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Meghana D
Research Scholar, Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India

Ramanamma P
Research Scholar, Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India

Rangaswamy V
Professor, Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India

Jaffer Mohiddin G
Department of Life Sciences and Agriculture, Universidad de las Fuerzas Armada, Sangolqui, Quito, Equador, South America

Correspondence
Meghana D
Research Scholar, Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India

Influence of novaluron and thiophanate methyl on microbial population in Groundnut (*Arachis hypogaea*. L) Soils

Meghana D, Ramanamma P, Rangaswamy V and Jaffer Mohiddin G

Abstract
Soil microflora is the key component of agricultural ecosystems that not only plays a significant role in the basic soil processes but is also actively involved in enhancing soil fertility and crop productivity. The present study was carried out to evaluate the effect of indiscriminate use of common pesticides especially insecticides and fungicides on the population of soil microflora in cultivated soil of ground nut field. Novaluron and Thiophanate methyl are the two pesticides used in the present research work to study the population of *Azospirillum*, bacteria and nitrifiers. These three microorganisms respectively has shown healthy growth upto 5.0 Kg/ha^{-1} pesticide application rate, whereas the growth of *Azospirillum*, bacteria, nitrifiers has decreased at 10.0 Kg ha^{-1} pesticide concentration. The important findings of the present study are that the effect on microbes by applying high amount of pesticides reflects the ecological imbalance in cultivated soil. The study focused on the uses of the pesticides as a plant protection agent occasionally hampers the growth of soil microflora in cultivated groundnut field.

Keywords: Groundnut soils, Microbial population–*Azospirillum*, bacteria, nitrifiers, Pesticides-Novaluron, Thiopahanate methyl

1. Introduction
Pesticides are important agrochemicals used for the prevention of crops from pests. Their use has been largely increased in last few decades. The application of pesticides starts from the pre sowing stage. Different treatments of pesticides include soil application, seed treatment, foliar spray etc., repeated application of pesticides contaminate the soil. 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 physico-chemical properties of soil like pH, salinity, and alkalinity leading to infertility of soil [1]. When these pesticides are applied at the possibilities exist that these chemicals may exert certain effects on non-target microorganisms [2]. The microbial biomass play an important role in the soil ecosystem where they fulfill a crucial role in nutrient cycling and decomposition [3]. Studies investigating effects of pesticides on soil microorganisms reported conflicting results [4]. Pesticides in the soil affect the non-target microorganisms [5] and their activities which are essential for maintaining soil fertility [6]. Microbial biomass in soil is considered to be an important feature of soil quality [7]. The use of pesticides to protect crops may alter the biological ability either by direct or indirect action, but the knowledge of soil microbial activity is still limited. Soil microbial biomass measurements has been reported to give an early indication of long-term changes in soil organic matter content, long before such changes could be measured by conventional techniques [8]. Microorganisms form a vital part of soil food web, therefore microbial biomass is considered to be a measure of potential microbiological and ecosystem functioning [9]. Soil respiration which is considered as one of the measures of microbial activity in soil has been reported as a criteria for evaluating pesticide toxicity [10]. *Azospirillum* is a free living micro aerophilic, heterotrophic diazotrophic bacterium that is actively involved in heterotrophic nitrogen fixation in several grass bacteria associations [11]. The occurance of Nitrogen fixing *Azospirillum* in rice roots and soils has also been reported [12]. The influence of several pesticides on the growth and Nitrogen fixation of *Azospirillum* sp has been investigated in pure culture systems by few workers [13-16]. Bacteria one of the major group of microorganisms in soil which have an important function in the chemical and photosynthetic production and in consumption and breakdown of organic matter and release of nutrients.

These are also involved in biofilmproduction that affects sediment stabilization, sediment clogging and deposition of sulphur. Additionally bacteria are important in process like nitrification, denitrification, nitrogen fixation, oxidation and reduction of sulpur and iron, degradation of organic components and precipitation of heavymetals [17]. For the present research studies we have selected Novaluron an Insecticide and Thiophanate methyl a fungicide for their effect on microbial population in the groundnut cultivated soils. Groundnut is a major oil seed cash crop which is cultivating in lakhs of hectares in Andhra Pradesh region. Due to the importance of growing ground nut crop in Ananthapuramu which is a semi arid region of Andhra Pradesh, those soils were selected for the present investigation of our research work.

2. Materials and methods

2.1 Soils used in the Present Study

Agriculture soils both red and black which were cultivating Groundnut crop in Anantapuramu district Andhra Pradesh, India were selected for the present study and soil was collected randomly near the rhizosphere zone using trowel at a depth of 0-12 cms and mixed thoroughly to prepare a homogenous composite sample. Soil sample was air dried at room temperature and they were cleaned to remove plant material and other debris and passed through 2 millimeter sieve and stored at 4ºC prior to analysis.

2.2 Physico chemical characteristics of soil samples:

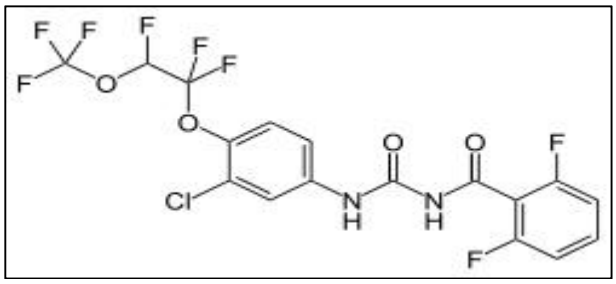
Mineral matter of soil samples was done by following the method [18]. Soil pH was determined by using 1: 1.25 soils to water ratio in systronic digital pH meter. Organic matter in soil samples was estimated by walkley-black oxidation. Total nitrogen content in soil samples was determined by Micro-Kjeldhal method [19]. Electrical conductivity was measured by Conductivity Bridge [20]. Contents of nitrite–nitrogen by Brucine method [21]. The important Physico-chemical properties of the two soils are presented in Table1.

Table1: Soil Phisyco- Chemical Properties

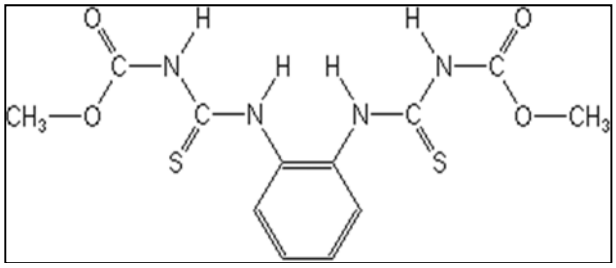
S.no	Properties	Black clay Soil	Red sandy soil
1.	Sand	65.8	55.3
2.	Silt	25.2	27.2
3.	Clay	9.0	17.5
4.	pH	7.2	6.2
5.	Water holding capacity (mg $^{-1}$soil)	0.47	0.27
6.	Electrical conductivity(m. mhos)	260	244
7.	Organic matter	1.33	0.72
8.	Total Nitrogen	0.082	0.046
9.	NH_4(µg g^{-1}soil)	7.93	7.92
10.	NO_2(µg g^{-1}soil)	0.54	0.43
11.	NO_3(µg g^{-1}soil)	0.86	0.62

2.3 Pesticides used in the present study

The pesticides used in the present research work are Novauron and Thiophanate methyl. Novaluron is relatively new benzoyl phenyl urea and insectgrowth regulator. The IUPAC name of novaluron is [1-3-chloro-4-(1, 1, 2-triflouro-2-triflouromethoxyethoxy) phenyl]-3-(2, 6-diflourobenzyol) urea]. It is a potent suppressor of important lepidopteran and coleopteran pests and provide control of several homeopteran and dipteran pests. No studies have specifically examined the mode of action of novaluron, but the general mechanisms and effects with benzoyl phenyl ureas apply. These compounds do not readily inhibit chitin synthesis in cell free systems, nor do they block the chitin biosynthetic pathway in intactive larvae [22]. The enzyme activities in soil were also influenced by the novaluron but the studies are less known about the detailed mechanism. Thiophanate methyl is a systemic fungicide belonging to benzimidazole fungicides used to control a broad range of fungal diseases such as mould, spot, mildew, scorch, rot and blight in variety of crops. The IUPAC name of thiophanate methyl is [dimethyl 4, 4'-(0-phylene) bis (3-thioallophanate). It is also applied in post harvest food storage, as a speed pre-planting treatment and as a timber treatment fungicide [23]. The antifungal activity of thiophanate methyl is based on the blockage of nuclear division during mitosis and destabilization of fungal cell structures. As a result of its action development fungal germ tube and formation of appressoria and mycelia growth were inhibited [24]. The literature reveals that the information regarding the effect of systemic fungicide thiophanate methyl on microbial activities in groundnut soils is limited. The chemical structures of novaluron and thiophanate methyl are shown in Figure 1.



Novaluron



Thiophanate methyl

Fig 1: Chemical structures of pesticides

3. Population of Azospirillum species

To determine the influence of Novaluron and Thiophanate methyl individually on population of Azospirillum species five gram portions of each soil were placed in 15x50 mm test tubes and were treated with different concentrations of pesticides (10, 25, 50, 75 and 100 µg g¯1 soil) which were equivalent to 1, 2.5, 5, 7.5 and 10 kg ha¯1 [25]. Soil samples without pesticides served as controls. The soils with and without pesticides were incubated at room temperature (28 ± 4 ˚C) in the laboratory. Moisture content was maintained at 60% WHC throughout the experimental period. After 7 and 14 days of incubation, triplicate soil samples were withdrawn for the estimation of the population of Azospirillum species following the most probable number (MPN) method [26].

3.1 MPN method for Azospirillum species

The population of Azospirillum species in soils was estimated by the MPN technique and it is followed by ten-fold serial dilutions and the numbers were calculated using the probability tables. 5 ml portions of sterile nitrogen-free semi solid malate medium (SSMM) [27] were taken five MPN tubes and was inoculated with 0.5ml aliquots of the suspensions from 10¯1 to 10¯5 soil dilutions and incubated at 37 ˚C. MPN tubes in which a typical white pellicle developed a few mm below the surface of the medium after 36 hours incubation were said to be positive for Azospirillum species. Characteristic rods with flat droplets and very active spiral movements were observed under the microscopic examination for confirmation.

3.2 Population of Nitrifiers

The influence of Novaluron and Thiophanate methyl on the population of nitrifiers is determined by placing ten grams portions of soil samples in test tubes (15x50 mm) and amended with pesticidal stock solutions. After 7 days and 14 days of incubation, triplicate soil samples were withdrawn for estimating the population density of nitrifiers, by following the most probable number (MPN) method.

3.3 MPN method for Nitrifiers

The amounts of nitrifiers were estimated by MPN method following two fold serial dilutions. Five MPN tubes each containing 5ml of steam sterilized mineral salts medium [28] received 0.5ml aliquots of the soil suspensions from dilutions ranging from 1:500 to 1:32000. Viable cells of nitrosofying and nitrifying bacteria were determined by estimating nitrite following diazotization method [29]. MPN values were calculated by referring to the tables of Fischer and Yates [30].

3.4 Population of Bacteria

The effect of Novaluron and Thiophanate methyl on the population of bacteria were determined by placing five gram portions of each soil in 15x50 mm test tubes and were treated with different concentrations of pesticides which were equivalent to 1, 2.5,5, 7.5 and 10 kg ha¯1. Soil samples receiving only distilled water were served as controls. Soil samples were then homogenized to distribute the pesticide and sufficient distilled water was added to 60% WHC and incubated at room temperature (28 ± 4 ˚C). After 10 days of incubation, triplicates of each treatment were withdrawn for the estimation of bacterial population. Aliquots were prepared from 10¯1 to 10¯7 from treated and untreated soil samples by serial dilution plate method on nutrient agar medium and subsequently incubated for 48 hours in an incubator at 37 ˚C. After incubation, bacterial colonies grown on nutrient agar medium were counted by Quebec colony counter. Bacterial populations were enumerated and expressed as number of colonies formed per gram soil (dry weight basis). Once the stimulatory concentrations of pesticides were determined, the soil samples were further incubated for 10, 20, 30 and 40 days for enumeration of bacterial populations.

Table 2: Population of *Azospirillum* (MPN x 10^3 g^{-1} soil) as influenced by the application of selected pesticides in black soil

Soil incubation in days, after pesticide application												
Pesticide	**7 days**						**14 days**					
	0*	1.0	2.5	5.0	7.5	10	0*	1.0	2.5	5.0	7.5	10
Novaluron	0.78d (100)	1.08c (138)	1.78a (228)	1.13b (145)	0.57e (73)	0.03f (4)	0.76d (100)	0.85c (122)	1.82a (239)	1.0b (131)	0.56e (74)	0.050e (6)
Thiophanate Methyl	0.78d (100)	1.06c (136)	1.61a (206)	1.48b (190)	0.69 (88)	0.04f (5)	0.76d (100)	0.85c (112)	1.72a (226)	1.3b (171)	0.42e (55)	0.033f (4)

*Insecticide concentration, Kg ha-1.

Figures, in parenthesis, indicate relative production percentages.

Means, in each row, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test

Table 3: Population of *Azospirillum* (MPN x 10^3 g^{-1} soil) as influenced by the application of selected pesticides in red soil

Soil incubation in days, after pesticide application												
Pesticide	**7 days**					**14 days**						
	0*	1.0	2.5	5.0	7.5	10	0*	1.0	2.5	5.0	7.5	10
Novaluron	0.73d (100)	0.80c (109)	1.3ba (178)	1.85a (253	0.75d (103)	0.07e (9)	0.18e (100)	0.72c (400)	0.92b (511)	1.13a (628)	0.68d (22)	0.04f (378)
Thiophanate Methyl	0.73d (100)	0.76c (104)	1.3b (178)	1.83a (251)	0.71 (97)	0.06f (8)	0.18d (100)	0.59c (328)	0.78b (433)	1.11a (617)	0.59c (327)	0.03e (17)

*Insecticide concentration, Kg ha-1.

Figures, in parenthesis, indicate relative production percentages. Means, in each row, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

Table 4: Population of nitrifiers (MPN x 10^3 g^{-1} soil) as influenced by the application of selected pesticides in black soil.

Soil incubation in days, after pesticide application												
Pesticide	**7 days**						**14 days**					
	0*	1.0	2.5	5.0	7.5	10	0*	1.0	2.5	5.0	7.5	10
Novaluron	0.78d (100)	1.08c (138)	1.78a (228)	1.13b (145)	0.57e (73)	0.03f (4)	0.76d (100)	0.85c (122)	1.82a (239)	1.0b (131)	0.56e (74)	0.050e (6)
Thiophanate Methyl	0.78d (100)	1.06c (136)	1.61a (206)	1.48b (190)	0.69 (88)	0.04f (5)	0.76d (100)	0.85c (112)	1.72a (226)	1.3b (171)	0.42e (55)	0.033f (4)

*Insecticide concentration, Kg ha-1.

Figures, in parenthesis, indicate relative production percentages. Means, in each row, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

Table 5: Population of nitrifiers (MPN x 10^3 g^{-1} soil) as influenced by the application of selected pesticides in black soil.

Soil incubation in days, after pesticide application												
Pesticide	**7 days**						**14 days**					
	0	1.0	2.5	5.0	7.5	10	0*	1.0	2.5	5.0	7.5	10
Novaluron	0.73d (100)	0.80c (109)	1.3ba (178)	1.85a (253	0.75d (103)	0.07e (9)	0.18e (100)	0.72c (400)	0.92b (511)	1.13a (628)	0.68d (378)	0.04f (22)
Thiophanate Methyl	0.73d (100)	0.76c (104)	1.3b (178)	1.83a (251)	0.71 (97)	0.06f (8)	0.18d (100)	0.59c (328)	0.78b (433)	1.11a (617)	0.59c (327)	0.03e (17)

*Insecticide concentration, Kg ha-1.

Figures, in parenthesis, indicate relative production percentages. Means, in each row, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

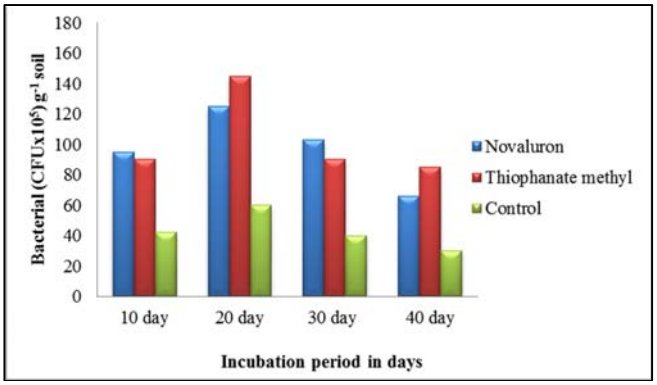


Fig 2: Influence of Novaluron and Thiophanate methyl at 2.5 kg ha^{-1} on the population of bacteria in black soil Means in each column followed by the same letter are not significantly different ($p \leq 0.05$) from each other according to Duncan's multiple range (DMR) test.

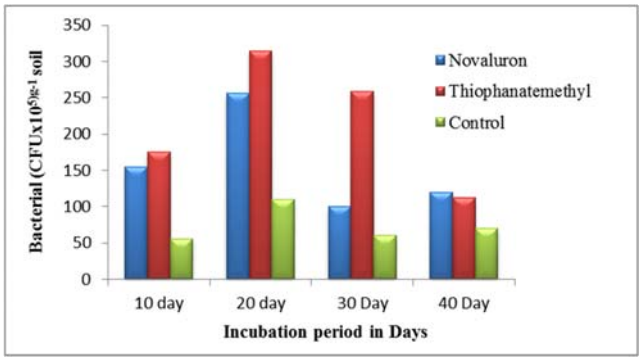


Fig 3: Influence of Novaluron and Thiophanate methyl at 2.5 kg ha $^{-1}$ on the population of bacteria in red soil. Means in each column followed by the same letter are not significantly different ($p \leq 0.05$) from each other according to Duncan's multiple range (DMR) test.

4. Discussion

The population of Azospirillum, Bacteria and Nitrifiers was low in both soils treated with Novaluron and Thiophanate methyl at low concentrations (1.0 $Kgha^{-1}$), Whereas the population of these three microorganisms was significantly enhanced in soils treated with Novaluron and Thiophanate methyl individually. When pesticides were applied at 2.5 $Kgha^{-1}$ to 5.0 $Kgha^{-1}$ the population of Azospirillum, Bacteria and nitrifiers was increased and by enhancing the pesticide concentration upto 7.5 $Kgha^{-1}$ to 10.0 $Kgha^{-1}$ the

population count of three organisms were decreased. A similar individual stimu-latory effect of monocrotophos and chlorpyrifos was previously demonstrated on the population of *Azospirillum* sp. [31]. Similar observations with other organophosphorus and pyrethroid insecticides and fungicides have also been reported [32, 33]. Increase in the population of *Azospirillum* sp. at high concentrations (100 ppm) of benomyl or 2-amino-benzimidazole (a hydrolysis product of benomyl) has been reported in paddy soil [34, 35]. Nayak & Rao (1980) have observed stimulation in population of *Azospirillum* sp. when treated with benomyl at lower concentration (5 ppm). The selected pesticides - monocro-tophos and chlorpyrifos, singly and in combination with mancozeb and carbendazim, respectively - at levels ranging from 1.0 to 5.0 kg ha^{-1} - significantly increased the population of *Azospirillum* sp. and the rate of ammonification in a vertisol and a laterite soil from a groundnut plantation. Further-more, these pesticides, singly and in combination, at levels of 1.0 to 10.0 kg ha^{-1} exerted synergistic, additive or antagonistic interactions toward population of *Azospirillum* sp. In groundnut soils. One of the insecticide novaluron used in this study has showed good antagonistic activity against Colorado potato beetle and low mammalian toxicity. A little number of researchers have studied the impact of pesticides on micro organisms [3, 4, 17] but knowledge about exotoxocological effects of novaluron is till now ignored especially towards microorganisms beaded with structural chemical targets.

5. Conclusion

The effect of pesticides decreases the population of bacteria, *Azospirillum* and nitrifiers at the high rates (10 kg ha^{-1}). Maximum growth of bacteria, *Azospirillum* and Nitrifiers was observed at field rates (5.0 kg ha^{-1}) whereas decrease in growth was noticed at 10 kg ha^{-1}. By this experiment we can conclude that the growth of bacteria, *Azospirillum* and nitrifiers was not harmed at recommended field rates.

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