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# Impact of Pesticide Applications in Cotton Agroecosystem and Soil Bioactivity Studies I: Microbial Populations

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**Abstract**: Field experiments were conducted to study the effect of pesticide applications on soil microbial populations in cotton agroecosystem. The pesticides were applied in test (research plot), treated (farmer) and control (without pesticides) fields. Soil samples were collected from sowing time, before and 2 days after each pesticide applications, at harvest and post harvest time during the crop seasons 1997-98. Soil samples were subjected to thawing before determining the microbial populations. Total bacterial counts (colony forming units; CFU) were determined by serially diluted samples into Luria broth media after 24 h while fungal counts were determined by Sabouraud media after 7 days incubation at 30°C. Endosulfan, profenophos + alphamethrin and methamidophos inhibited while monocrotophos and bifenthrin + acetamiprid enhanced the bacterial population. Fungal population was depressed with endosulfan while monocrotophos, methamidophos, endosulfan + dimethoate , fenpropathrin, bifenthrin + acetamiprid or with ethion or with a mixture of carbosulfan and chlorpyrifos and profenophos alone or with ethion or cypermethrin or alphamethrin stimulated. All the other applied pesticides did not cause any appreciable change in total bacterial and fungal populations. Samples collected after pesticide applications at harvest and post harvest time showed that the microbial population recovered to its normal levels at the end of each season.

**Key words:** Pesticides, soil, bacteria, fungal population

## Introduction

In modern agriculture, pesticides are frequently used in the field to increase crop production. Besides combating insect pests, insecticides also affect the population and activity of beneficial microbial communities in soil (Singh and Parsad, 1991 and Bhuyan *et al.*, 1992). Soil microorganisms have a major role in the metabolism of both organic and inorganic constituents in soils to form for plants. Possibility that insecticide residues in soil may have deleterious effects on soil microorganisms and their activities, has received a considerable attention (Edward, 1972). Excessive concentration of the insecticides in environment may shift microbial populations to temporarily favored group(s) of microorganisms, which may overpopulate the soil. Beneficial microorganisms could be suppressed provoking new and more complex problems from microorganisms, which have escaped competition with other suppressed groups (Tu and Mile, 1976). Because they occur in such mass large number and also participate in a wide range of conversion reactions, microorganisms are an important factor in the soil ecosystem and play a significant role in determining the fertility of soil. Therefore, it was inevitable that anthropogenically induced influences would attract a greater attention when farmers went over to more intensive forms of cultivation. Since excessive use has been made of pesticides in the last 40 years and presently the number of insecticides used is so great that relatively soil microbiologists have adequately investigated few. Organophosphorus, carbamate, pyrithroids are replacing most of the older organochlorine insecticides but little is known of the ecological side effects these newer materials may have in soils. It very soon became necessary to ask what effect they might have on the associated soil microorganisms under the heavy repeated applications of pesticides in cotton agroecosystem.

## Materials and Methods

The soil used was a clay loam, a typical agricultural soil in Punjab, Pakistan. Pesticides were applied at recommended rate to protect cotton crop under field conditions. Samples were collected randomly to a depth of 15 cm from all the plots and estimated for microbial populations.

**Insecticides**: The insecticides werepurchased locally and used individually or in combinations (mixtures). They were applied in the plots at the recommended rates of field application. Their general names and doses of application are given below:

**1st crop season**: Endosulfan + Dimethoate, Profenophos + Cypermethrin (polytrin C), Profenophos + Cypermethrin, Profenophos, Profenophos + Cypermethrin, Endosulfan, Carbosulfan + Fenvalerate, and Fenpropathrin were applied @ 1000 + 500, 600, 600, 1000, 600, 1000, 600 + 300 and 330 ml acre$^{-1}$ respectively.

**2nd crop season**: Methamidophos, Monocrotophos, Profenophos + Diafonthiuson, Profenophos + Alphamethrin, Bifenthrin + Endosulfan, Bifenthrin + Acetamiprid, Profenophos + Ethion, Bifenthrin + Ethion, Propargite, Bifen thrin + Carbosulfan + Chlorpyriphos were applied @ 500, 1000, 1000 + 250, 800 + 330, 250 + 1000, 250 + 125(g), 1000 + 600, 250 + 1500, 330, 250 + 500 + 1000 ml acre$^{-1}$ respectively.

**Field site and Insecticide spray schedule**: The field site was selected at NIAB experimental farm, Faisalabad. Three duplicate plots, size of 500 m$^2$, were made to layout the experiment; a) control plot without any pesticide treatment + fertilizers + cotton plants and treated plots; b) test plot (research plot) with pesticide treatment + fertilizers + cotton plant; c) treated plot from actual farm cotton plot (farmer plot). Cotton varieties NIAB-78 and CM-240 were sown @ 20 kg ha$^{-1}$ during 1997-99 crop seasons. Seeds were first delinted using commercial $H_2SO_4$ and washed thoroughly with tap water. Fertilizers, Nitrogen and Phosphorus from DAP and Potassium from Potash were applied @ 25 kg N ha$^{-1}$, 25 kg P ha$^{-1}$ and 56 kg K ha$^{-1}$ at the time of sowing and Nitrogen @ 58 kg ha$^{-1}$ (from urea) at the time of boll formation. The soil properties are given in Table 1.

Table 1: Soil physio-chemical properties

| Soil characteristics | Control soil | Treated soil |
|---|---|---|
| pH | 7.86 | 7.72 |
| Saturation (%) | 32.00 | 36.00 |
| Organic matter (%) | 0.48 | 0.79 |
| ECe ($dSm^{-1}$) | 0.39 | 0.21 |
| Total N (%) | 0.05 | 0.05 |
| Clay (%) | 22.79 | 23.03 |
| Silt (%) | 26.36 | 23.12 |
| Sand (%) | 50.58 | 53.86 |
| Textural class | Clay loam | Clay loam |

**Soil Sampling:** Soil samples (0-15 cm depth) were taken at random by soil auger from 12 – 15 cores (2.5cm diameter) from each field and mixed thoroughly to prepare composite sample. Plant material and other debris were removed from the soil sample and the soil was sieved using 2mm mesh. The soil samples were collected only at sowing time, before and after each pesticides application at harvest and post harvest during the crop seasons 1997-98. Samples were brought to the laboratory and stored at 4 $^{0}$C till analyses. Temperature, relative humidity and rainfall were recorded at sampling time throughout the experimental period.

**Soil microbial analysis**: Agar media were prepared for microorganisms, sterilized in autoclave at 1.05 Kg $cm^{2}$ and 120 $^{0}$C for 30 min. and cooled at a pouring temperature of about 40 $^{0}$C. Serial soil dilutions were made according to the desired microorganisms. 0.2 ml of desired dilution was spread evenly on the agar- media petri-plate to determine population per gram soil. Luria Bertoni agar media (Miller, 1972) was used for estimation of bacteria after 24 h. Sabouraud medium was used for estimation of fungi after incubation period of 7 days.

**Statistical analyses:** All results were calculated on the basis of the oven dry (105 $^{0}$C) weight of soil. Variables were tested for homogeneity of variance. The influence of pesticide applications and fields on microbial population was estimated with a simple two-factor factorial ANOVA including the factors pesticide application and fields. The Duncan's multiple range test was used to characterize significant differences between the pesticide application and fields.

## Results and Discussions

**Effect of pesticides on total number of bacteria:** Total number of bacteria in cotton fields, treated with normal agricultural rates of various pesticides, either alone or in combination, after 2 days of their application ranged from 11.00 x $10^{5}$ to 39.00 x $10^{5}$ (Table 2) CFU (colony forming units). The control and test fields (Table-2) showed the same number of bacteria in the fields ranged from 15.00 x $10^{5}$ to 22.00 x $10^{5}$ and 17.00 x $10^{5}$ to 23.00 x $10^{5}$ CFU respectively in 1997 crop season. While in farmer field greater variation was observed (11.00 x $10^{5}$ to 25.00 x $10^{5}$ CFU) in total number of bacteria. Endosulfan alone or with dimethoate decreased the total number of bacteria as compared to control field. Similar trend with endosulfan or dimethoate under field conditions was reported by Agarwal *et al.* (1997). TU C.M (1970) and Shamiyeh and Johnson (1973) have also reported inhibitory effect of insecticides on bacteria. In 1998, great variation was seen in total number of bacteria with pesticide application while field variation was non-significant. Pesticide x field interaction was highly significant (Table 3). Profenophos + alphamethrin and methamedophos reduced the total number of bacteria in farmer field as compared to control field. Significantly higher number of bacteria were found in the farmer field with the application of monocrotophos and bifenthrin + acetamiprid. Tu (1995) reported significant stimulation in bacteria population with pesticide application under laboratory conditions. All the other pesticide applications either alone or in combinations had no fluctuation (inhibition, stimulation or modification) in total number of bacteria in test and farmer fields. Hubbell *et al.* (1973) and Komal *et al.* (1999) reported similar non-significant effect on population of bacteria. Data(regarding total number of bacteria in samples) collected at sowing, before pesticide applications, after all pesticide applications, at harvest and at post harvest is shown in Tables 4 and 5. Variation in total number of bacteria in different fields at different intervals of sampling time in the crop season 1997 was non-significant while interaction between sampling time and fields was significant at 5 % level of critical difference (Table 4). Samples collected in 1998 at same sampling time showed variation among the total number of bacteria. Inhibition was seen in the total number of bacteria at 2 days after all pesticide applications that continued to the time of harvest but total number of bacteria recovered to the normal level as were at sowing time.

**Effect of pesticides on total fungi numbers:** Fungi are decay organisms in soil and have important role in providing essential element to higher plants. Although numerically much less abundant than bacteria, they are major contributors to soil biomass and account for as much as 70 % by weight of the

Table 2: Impact of pesticide application on total number of bacteria (CFU* x $10^{5}$ $g^{-1}$ dry soil) in untreated and treated cotton fields during 1997

| Pesticides | Field | | | |
|---|---|---|---|---|
| | Control | Test | Farmer | Mean |
| Endosulfan + Dimethoate | 19.75abcd** | 20.00abcd | 11.00e | 16.92B |
| Profenophos + Cypermethrin | 15.00cde | 18.00abcde | 19.00abcd | 17.33B |
| Profenophos + Cypermethrin | 22.00abc | 19.75abcd | 25.00a | 22.25A |
| Profenophos + Cypermethrin | 18.00abcd | 19.00abcd | 16.00bcde | 17.67B |
| Profenophos + Cypermethrin | 20.00abcd | 23.00ab | 22.00abc | 21.67A |
| Endosulfan | 22.00abc | 17.00bcde | 14.00de | 17.67B |
| Carbosulfan + Fenvalerate | 17.00bcde | 22.75ab | 19.00abcd | 19.58AB |
| Fenpropathrin | 17.00bcde | 19.00abcd | 22.00abc | 19.33AB |
| Mean | 18.48A | 19.44A | 18.88A | |

*Colony forming units **Means followed by same letters are statistically non-significant LSD (5%): For pesticides (P) 3.54; for fields (F) ns; for interaction (PxF) 6.13

Table 3: Impact of pesticide applications on total number of bacteria (CFU x $10^5 g^{-1}$ dry soil) in untreated and treated cotton fields during 1998

| Pesticides | Control field | Test field | Farmer field | Mean |
|---|---|---|---|---|
| Methamidophos | 23.00cdefgh | 13.00ij | 12.00j | 16.00E |
| Monocrotophos | 27.00bcd | 23.00cdefgh | 39.00a | 29.67A |
| Profenophos + Diafonthiuron | 19.00efghij | 23.00cdefgh | 25.00bcdef | 22.33CD |
| Profenophos + Alphamethrin | 32.00ab | 28.00bc | 20.00defghi | 26.67AB |
| Bifenthrin + Endosulfan | 24.00cdefg | 26.00bcdef | 23.00cdefgh | 24.364BC |
| Bifenthrin + Acetamiprid | 26.75bcde | 25.25bcdef | 38.00a | 30.00A |
| Profenophos + Ethion | 16.50ghij | 18.75fghij | 16.00hij | 17.08E |
| Bifenthrin + Ethion | 19.00defghij | 14.25ij | 17.00ghij | 16.83E |
| Propargite | 20.00defghi | 23.00cdefgh | 22.75cdefgh | 21.92CD |
| Bifenthrin + Carbosulfan + Chlorpyrifos | 19.00defghij | 17.00ghij | 18.50fghij | 18.33DE |
| Mean | 22.70A | 21.60A | 22.65A | |

LSD (5%) For pesticides (P) 3.856; for fields (F) ns; for interaction P x F) 6.679. LSD (1%). For pesticides (P) 6.679; for fields (F) ns; for interaction PxF) 9.001, for other explanation see Table 1.

Table 4: Total number of bacteria in soil untreated or treated with normal agricultural rate of pesticides in cotton fields at different time interval

| Days | Sampling time | CFU x 105g-1 dry soil | | | |
|---|---|---|---|---|---|
| | | Control | Test | Farmer | Mean |
| 0 | Sowing | 19.50abc | 9.00abc | 16.50cd | 18.33A |
| 51 | BPA* | 22.350a | 17.00bcd | 12.00d | 17.17A |
| 137 | 2 days AAPA** | 17.00bcd | 19.00bcd | 22.00ab | 19.33A |
| 210 | Harvest | 18.50abc | 17.00bcd | 20.50abc | 18.67A |
| 251 | Post harvest | 21.80ab | 18.50abc | 19.50abc | 19.93A |
| | Mean | 19.86A | 18.10A | 18.10A | |

*Before pesticide applications **After all pesticide applications, LSD (5%). For sampling time (S) ns; for fields (F) ns; for interaction (SxF) 4.567, for other explanation see Table 1.

Table 5: Total number of bacteria in soil untreated or treated with normal with normal agricultural rate of pesticides in cotton fields at different intervals of time, 1998

| Days | Sampling time | CFU x 105g-1 dry soil | | | |
|---|---|---|---|---|---|
| | | Control | Test | Farmer | Mean |
| 0 | Sowing | 19.50abcd | 15.00d | 25.50a | 20.00AB |
| 51 | BPA* | 15.50d | 16.25cd | 23.00ab | 18.25B |
| 137 | 2 days AAPA** | 19.50abcd | 17.00bcd | 8.50e | 15.00C |
| 210 | Harvest | 16.25cd | 15.00d | 9.25e | 13.50C |
| 251 | Post harvest | 23.25a | 20.00abcd | 21.75abc | 21.67A |
| | Mean | 18.80 | 16.65 | 17.60 | |

*Before pesitice applications **After all pesticide applications LSD (5%): For sampling time (S) 1.029; for fields (F) ns: for interaction (S x F) 5.406. LSD (1%). For sampling time (S) 4.332; for fields (F) ns; for interaction (S x F) 7.504. For other explanation see Table 1.

Table 6: Impact of pesticide applications on fungal population (CFU x $10^3 g^{-1}$ dry soil) in untreated and treated cotton fields, 1997

| Pesticides | Control field | Test field | Farmer field | Mean |
|---|---|---|---|---|
| Endosulfan + Dimethoate | 50.00d | 60.50c | 59.75c | 56.75BC |
| Profenophos + Cypermethrin | 51.50d | 59.50c | 51.50d | 54.17BC |
| Profenophos + Cypermethrin | 49.50d | 61.00c | 60.00c | 56.83BC |
| Profenophos + Cypermethrin | 60.50c | 79.50b | 87.50a | 75.83A |
| Profenophos + Cypermethrin | 60.00d | 52.00c | 61.50c | 57.83B |
| Endosulfan | 61.00c | 49.50d | 49.00d | 53.16C |
| Carbosulfan + Fenvalerate | 50.00d | 52.00d | 51.50d | 54.50BC |
| Fenpropathrin | 50.50d | 59.50c | 61.00c | 57.00BC |
| Mean | 54.13C | 59.18B | 61.47A | |

LSD (5%): for pesticides (P) 3.629; for fields (F) 2.222; for interaction (PxF) 6.286. LSD (1%): For pesticides (P) 4.925; for fields (F): 3.016; for interaction (PxF) 8.530. For other explanation see Table 1.

Table 7: Impact of pesticide applications on fungal population (CFU x $10^3 g^{-1}$ soil) in untreated and treated cotton fields during 1998)

| Pesticides | Control field | Test field | Farmer field | Mean |
|---|---|---|---|---|
| Methamidophos | 40.00c | 60.00a | 50.00b | 50.00C |
| Monocrotophos | 29.75d | 60.50a | 60.50a | 50.25C |
| Profenophos + Diafonthiuron | 50.00b | 50.00b | 50.50b | 50.17C |
| Profenophos + Alphamethrin | 40.50c | 49.75b | 49.50b | 46.50D |
| Bifenthrin + Endosulfan | 50.50b | 50.25b | 50.00b | 50.25C |
| Bifenthrin + Acetamiprid | 40.25c | 49.25b | 49.75b | 46.40D |
| Profenophos + Ethion | 39.75c | 59.50a | 59.50a | 52.75C |
| Bifenthrin + Ethion | 50.50b | 59.00a | 60.00a | 56.50B |
| Propargite | 60.50a | 60.00a | 60.25a | 60.25A |
| Bifenthrin + Carbosulfan + Chlorpyrifos | 50.25b | 61.00a | 59.75a | 57.00B |
| Mean | 45.20B | 55.90A | 54.90A | |

lsd (5%): FOR PESTICIDES (p) 2.700; FOR FIELDS (f) 1.479; For interaction (P x F) 4.576. LSD (1%): For pesticides (P) 3.639; for fields (F) 1.993' fpr omteractopm (PxF) 6.302.

Table 8: Fungal population in soil untreated or treated with normal agricultural rate of pesticides application in cotton fields at different time intervals during 1997.

| Days | Sampling time | CFU x 105g-1 dry soil | | | |
|---|---|---|---|---|---|
| | | Control | Test field | Farmer field | Mean |
| 0 | Sowing | 47.00e | 55.00bc | 54.00cd | 52.00B |
| 51 | BPA* | 49.50de | 59.00ab | 59.50ab | 56.00A |
| 137 | 2 days AAPA** | 50.50cde | 59.50ab | 61.00a | 57.00A |
| 210 | Harvest | 51.50cde | 51.50cde | 50.00cde | 51.00B |
| 251 | Post harvest | 52.00cde | 53.00cde | 53.50cd | 52.83B |
| | Mean | 50.10B | 55.60A | 55.60A | |

*Before pesticide applications **After all pesticide applications, LSD (5%): For pesticides (P) 2.745; for fields (F) 2.126; for interaction (P x F) 4.754. LSD (1%): For pesticides (P) 3.810; for fields (F) 2.951; for interaction (P x F) 6.599.

Table 9: Fungal population in soil untreated or treated with normal agricultural rate of pesticides in cotton fields at different time intervals during 1998

| Days | Sampling time | CFU x 105g-1 dry soil | | | |
|---|---|---|---|---|---|
| | | Control | Test field | Farmer field | Mean |
| 0 | Sowing | 47.00cde | 46.00de | 45.00e | 46.00C |
| 51 | BPA* | 50.00bcd | 39.50f | 32.00g | 40.50D |
| 137 | 2 days AAPA** | 49.50bcde | 60.00a | 60.50bcd | 56.67A |
| 210 | Harvest | 46.50cde | 54.00b | 50.50bcd | 50.33B |
| 251 | Post harvest | 47.50cde. | 51.00bc | 50.00bcd | 49.50B |
| | Mean | 48.10B | 50.10A | 47.60B | |

*Before pesticide applications **After all pesticide applications, LSD (5%): For pesticides (P) 2.51; for fields (F) 1.944; for interaction (PxF) 4.348. LSD (1%): For pesticides (P) 3.484; for fields (F) NS; for interaction (P x F) 6.034.

biomass (Lynch, 1983). The data regarding fungal population ranged from 32 to 88 x $10^3$ CFU in cotton fields during both the crop seasons. In 1997 (Table 6) total number of fungi showed highly significant variation in all the fields. Endosulfan + dimethoate, profenophos + cypermethrin and fenpropathrin increased while endosulfan alone inhibited the fungal population. Similar temporary decrease with endosulfan in cotton fields was also reported by Komal *et al.* (1999). h 1998 methamedaphos, monocrotophos, bifenthrin + acetamiprid, profenophos with ethion or with alphamethrin and bifenthrin with ethion or with a mixture of carbosulfan and chlorpyrifos stimulated the fungal population as compared to control soil (Table 7). All other pesticides alone or a mixture of pesticides did not effect the fungal population. Similar no effect of insecticide on fungi have been reported by many workers Tu (1995), Richardson and Miller (1960), Ambrogioni *et al.* (1987), Gunner (1970) and Sivasthamparam (1969). Stimulatory effect have been confirmed by Naumann K, 1970a and Xu *et al.*, 1996. Variation in fungal population at different interval of time (Table 8, 9) showed that adverse effect (inhibition or stimulation) on fungal numbers were nullified by the end of the crop season in all the fields.

After application of pesticides at recommended normal agricultural field rates, both inhibitory and stimulatory effects were observed, in general being very weak and transient and resulting in a recovery of microflora and its functions.

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