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Influence of fungicide (Carbendazim) and herbicides (2, 4-D and Metribuzin) on non-target beneficial soil microorganisms of Rhizospheric Soil of Tomato Crop

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Abstract: *In present study pot experiments for Tomato plant were conducted to evaluate the effect of Carbendazim (fungicide), 2,4-D and Metribuzin (herbicides) on certain beneficial microorganisms of rhizosphere. Total counts of bacteria, actinomycetes and fungi were lower in the treated soil than the untreated soil. The significant negative effect of the herbicides and fungicide on counts of Azotobacter, Azospirillum and PSB were observed. The period of retardation or stimulation in growth differed according to the type of pesticide and type of micro-organisms under study.*
Key words: *Carbendazim, 2,4-D, Metribuzim, Non-target Microorganisms, Azotobacter, Azospirillum.*

I. Introduction

The word pesticides encompass a heterogeneous group of chemicals developed to control a variety of pests. According to Miligi [1] *et al.,* 2006 pesticides are generally categorized as insecticides, herbicides and fungicides according to the type of pest which they have shown efficacious action. The use of herbicides has increased in both developed and under developing countries during the last decades.

Microorganisms play an important role in many soil biological processes, including nitrogen transformations, organic matter decomposition, nutrient release and their availability, as well as stabilize the soil structure and affect its fertility, investigated by Vyas [2] 1988; Edwards [3] and Bater, 1990; Khan [4] and S cullion, 2000. Soil microflora is the first biota that undergoes direct and indirect impacts of toxic substances introduced to soil. Due to its fast response to contaminants, ubiquity, size and recycling of elements soil microflora is suitable to act as a "biomarker" reflecting the negative effects of pesticides treatment and is commonly used in ecotoxicological tests to evaluate the influence of chemicals on soil system as concluded by Doelman [5] and Vonk, 1994; Edwards [6] *et al*., 1996; Doran [7] and Zeiss.

Soil is the most important site of biological interactions. The indiscriminate use of pesticides disturbs the soil environment by affecting flora and fauna including microflora of soil, and also the physicochemical properties of soil like PH, salinity, alkalinity leading to infertility of soil. The important microflora, beneficial for the growth of plants includes nitrogen fixing bacteria and phosphate solubilizing bacteria, present in the rhizosphere of the plant. The rhizosphere is the zone of soil surrounding the root which is affected by it. The significance of the rhizosphere arises from the release of organic material from the root and the subsequent effect of increased microbial activity on nutrient cycling and plant growth. The excess application of these pesticides may adversely affect the function of these rhizospheric microorganisms. Since the fertility of the soil depends on the number and type of microorganisms present in the soil. Although, pesticides are intended to protect crops, they may affect non-target organisms and contaminate soil environment resulting in alterations the equilibrium of soil processes for shorter or longer periods. TU, CM [8] and Miles, J.R.W., 1976 studied that pesticides reaching the soil affect non target organisms and their activities. To examine these side effects, several investigations are necessary to identify possible changes in the bioactivity of soil organisms contributing to soil fertility. These organisms especially bacteria, fungi and actinomycetes decompose root residues and bring about many reactions necessary for plant growth and crop production. According to Chisholm [9] *et al*., 1950 pesticide residues generally will remain in the top 15 cm layer of the soil which is the region of greatest activity of soil microflora thus favoring the interaction of pesticide residues with the flora of the soil ecosystem, Alexander, M., [10] 1961. Most of studies on the effect of pesticides on soil microbial activity have been laboratory also studied by Kale [11] and S.P. Raghu, 1989; TU, C.M., [12] 1992; Zelles, L.[13] *et al.,* 1985 with single applications of pesticides for short periods. The effect of repeated application in the field on activities of soil microbes and enzymes has so far received little attention. Margni [14] *et al.,* 2002 concluded that the observed changes in the soil activity depend on the intensity and spectrum of activity as well as persistence of the parent chemicals or its metabolites. Pesticides might affect microorganisms by reducing their numbers, biochemical activity, diversity and changing the microbial community structure, Martinez-Toledo [15] *et al*., 1998; Smith [16] *et al*., 2000; Cycoń [17] and Kaczyńska, 2004. The toxic effect of pesticides may be toxic to many soil microorganisms because they can penetrate the cell, disturb the microbial metabolism and

often cause the death of sensitive part of microbial populations. In 2008 Doignon [18] and Rozes reported that the triazole fungicide (Flusilazole) modified the sterol content of *Saccharomyces cerevisiae.* The plasma membrane fluidity was altered by the presence of methyl sterol which increased with the flusilazole concentration, thus affect the microorganisms.

The present study was under taken, as tomato crop regularly grown in India around the year almost in every part of the country to meet the demand. Hence farmers are indiscrimately using the herbicides and fungicides to protect their tomato crops. The present study was undertaken to investigate the impact of herbicides and fungicide (which is commonly applied) on tomato crop rhizospheric soil and ultimately impact on soil microorganisms which directly and also indirectly affect crop yield.

II. Material And Methods

1. Setting up of Pot experiments:

Soil samples were collected in bulk from rhizosphere of different Tomato crop fields from rural area of Hyderabad metropolis, India. Collected soil samples were mixed and prepared composite soil for the experiments. 5kgs of soil was taken from the composite soil for each pot. Soil was gently air dried to the point of soil moisture suitable for sieving, and sieved through 2mm sieved and checked the following initial physical and chemical parameters i.e., Organic carbon content, Electrical Conductivity, PH, Sodium and Calcium, the total nitrogen content, Phosphorus and Potassium to know the initial soil condition suitable for tomato crop.

2. Fungicide and Herbicides

In the study one fungicide and two herbicides were used, a fungicide Carbendazim ($C_9H_9N_3O_2$) a widely used broad-spectrum benzimidazole fungicide and Herbicides 2,4-D ($C_8H_6Cl_2O_3$) and Metribuzin ($C_8H_{14}N_4OS$) a (triazinone herbicides) are the active ingredients of the fungicide and herbicides, respectively. The recommended dose of fungicide and herbicides and method for application was adopted.

3. Plants

Tomato plant, *Lycopersicon esculentum*, of S-22 variety was used in the experiments; three seeds were sown directly in pots filled with 5 kg soil. The seedlings were thinned to one plant per pot during the second week, giving due consideration to uniformity of seedling sizes.

4. Isolation of microorganisms and analysis

Soil samples were collected from rhizosphere of tomato crop plants pots in triplicates frequently after 7, 14, 21, 28 and 35 days of plantation, the numbers of microorganisms were determined using the dilution plate methods and appropriate agar media. The numbers of colony forming units (CFU) in the selective media were determined by means of the serial dilution technique and the spread plate method. The total numbers of colonies were counted frequently after 7, 14, 21, 28 and 35 days and average number of colonies were calculated for 1g of dry soil (CFU. g^{-1} DM). For enumeration of microorganisms, 10g of soil (dry basis) was placed in a sterile Erlenmeyer flask with 90 ml sterile water and shaked on rotary shaker for 30 minutes. Transferred 10ml of soil suspension to 90ml sterile water and shaked vigorously for few minutes, serially diluted 10^{-5} for bacteria and Actinomycetes and also for Azotobacter, Azospirillum and Phosphate Solubilizing Bacteria and 10^{-3} for fungi were obtained. Soil suspension, 0.1 ml was spreaded with a flamed "L-shaped glass rod" and Plates were incubated. After incubation colonies counted and isolated to obtained pure culture for further investigation in laboratory.

Total number of colonies of Bacteria, Fungi, Actinomycetes, Azotobacter, Azospirillum and Phosphate Solubilizing Bacteria (PSB) were inoculated and counted on appropriate and suitable media like Nutrient Agar, Martin's Medium, Kenknight's Agar media, Jensen's medium, Semi-solid sodium malate medium, Pikovskaya's solid medium respectively.

III. Results And Discussion

The results clearly indicating that in initial days application of Pesticides i.e., Fungicide - Carbendazim, Herbicides 2,4-D and Metribuzim) used drastically reduced the number of Bacteria, fungi, actinomycetes, and beneficial microflora i.e., azotobacter, azospirillum and Phosphate Solubilizing Bacteria (PSB) in treated soil compared with the untreated (Control) soil. Colonies decreased with application of fungicide and herbicides.

The herbicides and fungicide were most effective in reducing the number of microorganisms at the first application time (1 – 20 days). The results obtained are given in table 1, 2, 3, 4, 5 and 6. The inhibition in growth in microorganisms was gradually increased in presence fungicide (Carbendazim) and herbicides (2,4-D and Metribuzin). The maximum inhibition in growth of microorganisms was recorded on 14^{th} day. The inhibition in growth gradually reduced from 21^{st} day and the total number of Bacteria, fungi, actinomycetes, azotobacter, Azospirillum, and Phosphate Solubilizing Bacteria (PSB) gradually increased from 21^{st} day. This

condition indicating that the application of herbicides (2,4-D, Metribuzin and Atrizin) and fungicide (Carbendazim) in agricultural soil particularly tomato crop cultivating soil leads to decrease the total numbers of soil microorganisms at initial period. Noval conditions i.e., after gradually diluted, the pesticides may not much affect on soil microbial populations and lead to selection pressure in existing microorganisms to promote the functional groups of microorganisms adapted to the new conditions (Marrone [19] 1993.

TABLE 1. Number of Bacterial (Colony forming units) in soil treated with herbicides and fungicide.

Application Period:	Mean Number of colonies forming units (CFU) Bacteria x 10^{-5}/g-1 soil treated with herbicides and fungicide at different application Period							
	Control	I/% *	Carbendazim	I/% *	2,4-D	I/% *	Metribuzin	I/% *
1st day	127.3	0	224.6	0	148.6	0	66	0
7th day	151.3	18.85	191	-14.96	134.6	-9.421	3.6	-94.55
14th day	215.6	69.36	50	-77.74	60	-59.62	4.6	-93.03
21st day	321.3	152.4	116.6	-48.09	46.3	-68.84	7	-89.39
28th day	332	160.8	159.6	-28.94	78	-47.51	11.3	-82.88
35th day	454.6	257.1	216.6	-3.562	115.6	-22.21	49.6	-24.85

*Percentage of Inhibition

TABLE 2. Number of Fungi (Colony forming units) in soil treated with herbicides and fungicide.

Application Period:	Mean Number of colonies forming units (CFU) Fungi x 10^{-3}/g-1 soil treated with herbicides and fungicide at different application Period							
	Control	I/%*	Carbendazim	I/%*	2,4-D	I/%*	Metribuzin	I/%*
1st day	11.3	0	25	0	30.6	0	23.6	0
7th day	18	59.29	6	-76	27	-11.76	13.6	-42.37
14th day	26.6	135.4	2.3	-90.8	11.6	-62.09	2	-91.53
21st day	33.6	197.3	4	-84	14.6	-52.29	3.6	-84.75
28th day	46.3	309.7	4.3	-82.8	18.6	-39.22	8	-66.1
35th day	29	156.6	3	-88	13	-57.52	7	-70.34

*Percentage of Inhibition

TABLE 3. Number of Actinomycetes (Colony forming units) in soil treated with herbicides and fungicide.

Application Period:	Mean Number of colonies forming units (CFU) Actinomycetes x 10^{-5}/g-1 soil treated with herbicides and fungicide at different application Period							
	Control	I/%*	Carbendazim	I/%*	2,4-D	I/%*	Metribuzin	I/%*
1st day	101.3	0	100	0	119.3	0	97	0
7th day	113.6	12.14	67	-33	72	-39.65	60.6	-37.53
14th day	163.6	61.5	95	-5	64.6	-45.85	54	-44.33
21st day	171	68.81	98.6	-1.4	64.3	-46.1	86.6	-10.72
28th day	197.6	95.06	156.6	56.6	95	-20.37	110.6	14.021
35th day	223.6	120.7	171	71	97.3	-18.44	121.3	25.052

*Percentage of Inhibition

TABLE 4. Number of Azotobacter (Colony forming units) in soil treated with herbicides and fungicide.

Application Period:	Mean Number of colonies forming units (CFU) Azotobacter x 10^{-5}/g-1 soil treated with herbicides and fungicide at different application Period							
	Control	I/%*	Carbendazim	I/%*	2,4-D	I/%*	Metribuzin	I/%*
1st day	93.3	0	96.3	0	90.6	0	91.6	0
7th day	117.6	26.05	50.3	-47.77	55.2	-39.07	48.6	-46.94
14th day	122.6	31.4	44.6	-53.69	40.6	-55.19	35.6	-61.14
21st day	135.3	45.02	43.3	-55.04	37.6	-58.5	31.6	-65.5
28th day	166.6	78.56	53.3	-44.65	51.6	-43.05	41.6	-54.59
35th day	150.1	60.88	50	-48.08	43	-52.54	40.6	-55.68

*Percentage of Inhibition

TABLE 5. Number of Azospirillum (Colony forming units) in soil treated with herbicides and fungicide.

Application Period:	Mean Number of colonies forming units (CFU) Azospirillum x 10^{-5}/g-1 soil treated with herbicides and fungicide at different application Period							
	Control	I/%*	Carbendazim	I/%*	2,4-D	I/%*	Metribuzin	I/%*
1stday	19	0	19	0	17.3	0	17	0
7th day	23.6	24.21	15.6	-17.89	13.3	-23.12	16.6	-2.353
14th day	32.3	70	18.6	-2.105	8.3	-52.02	18.6	9.4118
21st day	37	94.74	20.3	6.8421	12.3	-28.9	24.6	44.706
28th day	41.3	117.4	26	36.842	16.6	-4.046	34.6	103.53
35th day	38.4	102.1	23	21.053	11.1	-35.84	61.3	260.59

*Percentage of Inhibition

TABLE 6. Number of Phosphate Solubilizing Bacteria (Colony forming units) in soil treated with herbicides and fungicide.

Application Period:	Mean Number of colonies forming units (CFU) Phosphate Solubilizing Bacteria x 10^{-5}/g- treated with herbicides and fungicide at different application Period							
	Control	I/%*	Carbendazim	I/%*	2,4-D	I/%*	Metribuzin	I/%*
1st day	14.6	0	15.6	0	15.3	0	15.3	0
7th day	19.6	34.25	9.3	-40.38	6.3	-58.82	10.3	-32.68
14th day	24.3	66.44	2.3	-85.26	5.6	-63.4	8	-47.71
21st day	31.6	116.4	7.6	-51.28	10	-34.64	13	-15.03
28th day	40.3	176	16.6	6.4103	18	17.65	21.6	41.176
35th day	45.3	210.3	14.6	-6.41	11	-28.1	23.6	54.248

*Percentage of Inhibition

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