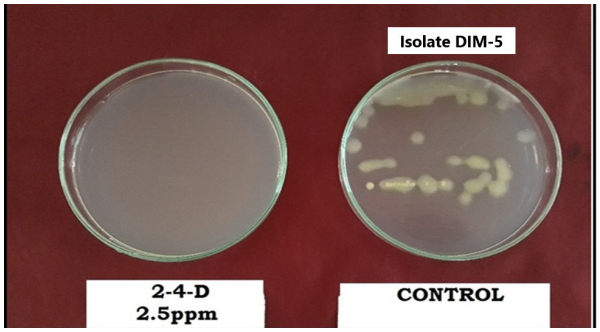

Plate 3. Sensitivity of Bacteria at 2.5 ppm concentration of 2-4-D

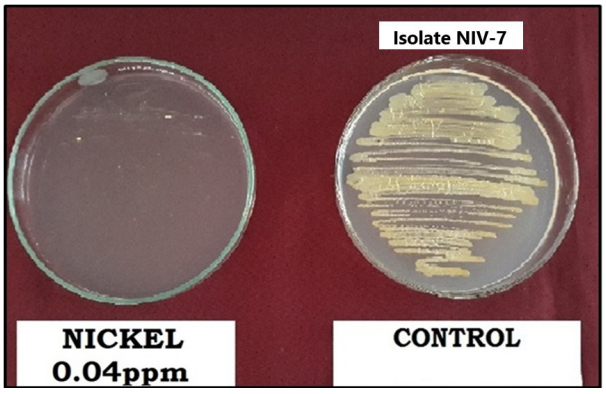

Plate 4. Sensitivity of Bacteria at 0.04 ppm concentration of Nickel

et al., 2011). At0.01 and 30 ppm concentration of Copper 16 isolates were screened, out of them COM-2 showed sensitivity to Copper at 0.01 ppm (Plate 5). *Paenibacillus* spp isolated from pristine soil showed highest sensitivity to Cu^{2+} at concentration 0.011mg.L^{-1}, i.e.0.01 ppm of Cu^{2+} (Rathnayake *et al.*, 2009). At 0.5 and 2.5 ppm concentration of Lead 14 isolates were screened, out of them no any isolate showed sensitivity to Lead, same results were seen at 0.07 and 1.5 ppm concentration of Cadmium, at 0.07 and 1.5 ppm concentration of Chromiumand at 0.05 and 1.1 ppm concentration of Arsenic.

CONCLUSION

This study revealed that many of bacteria isolated to detect the sensitivity for pesticides and heavy metal residue found in marketable fruits and vegetables showed normal growth on a nutrient agar medium. These isolates when used to detect sensitivity for respective pesticide and heavy metal residue at low concentration out of them very few Isolates CAR-8,CTG-10, DIM-5 showed sensitivity to Carbendazim at 2.5 ppm, Captan at 1ppm and 2-4-D at 2.5 ppm respectively and NIM-7 and COM-2 showed sensitivity to heavy metals like Nickel at 0.04 ppm and Copper at 0.01ppm concentration. Hence these finding suggest that there are sensitive bacteria found at low concentration of pesticides and heavy metals in soil and water sample collected from western railway track area of Mumbai.

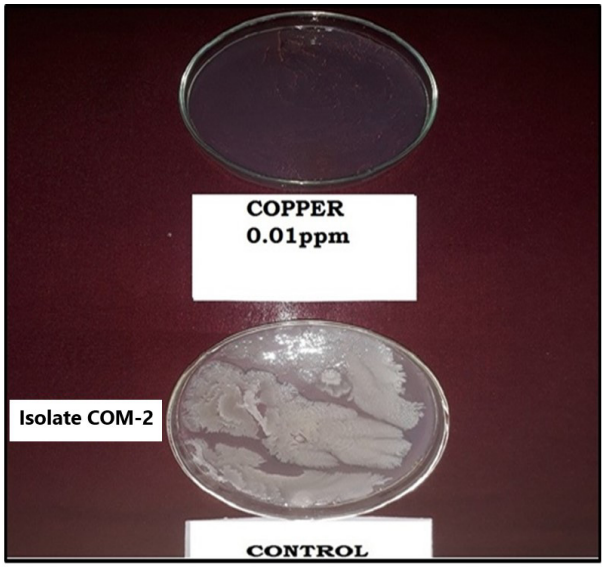

Plate 5. Sensitivity of Bacteria at 0.01ppm concentration of Copper

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