# Effect of Systemic Soil Insecticides and a Plant Product on Microbial Load of Soil in Root (wilt) Affected Coconut Monocropping Ecosystem

**By**

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## Abstract

The changes undergoing in the microorganism population due to the application of phorate 10G, carbofuran 3G (@ 10 g a.i./ palm) and neem oil cake (@ 1.5 kg / palm) in the basin region of coconut growing in root (wilt) affected area was studied. Generally, a high microbial population was observed in the control plot. Carbofuran proved to be more toxic as compared to phorate as it suppressed the bacterial, actinomycetal, and free-living $N_2$ -fixer's number significantly. Against *Nitrosomonas* and *Nitrobacter* (nitrifiers), the influence was inconsistent. Neem oil cake enhanced bacterial and free-living $N_2$-fixer count; against actinomycetes and fungi there was an initial stimulatory, and then antagonistic impact, whereas, it proved detrimental to the nitrifiers. From the six soil samplings done, spread over a period of six months, the microbial load was recorded to be high whenever there was moderate rainfall and medium temperature. Application of neem oil cake produced positive effect on the beneficial microorganisms as compared to the systemic insecticides.

## Introduction

India stands first in the world in coconut production (APCC, Statistical Year Book, 1996) and approximately 50% is contributed by Kerala State. Yet the productivity level in this state is low, one of the major reasons being the prevalence of root (wilt) disease. This disease is caused by a phytoplasma (Solomon *et al*., 1983) which is carried by insect vectors *Stephanitis typica* and *Proutista moesta* (Mathen, *et al.,* 1990). A study was undertaken to evaluate the effect of soil application of systemic granular insecticides and a natural plant product on vector control and its influence on regulation/incidence of root (wilt) disease on underplanted coconut seedlings in the diseased area. From the same experimental area, we collected soil samples to record the response of the soil microflora towards the insecticides and plant product. Exhaustive work had been done by Radha and Menon (1954), Rawther and Radha (1963), Potty (1977) and Thomas (1987), on the rhizosphere microflora in coconut monocropping and coconut based multi-storeyed cropping systems in root (wilt) affected area and similar work in root (wilt) free soils had been conducted by Nair and Subba Rao (1977), Ghai and Thomas (1989), Bopaiah and Shetty (1991) and Bopaiah (1994). However, this is the first attempt in describing the changes occurring in soil microflora in root (wilt) prevalent area as a consequence of application of carbofuran, phorate and neem oil cake.

To assess the effect of introduced pesticides, enumeration of microbial population like bacteria, actinomycetes and fungi is utmost important (Atlas, *et.al*., 1978). The nitrifying organisms are extremely sensitive to environmental changes. Therefore the nitrogen transformation is one of the most used parameters, to study the effect of agrochemicals (Parr, 1974).

We investigated the impact of phorate 10 G (@ 10 g a.i. palm), carbofuran 3G (@ 10g a.i./ palm) and neem oil cake (@ 1.5 kg/ palm) on the number of bacteria, actinomycetes, fungi, free living nitrogen fixers and nitrifying bacteria *Nitrosomonas* and *Nitrobacter*, in the basin region of coconut palm.

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## Materials and Methods

For the enumeration of the microflora, soil samples were collected from the coconut palm basin before the application of the pesticide during last week of December 1996 and on 3rd, 15th, 75th, 135th and 180th days after pesticides were applied on 23rd January 1997. Generally, pesticide residues will occur in the top 6-8 inches of soil (Lichtenstein *et al*., 1962). This also is the region of greatest activity of soil flora and fauna (Alexander, 1961); thus, setting the stage for interaction of pesticides with soil life system. Using an auger, soil from top to a depth of 25cm was collected from 3 spots of the basin. This was pooled, homogenised and air-dried. Collections were done from three palms at random from different replicates of each treatment. Soil samples were drawn from this material for the microbial count.

The population count of bacteria, actinomycetes, fungi and free-living nitrogen fixers was done by serial dilution technique (Nair and Subba Rao, 1977). One ml of 1:1,000,000 dilution for bacteria, 1:10,000 for fungi and actinomycetes and 1:1,000 for $N_2$-fixers were plated. Five replications were done. The petri-plates were incubated at 28 ± 2°C for 4-7 days. Colonies developing on the agar medium were scored. Total counts of bacteria, actinomycetes, fungi and free - living $N_2$- fixers were made on nutrient agar (Allen, 1959) Kenknights and Munaier agar (Allen, 1959), Martin's rose bengal agar (Martin, 1950) and Waksman medium 77, respectively. The results were expressed on a dry weight soil basis. The impact of the systemic insecticides and neem oil cake on the population of nitrifiers in the rhizosphere was determined by the most probable number (MPN) method of Alexander and Clark (1965). Ten grams of soil samples were taken from all the treatments and serially diluted up to $10^{-6}$ fold in sterile water. One ml aliquot was transferred from all dilutions to each of five test tubes containing 3 ml of sterile ammonium calcium carbonate medium (for *Nitrosomonas* detection) and to a similar set of five test tubes containing nitrite calcium carbonate medium (for *Nitrobacter* detection). The tubes were incubated at 30 ± 1°C for a period of 21 days. The presence of nitrite and nitrate was tested by the addition of three drops of Griess Illosvay's reagent. The test tubes which gave purplish red color were recorded as positive for *Nitrosomonas* (i.e. indicating the presence of nitrite). In case of *Nitrobacter*, tubes which failed to develop purplish red colour were recorded positive. Through a table of most probable numbers prepared by Cochran (1950), the population of the nitrifiers was computed using the positive results noted in the above experiment.

Analysis of variance for two way classification was followed for the analysis of data.

## Results

The population of the bacteria reduced significantly in all the treatments after initial sampling and increased during the last sampling (180th day), except in the case of carbofuran (Table 1). The beneficial effect of neem oil cake became apparent from the 135th day onwards and the maximum bacterial count of 233.4 x $10^6$ cfu/g dry soil was recorded on 180th day sample. Compared to control, neem oil cake improved the bacterial population; carbofuran proved toxic; whereas, phorate granules was neutral.

The total population of actinomycetes was stimulated by phorate and neem oil cake upto 75th day as compared to control (Table 2). Carbofuran again proved deleterious throughout the sampling period, with the lowest population being registered on 75th day (14 x $10^4$ / g dry soil). Whereas, the highest number was recorded on 135th day in control (193.6 x $10^4$ /g dry soil).

The fungal population was high 3 days after application of the pesticides and neem oil cake when compared to the control. The systemic insecticides suppressed the population from 135th day with carbofuran being more intense. Neem oil cake had adverse effect on the fungi during 75th day of the sampling (Table 3). Overall, there was no significant difference among the treatments.

The count of free-living $N_2$-fixers was enhanced significantly by the application of neem oil cake with maximum number being recorded on the 75th day in this treatment (180.2 x $10^3$). It is evident from Table 4 that ill effect of both the synthetic pesticides was maximum on 75$^{th}$ day when compared to the control.

*Nitrosomonas* which acted in the first step of nitrification i.e. conversion of ammonium to nitrite ($NH_4^+$ ? $NO_2^-$ ) was observed to be very high during the last sampling from control plot (Table 5). A clear picture that emerged from this table is that synthetic pesticides improved the *Nitrosomonas* immediately after application, neem oil cake proved to be antagonistic. *Nitrobacter* which completed the nitrification in the second step by oxidizing nitrite to nitrate ($NO_2^-$ ? $NO_3^-$ ) was also inhibited by neem oil cake application as evident from Table 6. The influence of phorate and carbofuran was also adverse but inconsistent when compared to control during respective sampling period.

**Discussion**

The initial sampling for this experiment was done during last week of December 1996. With copious rainfall up to November (Table 7) and application of 333 + 300 + 666 g of NPK during September-October 1996 the soil condition had been very favorable for healthy microbial activity as observed from the result data tables. From December 1996 onwards, the rainfall stopped to nil with increase in the maximum temperature (Table 7). These factors caused drying of the top soil resulting in lowered microbial activity during subsequent samplings. Fungal count was an exception which was observed to be high even during summer. The reason was the ability of the fungi to be present in dormant stage during unfavorable conditions (Alice *et al.*, 1980). By the time the second last and last samples of soil were collected (during June/ July 97) the rainfall had started again providing sufficient moisture to improve the microflora population. This rainfall, temperature relationship with microbial activity was clearly observed in the results of control palms.

The bacterial count was much higher than any other type of microbe as this has the capacity to thrive vigorously on the root exudates (Alice *et al*., 1980). Among various treatments, carbofuran negatively influenced the bacteria in the basin region of coconut palm in root (wilt) affected area. Our results corroborated of Tu's (1972) wherein he found that 1 and 5 ppm carbofuran inhibited population of bacteria at 28$^o$C. Significant reduction in bacterial population was also reported by Oblisamy, *et al*. (1979) when carbofuran was applied at 1, 10 and 500 ppm a.i. in sandy soil. Nimbalkar, *et al*., 1989 reported that phorate when applied at 1, 1.5 and 2 kg a.i./ ha for control of sucking pests in cotton reduced the population of bacteria initially but at harvest the number of microorganisms increased. Our study though carried out in an perennial plantation crop, also gave similar results when phorate was applied @ 10g a.i./ seedling in the basin region. In the case of neem oil cake treatment; the results showed improved bacterial population when compared to control. Our observation is supported by the report of Manibhushan *et al*., (1987) and Mukherjee *et al*., (1991) who found that bacterial number increased two fold on 60$^{th}$ day over the 40$^{th}$ day sampling.

Actinomycetes population was generally suppressed by carbofuran in the basin region of coconut palm throughout the experimental period except on the 15$^{th}$ day sampling. But Mathur *et.al.*, (1980) published that actinomycetes either increased or showed neutral response to carbofuran application in paddy fields. Varshney and Rana (1987) and Nimbalkar, *etal*.(1989) reported that phorate was non-deletirious towards actinomycetes as we had noticed the same in coconut monocroping ecosystem too. Rather there was stimulation in activity of actinomycetes during the initial stages. The interaction of neem oil cake with actinomycetes was positive up to 75$^{th}$ day after which the number declined. Our results are comparable with the findings of Manibhushan *et al.* (1989) and Mukherjee *et,al.,* (1991). Toxicity against fungal population by carbofuran and phorate had been reported by many workers (Kandaswamy *et al.* 1977; Singh and Prasad, 1979; Cowley and Lichtenstein, 1970) as we had recorded in our experiment. Neem oil cake had shown neutral effect towards the fungal population. Though carbofuran stimulates free-living $N_2$-fixers in sandy-loam soil

(Backman and clark, 1977), but we noted it to be detrimental to free -living $N_2$ -fixers in coconut palm basin. Phorate showed inconsistent stimulation and bacteriostatic effect on free-living $N_2$-fixers, whereas such organophosphorus had been reported to be inhibiory to aerobic $N_2$-fixing population (Sivasithamparam, 1970). Organically rich neem oil cake significantly improved the asymbiotic $N_2$ -fixers in coconut root basins. Similar observations had been made by Gopal (1995) in case of *Azotobacter* from sandy loam soil where 10% a.i. neem-based granule was applied @ 20kg ha. Eppler (1996) also made similar reports of useful microflora being stimulated by neem "actives" in soil.

Application of carbofuran and phorate had no adverse effect on nitrification by *Nitrosomonas* and *Nitrobacter* in this study. Such an observation was also reported by Ramakishna *et al.,* (1978). Studies conducted by Patel and Desai (1985) also gave similar observations with organophosphorus pesticides. Neem oil cake showed significant suppression of both the chemoautotrophs which is in agreement with the observations of Mishra *et al.,* (1975), Santhi *et al.,* (1986) and Gopal (1995). It is also a well known fact that neem extracts are being used with urea prills as nitrification retarders (Waghray, 1997).

## Conclusion

A very high microbial load had been observed by us in the basin region of coconut palm growing in heavily root (wilt) affected area. This observation is in consonance with the report of Potty (1977). The population of these microorganisms changes with fluctuation in the abiotic as well as biotic conditions. Our findings, in general, indicate that application of organically abundant neem oil cake improved the soil health by activating the beneficial soil microorganisms. The chemical pesticides showed some adverse effect with carbofuran being more severe than phorate.

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**Table 1: Effect of systemic insecticides and plant product on bacterial population in coconut basin (n x $10^6$ CFU/g dry soil) (Average of 5 replications)**

| Treatment | Day of sampling | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 15 | 75 | 135 | 180 | |
| Control | 176.2 | 7.2 | 3.8 | 19.0 | 64.2 | 200.2 | 78.4 |
| Phorate | 274.4 | 31.6 | 4.4 | 4.4 | 13.0 | 163.0 | 81.8 |
| Carbofuran | 170.0 | 20.0 | 5.4 | 3.4 | 5.2 | 52.0 | 42.6 |
| Neem oil cake | 220.4 | 32.8 | 3.0 | 11.2 | 188.2 | 233.4 | 114.6 |
| Mean | 210.2 | 22.9 | 4.1 | 9.5 | 67.6 | 162.1 | |

CD for treatment: 22.6, CD for day of sampling: 27.7, CD for interactions: 55.4

**Table 2: Effect of systemic insecticides and plant product on actinomycetes population in coconut basin (n x $10^4$ CFU/g dry soil) (Average of 5 replications)**

| Treatment | Day of sampling | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 15 | 75 | 135 | 180 | |
| Control | 120.6 | 27.8 | 28.4 | 33.8 | 193.6 | 149.2 | 92.2 |
| Phorate | 152.4 | 32.2 | 46.2 | 36.4 | 55.2 | 55.4 | 62.8 |
| Carbofuran | 116.0 | 20.8 | 38.4 | 14.4 | 48.0 | 42.8 | 46.7 |
| Neem oil cake | 82.2 | 48.2 | 118.8 | 88.2 | 62.2 | 445.6 | 74.2 |
| Mean | 117.8 | 32.2 | 57.9 | 43.2 | 89.7 | 73.2 | |

CD for treatment: 12.8, CD for day of sampling: 15.7, CD for interactions: 31.4

**Table 3: Effect of systemic insecticides and plant product on fungal population in coconut basin (n x $10^4$ CFU/g dry soil) (Average of 5 replications)**

| Treatment | Day of sampling | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 15 | 75 | 135 | 180 | |
| Control | 7.0 | 19.0 | 2.8 | 15.0 | 11.4 | 12.2 | |
| Phorate | 8.2 | 17.6 | 7.6 | 16.0 | 8.0 | 9.4 | |
| Carbofuran | 9.4 | 15.2 | 6.2 | 6.8 | 7.4 | 8.0 | |
| Neem oil cake | 11.8 | 13.4 | 8.0 | 8.4 | 12.8 | 13.2 | |
| Mean | 9.1 | 16.3 | 6.1 | 11.5 | 9.9 | 10.7 | |

CD for Treatment : N.S, CD for day of sampling : 2.6, CD for interactions : 5.2

**Table 4: Effect of systemic insecticides and plant product on free living $N_2$ fixer population in coconut basin (n x $10^3$ CFU/g dry soil) (Average of 5 replications)**

| Treatment | Day of sampling | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 15 | 75 | 135 | 180 | |
| Control | 70.6 | 25.0 | 36.4 | 42.6 | 44.8 | 55.6 | 45.8 |
| Phorate | 116.0 | 19.8 | 41.6 | 16.2 | 38.4 | 28.8 | 43.4 |
| Carbofuran | 53.6 | 18.2 | 40.8 | 18.0 | 46.4 | 24.0 | 33.5 |
| Neem oil cake | 104.4 | 51.2 | 178.8 | 180.2 | 106.8 | 107.8 | 121.5 |
| Mean | 86.1 | 28.5 | 74.4 | 64.2 | 59.1 | 54.0 | |

CD for treatment: 7.9, CD for day of sampling: 2.6, CD for interactions: 19.3

**Table 5: Effect of systemic insecticides and plant product on *Nitrosomonas* population in coconut basin (n x $10^3$/g dry soil) (results of 10 fold dilution with 5 tubes per dilution)**

| Treatment | Day of sampling | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 15 | 75 | 135 | 180 | |
| Control | 28.0 | 33.0 | 40.0 | 11.0 | 13.3 | 18.0 | 23.8 |
| Phorate | 22.0 | 30.0 | 13.0 | 14.0 | 7.9 | 7.0 | 15.6 |
| Carbofuran | 95.0 | 17.0 | 11.0 | 7.0 | 9.5 | 2.2 | 23.6 |
| Neem oil cake | 22.0 | 1.7 | 1.4 | 7.9 | 2.2 | 7.0 | 7.0 |
| Mean | 41.7 | 20.4 | 16.3 | 9.9 | 8.2 | 8.5 | |

CD: Non – Significant

**Table 6: Effect of systemic insecticides and plant product on *Nitrobacter* population in coconut basin (n x $10^3$ /g dry soil) (results of 10 fold dilution with 5 tubes per dilution)**

| Treatment | Day of sampling | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 15 | 75 | 135 | 180 | |
| Control | 22.0 | 25.0 | 32.0 | 12.0 | 14.0 | 9.5 | 19.0 |
| Phorate | 33.0 | 31.0 | 59.0 | 12.0 | 14.0 | 1.1 | 25.0 |
| Carbofuran | 17.0 | 43.0 | 35.0 | 2.7 | 1.7 | 3.8 | 17.2 |
| Neem oil cake | 13. | 6.1 | 2.8 | 3.0 | 2.4 | 5.2 | 5.4 |
| Mean | 21.25 | 26.2 | 32.2 | 7.4 | 8.0 | 4.9 | |

CD : Non – Significant

**Table 7: Rainfall, Temperature and Relative Humidity (RH) Chart**

| Year | Month | Temperature ($^{O}$C) | | RH (%) | Rainfall (mm) |
|---|---|---|---|---|---|
| | | Max. | Min. | | |
| 1996 | July | 30.3 | 23.5 | 95 | 358.1 |
| | August | 29.9 | 23.3 | 95 | 123.2 |
| | September | 29.9 | 23.5 | 95 | 261.1 |
| | October | 30.9 | 22.9 | 95 | 266.2 |
| | November | 31.5 | 22.9 | 95 | 153.4 |
| | December | 31.9 | 21.2 | 94 | 58.5 |
| 1997 | January | 33.3 | 20.5 | 92 | 0.0 |
| | February | 33.4 | 21.9 | 92 | 4.9 |
| | March | 34.2 | 23.5 | 91 | 47.9 |
| | April | 33.8 | 23.7 | 93 | 109.8 |
| | May | 33.1 | 24.1 | 93 | 84.3 |
| | June | 31.8 | 23.5 | 93 | 551.6 |
| | July | 30.4 | 23.3 | 96 | 579.6 |