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Effect of agrochemicals on microflora in soybean rhizospheric soil

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ABSTRACT

After application of agrochemicals at 1, 30 DAS and at harvest the total number of fungi, bacteria, actynomycetes, *pseudomonas*, *azatobactor*, *rhizobium* were counted.The result with regard to bacterial population in soybean field were significantly influenced by bioinoculant viz., r*hizobium*. The bacterial populations were inhibited by herbicides Alachlor in soybean and fungicides i.e. thiram and mancozeb in soybean. After 30 days of spraying of chemicals the bacterial populations were restored. With regard to r*hizobium* population, the bioinoculant were significantly influenced the population soybean field. The herbicides and fungicides were significantly decreased bioinoculant population, maximum inhibition was observed in mancozeb treated plot at 30 DAS. The results with regards to pseudomonas, actinomycetes and fungi population were influenced by bioinoculant viz., *rhizobium*. While population were inhibited by alachlor, thiram and mancozeb in soybean field. The *Pseudomonas*, actinomycetes and fungi population were restored after 30 days of spraying. With regard to yield of soybean were significantly influenced by bioinoculant in combination with herbicide and fungicide.

1. Introduction

Soil is a dynamic living system and consists of a variety of micro flora *viz.* bacteria, actinomycetes, fungi, *etc*. Modern agriculture is really associated with the use of different agrochemicals. Different classes of agrochemicals like fungicides, herbicides and insecticide are being used in interated crop management. Taking in to account the

present investigation is an attempt to see the effect of agrochemicals on soil micro flora in soybean rhizospheric soil. Agrochemical residues usually occur in the top fifteen cm layer of soil. It is also the region of greatest activity of soil micro flora. *Trichoderma, pseudomonas* helps for controlling harmful soil borne plant pathogens *viz. pythium, phytopthora, fusarium, rhizoctonia* etc. Agrochemicals are a potential threat for soil microorganisms and in the long term may alter their productive, protective and adaptive capacities (Soulas and Lors, 1999). In India, the total agrochemicals consumption is about 80,000 million tones per year. Among these insecticides occupies 63 %, fungicides 18%, herbicides 16% and other chemicals 3 %. Agrochemicals 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 period. The observed changes in the soil activity depend on the intensity and spectrum of activity as well as persistence of the chemicals (Margni *et al.*., 2002). Area, production and productivity of soybean in India during *kharif* 2007 was 87.5 lakh ha., 88.65 lakh tones and 1018 kg per ha respectively. In case of Maharashtra it was 32.628 lakh ha with average yield of 1200 kg/ha and total production of 31.54 lakh tones.

2. Materials and methods

During the course of present investigation a series of experiments were carried out in the field at All India Coordinated Research Project on Weed Control, Marathwada Agricultural University, Parbhani. The seeds of Soybean (JS-335) used for sowing in field experiment. The bioinoculants used for seed treatments i.e. *rhizobium and*t he herbicide alachlor used in soybean crop at different dosage.

2.1. Media used

Different semi-solid media were used for different microorganism. These are Thronton's agar media used for estimation of bacteria, Martin's Rose Bengal agar media used for estimation of fungi (Pepper *et al.*., 1995). Ken Knight's agar media for Actinomycetes, Kings B media for *Pseudomonas*, Yeast extract mannitol agar media for *Rhizobium* (Anarson, 1970) and Ashby's mannitol agar media used for *Azotobacter* estimation (Monkiedje *et al*., 2002).

2.2. Soil sampling

Soil samples were taken at randomly by soil sampling auger from 4-5 places from each plot and mixed thoroughly to prepare one composite sample. The plant material and other debris were removed from the sample by hand, the samples were dried in shade and then ground with wooden mortar and pastle and the soil was sieved using 4 mm mesh. The soil samples were collected from top 15 cm. Soil samples were collected at 1 DAS, 30 DAS and at harvesting in soybean crop.

2.3. Microbial analysis

After application of agrochemicals at 1, 30 and at the time of harvest in treated plots the total number of fungi, bacteria, actinomycetes, p*seudomonas,* nitrogen fixing bacteria i.e. r*hizobium* was counted. The number of colony forming unit (cfu) in the selective media were determined by means of the serial dilution technique and the pour plate method (Salle, 1973). Analyses were performed in three replicates.

2.3. Serial dilution

First prepared a series of dilution blanks consisting of sterile water for each series 250 ml conical flasks containing 90 ml of sterile water and 8-10 test tubes containing 9 ml distilled water are required. Transfer 1 g of soil sample in 10 ml sterile distilled water in test tube (1:10) and shake properly. Transfer 1 ml of suspension from this tube to another tube containing 9 ml of sterile distilled water (1:100). Against transfer 1 ml of suspension from this tube to containing 9 ml sterile distilled water (1:1000). Similarly dilution process is continued as per requirement

2.4. Pour plate method

Using fresh sterile micro pipette, pour 1 ml aliquot from the last dilution into each of the three Petri dishes. Using the same pipette, similar aliquots from next two dilutions in three Petri dishes for each dilution, after this poured approximately 20 ml volume of molten medium in each of the plates. Immediately after pouring, gently

move the plates in a whirling motion to mix the contents, than allow the medium to solidify and incubate in BOD incubator at $30^{o} \pm 2^{o}$ C in inverted position.

2.5. Calculation of total viable count

After appropriate incubation period calculate the number of colonies per plate and record by using digital colony counter. Total viable counts are calculated from the following formula.

Total viable count = Average number of colonies x size of aliquot x dilution factor.

3. Results

3.1. Bacterial population

The data on bacterial population are presented in Table 1. The result at 1 day after spray and bioinoculant treatment shows that the T_1 i.e. seed treatment of *Rrhizobium* @ 25 g/kg seed and T_2 i.e. Thiram @ 2 g/kg seeds not much shows adverse effect on bacteria population. These population were in T_1 (48×10^6)and T_2 (46.53×10^6) were at par with control T_6 (49.33×10^6). The 25 % decreased population observed in T_5 (38.66×10^6) which contents seed treatment of *Rhizobium* @ 25 g/kg seed + Thiram @ 2 g/kg seed + pre-emergence spray fo Alachlor @ 2 kg/ha as compared to control i.e. 49.33×10^6. In T_3 i.e. pre-emergence spray of herbicide i.e. Alachlor @ 2 kg/ha has 13 % reduced bacteria population as compared to control. At 30 DAS and 1 day after spraying of Mancozeb @ 0.02 %, the significantly lowest bacterial count was observed (40×10^6). This was followed by T_5 (45.33×10^6) and T_3 i.e. Alachlor spray @ 2 kg/ha (49.66×10^6). The highest bacterial count was observed i.e. *Rhizobium* seed treatment (68×10^6). Recovered bacterial population was noticed at harvest in all treatments

3.2. *Azotobacter* population

The data regarding *Azotobacter* population of soil is presented in Table 10 (Fig 10). The lowest *Azotoabcter* count was observed in T_5 i.e. seed treatment with *Rhizobium* @ 25 g/kg seed + Thiram @ 2 g/kg seed + pre-emergence spray of Alachlor @ 2 kg/ha (18.33×10^6) followed by T_3 i.e. pre-emergence spray of Alachlor @ 2 kg/ha (21×10^6). At 30 DAS and 1 day after spraying of Mancozeb 0.02 % decrease 50% *Azotobacter* population from 41.53×10^6 in control (T_7) to 19.66×10^6 (T_3) followed by T_5 i.e. seed treatment with *Rhizobium* @ 25 g/kg seed + Thiram @ 2 g/kg seed + pre-emergence spray Alachlor @ 2 kg/ha + Mancozeb @ 0.02 % (32×10^6), while in other treatment i.e. pre emergence spray of Alachlor @ 2 kg/ha T_3 and seed treatment with Thiram @ 2 g/kg seed. *Azotobacter* population was recovered after 30 days.

3.3. *Rhizobium* population

The Alachlor @ 2 kg/ha in combination with Thiram seed treatment @ 3 g/kg seed and *Rhizobium* seed treatment @ 25 g/kg seed decreased 49% *Rhizobium* population at 1 DAS (16.33×10^6) as compared to control (30.66×10^6) followed by T_3 i.e. pre-emergence spray of Alachlor @ 2 kg/ha showed *Rhizobium* population 23.33×10^6 Table 11, Fig 11 (Plate 6). *Rhizobium* population recovered after 30 DAS in Alachlor treated plot. While 1 day after spraying of Mancozeb @ 0.02 % in T_4 decreased 49 % *Rhizobium* population i.e. 20.66×10^6 as compared to control (40.66×10^6). At the time of harvest the *Rhizobium* population in Mancozeb treated plots also recovered (43×10^6).

3.4. *Pseudomonas* population

The data regarding *Pseudomonas* population are presented in Table 1. The lowest *Pseudomonas* count was recorded in T_5 i.e. Alachlor spray @ 2 kg/ha + Thiram seed treatment @ 2 g/kg seed + *Rhizobium* seed treatment @ 25 g/kg seeds at 1 DAS (30.53×10^6). This was followed by Alachlor spray (37×10^6). The *Pseudomonas* population was recovered after 30 days of spraying in T_3 i.e. Alachlor @ 2 kg/ha (52.33×10^6) it was at par with control (59×10^6) while 1 day after spraying of Mancozeb @ 0.02 % in T_4 indicate lowest microbial count (34.33×10^6) which was significantly lowest than rest of the treatment. At harvest significantly highest count observed in T_1 i.e. seed treatment with *Rhizobium* @ 25 g/kg seed (68×10^6) which was at par with T_5 (66.66×10^6) and in all treatment population was restored.

Table 1

Effect of Agrochemicals on Bacteria, Actinomycetes ,Fungi, Azatobactor, Rhizobium and Pseudomonas in soybean rhizospheric soil

Tr. No.	Total bacterial population (cfu x 10^6)			Total Actinomycetes population (cfu x 10^4)			Total Fungal population (cfu x 10^3)			Total *Azotobacter* population (cfu x 10^6)			Total *Rhizobium* population (cfu x 10^6)			Total *Pseudomonas* population (cfu x 10^6)		
	1 DAS	30 DAS	At harvest	1 DAS	30 DAS	At harvest	1 DAS	30 DAS	At harvest	1 DAS	30 DAS	At harvest	1 DAS	30 DAS	At harvest	1 DAS	30 DAS	At harvest
T_1	48.00	68.00	76.00	47.66	65.00	70.66	40.00	54.00	64.00	27.00	42.66	47.66	28.66	49.33	57.00	41.00	60.33	68.00
T_2	46.33	54.66	70.00	46.33	60.66	68.00	39.66	48.33	60.00	2.366	40.00	44.66	28.00	39.00	46.33	40.33	56.66	64.33
T_3	42.33	49.66	69.66	44.66	58.33	67.66	37.33	47.00	59.66	21.00	37.66	42.66	23.33	37.00	44.33	37.00	52.33	63.66
T_4	48.00	40.66	68.33	47.00	40.66	66.66	40.00	35.00	59.66	26.00	19.66	40.33	30.00	20.66	43.00	42.00	34.33	62.00
T_5	38.66	45.33	72.33	38.00	53.66	69.00	33.00	43.33	63.33	18.33	32.00	46.33	16.33	32.00	51.00	30.33	48.66	66.66
T_6	49.33	66.66	72.66	48.00	62.00	68.33	40.66	50.33	62.33	26.33	41.33	45.00	30.66	40.66	47.33	42.33	59.00	65.00
SE ±	1.41	2.78	1.43	2.47	3.47	1.78	2.68	2.95	1.90	0.90	1.52	2.19	1.24	1.83	1.61	3.56	2.25	1.63
CD at 5%	4.61	8.74	4.01	8.07	10.92	5.60	8.74	9.30	6.00	2.93	4.79	6.88	4.06	5.75	5.09	11.61	7.07	5.15

T_1 Seed inoculation with *Rhizobium* @ 25 g/kg seed T_2 Seed treatment with Thiram @ 2 g/kg seed

T_3 Pre-emergence spray of Alachlor @ 2 kg/ha T_4 Mancozeb spray @ 0.02 %

T_5 Seed inoculation with *Rhizobium* @ 25 g/kg seed + Seed treatment with Thiram @ 2 g/kg seed + Pre-emergence spray of Alachlor @ 2 kg/ha + Mancozeb spray @ 0.02 %

T_6 Control

Table 2

Effect of Agrochemicals and Bioinoculants on yield of Soybean.

Tr. No.	Treatment	No. of branches	No. of pods	No. of nodules	Test weight (100 seed) g	Yield (q/ha)
T_1	Seed inoculation with *Rhizobium* @ 25 g/kg seed	3.44	23.11	40.67	14.25	21.20
T_2	Seed treatment with Thiram @ 2 g/kg seed	2.70	21.66	29	13.58	21.14
T_3	Pre-emergence spray of Alachlor @ 2 kg/ha	2.44	19.33	35	13.07	19.58
T_4	Mancozeb spray @0.02 %	2.88	20.44	32.67	13/20	20.74
T_5	Seed inoculation with *Rhizobium* @ 25 g/kg seed + Seed treatment with Thiram @ 2 g/kg seed + Pre-emergence spray of Alachlor @ 2 kg/ha + Mancozeb spray @0.02 %	3.00	22.36	39.67	14.07	23.70
T_6	Control	2.11	18.66	24	12.01	18.50
SE ±		0.82	0.17	0.20	0.45	0.63
CD at 5%		2.29	0.53	0.61	1 .37	1.88

3.5. Actinomycetes population

The pre-emergence spray of Alachlor @ 2 kg/ha (T_3) decreased actinomycetes population from 48 x 10^4 to 44.66 x 10^4 while Alachlor in combination with Thiram and *Rhizobium* indicate lowest microbial count (T_3) i.e.38 x 10^4 (Table 1). The actinomycetes population in Alachlor treated plot was recovered at 30 DAS. After Mancozeb application when disease incidence was occurred after 30 days of sowing, 31 % reduced actinomycetes population (40.66 x 10^4) as compared to control (62 x 10^4) while it was restored at time of harvest. At the time of harvest in all treatment statistically significant increased Actinomycetes population.

3.6. Fungal population

The data regarding fungal population of soil in Soybean is presented in Table1. The result at 1 DAS revealed that T_3 i.e. combined effect of seed treatment of Thiram @ 3 g/kg seed, seed treatment of *Rhizobium* @ 25 g/kg seed and pre emergence spray of Alachlor @ 2 kg/ha reduced fungal population from 40.66 x 10^3 in control to 33 x 10^3 in treated plot. This was followed by T_3 i.e. pre-emergence spray of Alachlor @ 2 kg/ha showed reduced fungal population i.e. 37.33 x 10^3 after 30 days the fungal population in Alachlor treated plots was restored while in Mancozeb treated plot @ 0.02 % when disease incidence was occurred showed reduced in number i.e. 35 x 10^3 this was followed by T_5. The significantly highest count were recorded in T_1 i.e. seed treatment of *Rhizobium*. At the time of harvest in the Mancozeb @ 0.02 % treated plot (T_4) also restored the fungal population it showed that the herbicides and fungicides effects was only temporary i.e. about 30 days.

3.7. Effect on number of branches, pods, nodules per plant, test weight and yield

Seed inoculation with *Rhizobium* recorded maximum number of branches i.e. 3.44 followed by *Rhizobium* + Thiram + Alachlor + Mancozeb i.e. 3.00. Minimum branches recorded in control 76.00 i.e. 2.11. The data presented in Table 20 clearly indicated that the inoculation of *Rhizobium* has brought about maximum increase in pods i.e. 23.11 and which was followed by *Rhizobium* + Thiram + Alachlor + Mancozeb i.e. 22.36. Minimum pods recorded in control T_6 i.e. 18.66. Nodules are the niches of microorganism especially where *Rhizobium* lives and fixes atmospheric nitrogen. *Rhizobium* lives symbiotically in the roots of leguminous plants and significantly increases number of nodules. It is evident from the data presented in Table 20 that maximum number of nodules 40.67 per plant were recorded with seed inoculation with *Rhizobium*. The seed treatment with *Rhizobium*, Thiram and spray of Alachlor, Mancozeb also showed 39.67 effective number of nodules per plant. Minimum number of nodules per plant showed in control i.e. 24. The data presented in Table 20 clearly indicated that the inoculation of *Rhizobium* has brought about maximum increase in test weight i.e. 14.25 g which was followed by *Rhizobium* + Thiram + Alachlor + Mancozeb i.e. 14.07 g. Minimum test weight recorded in control i.e. 12.01 g. The grain yield (Table 16and Fig 16) as influenced by different agrochemicals and bioinoculants was ranged from 18.50 to 23.70 q/ha. The treatment (T_5) receiving combination of seed treatment with *Rhizobium* @ 25 g/kg seed + seed treatment with Sulphur @ 2 g/kg seed + pre-emergence spray of Alachlor @ 2 kg/ha + Mancozeb spray @ 0.02 % (30 DAS) when disease incidence observed, showed highest yield (23.70 q/ha) superior to other treatments followed by T_1, T_2, T_4, T_3 and T_6 in the descending order of yield which were at par with each other.

4. Discussion

In present investigation, the soil samples of agrochemicals and bioinoculant treated plots of Soybean showed that bacterial population in seed treatment with *Rhizobium* and Thiram not much showed any adverse effect while pre-emergence spray of Alachlor @ 2 kg/ha had 13 % reduced bacterial population. 25% decreased population observed in combined treatment of seed treatment with *Rhizobium* + Thiram + pre-emergence spray of Alachlor. At 30 DAS the significantly lowest bacterial count was observed in Mancozeb treated plot while highest bacterial countwas observed in *Rhizobium* treated plot at the time of harvest in all the treatments bacterial population were recovered. Similar results were obtained by several research workers (Votes *et al..*, 1974; Kole *et al..*, 1987; Vig *et al..*, 1990; Shukla *et al..*, 1997; Khan and Ahmed, 2002). In regard to *Azotobacter* population the lowest count was observed in combined effect of pre-emergence spray of Alachlor with seed treatment of Thiram and *Rhizobium*. At 30 DAS in Mancozeb treated plot when disease incidence was occurred, decrease 50% *Azotobacter* population while in other treatment i.e. pre-emergence spray of Alachlor and seed treatment with Thiram recovered *Azotobacter* population in Mancozeb treated plot recovered population at the time of harvest of Soybean. Similar

results were found by several research workers (Votes *et al.*., 1974; Ferrer *et al.*., 1986; Vig *et al.*., 1990; Khan and Ahmed, 2002 and Cycon *et al.*., 2007). In regard to *Rhizobium* population, combined effect of Alachlor and seed treatment with Thiram and *Rhizobium* decreased 49 % *Rhizobium* population at 1 DAS followed by pre-emergence spray of Alachlor but it recovered after 30 days while one day after spraying of Mancozeb when disease incidence was observed decreased 45% *Rhizobium* population. At the time of harvest the *Rhizobium* population in Mancozeb treated plots also recovered. Simialr results were obtained by Votes *et al.*. (1974) Moiroud *et al.*. (1985), Gadkari (1988), Shetty and Magu (2000), Khan and Masood (2002), Mishra and Chandrabhanu (2006) and Cycon *et al.*. (2007). In regard to *Pseudomonas* , actinomycetes and fungi population the lowest microbial count was recorded in combined effect of pre-emergence spray of Alachlor @ 2 kg/ha, seed treatment with Thiram @ 2 g/kg seed, *Rhizobium* seed treatment @ 25 g/kg seed at 1 DAS. This was followed by Alachlor spray. After 30 DAS population *Pseudomonas,* Actinomycetes and Fungi were recovered. At the time of disease occurrence (30 DAS) in Mancozeb treated plot the populations were decreased 25 %, 31 % and 27 % in *Pseudomonas*, Actinomycetes and fungi respectively, while it was restored at the time of harvest. The result of effect of herbicides, fungicides and bioinoculant on *Pseudomonas*, Actinomycetes and fungi correlates with report of Comper *et al.*. (1973), Khalifa *et al.*. (1987), Macedo *et al.*. (1988), Kole *et al.*. (1989), Shukla *et al.*. (1990), Thopate *et al.*. (1990), Vig *et al.*. (1990), Donderski *et al.*. (1992), Martienz Toledo *et al.*. (1998), Teixido *et al.*. (1999) and Khan and Ahmed (2007). The result with regard to the plant height, yield and test weight of Sorghum influenced by *Azotobacter* inoculation have brought about the significant increase in plant height, test weight and yield. Bopaiah and Khader (1984) reported the increased plant height when inoculated with *Azotobacter*. Gopal Reddy *et al*. reported seed inoculation with *Azotobacter* increased 45 per cent yield. The similar results have been reported by Kubsad *et al*. (2003) and Sumarjeet Singh (1998). The result obtained with regard to nodulation, number of pods, number of branches, yield, test weight of Soybean clearly indicated that the *Rhizobium* inoculation have brought about the maximum number of nodules, pods, branches , test weight and yield of Soybean. The results obtained in present investigation are in full agreement with those reported in the past. Wunze *et al.*. (1997) reported microbial inoculation increased number of pods, bunches, test weight and yield. Singh *et al.*. (1998) found that seed inoculation of *Rhizobium* increased number of nodules and yield of Soybean. Similar results have been reported by Kubsad *et al.* (2003), Sumarjeet Singh (1998), Patil *et al*. (2004). The germination per cent recorded at 10 days was significantly influenced by different bioinoculants. The inoculation of *Azotobacter* (Sorghum) and *Rhizobium* (Soybean) brought about the increase in germination. With regard to shoot and root length, fresh and dry weight of shoot and root and vigour index in Sorghum and Soybean in both the pot culture method and field condition. Bopaiah and Khader (1989) reported increased plant height when inoculated with bioinoculant. Terry *et al.*. (1996) reported that application of *Azotobacter chrococcum* to tomato significantly increased the plant height, root length, fresh and dry weight of above ground parts and of shoot system. The similar results have also been reported by Wange and Ranawade (1997), Sumarjeet Singh (1998) and Singh *et al.* (1998).

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