

The Effect from the Pesticides on Bacteria Involved in Nitrogen Fixation

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Abstract - Plants may be protected against pests with the use of pesticides, however during the last forty years, widespread pesticide usage has disrupted the natural biological system. Some agricultural herbicides have the potential to damage microorganisms that fix nitrogen. Studying how pesticides affect microorganisms that fix nitrogen was the goal of the current investigation. Actually, the purpose of the research was to determine how the herbicides affected the desirable nitrogen-fixing bacteria, which are crucial for plant development and higher yields. In comparison to the growth of herbicide significantly reduced both nitrogen-fixing bacteria. This study's findings support the notion that pesticides operate differently at various areas and have varying effects on the development of bacteria that fix nitrogen. Evidence suggests that the pesticides used in field conditions may have had a negative impact on the population of these bacteria because of their high toxic nature.

Keywords - Pesticides, Nitrogen Fixing Bacteria

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INTRODUCTION

Pests that impede crop development are eliminated from agricultural fields using pesticides, which are synthetic organic substances. Pesticides are chemicals used to kill or deter various pests, including insects, weeds, rodents, birds, bacteria, nematodes, and plant parasite viruses. Pesticides assist in eliminating or delaying insect activity, saving the crop at various phases of development. Thus, pesticides assist impoverished farmers in producing a healthy crop with less labor expenditure. It decreases needless time spent looking for, clearing weeds from, or getting rid of pests' known or unknown feeding grounds. As everyone is aware, a farmer must spend a significant amount of money on pest control throughout the year. As a result, individuals have been looking for appropriate and particular substances.[1]

Decoction, combination, and fume were also referenced in historical and legendary literature as methods of insect control for plants. Around 4500 years ago, elemental sulphur dusting was used to ward off pests in ancient Mesopotamia, marking the beginning of pesticide use. The Rig Veda, a revered Hindu literature that is more than 4,000 years old, also made reference to the usage of toxic plants to ward off hazardous pests. Chemicals like arsenic, mercury, or lead were utilized to eradicate pests throughout the 15th century. According to the literature, nicotine sulfate, an insecticide made from tobacco plant leaves, was regularly used in agricultural fields in the 17th century AD. Pyrethrum and rotenone, two natural insecticides, were first used in the 19th century.

Pesticide usage has been increasing steadily and rapidly over the world since the latter half of the 19th century, which has resulted in a significant rise in pesticide contamination.[2-4]

The United States' first pesticide governing body was established by law in 1910, taking into account the effects on the environment. However, application continued on a worldwide scale. In reality, it surged in the first half of the 20th century, which led to the introduction of several synthetic pesticides starting in 1940 all over the globe. Thus, the 1940s to 1950s have been seen as the start of the modern age of pesticide use (Murphy, 2005). The third agricultural revolution, sometimes known as the "green revolution," started in the late 1960s as a consequence of the introduction of synthetic pesticides. This revolution included new technology, such as the use of hybrid crop varieties together with herbicides and chemical fertilizers, as well as the usage of contemporary farm equipment. The method has enabled remarkable agricultural productivity all around the world.[5]

Prior to being made aware of their negative effects on human health and connection to certain environmental problems, people began to see pesticides as a crucial component in the food production process (Hazarika, 2010). However, the fact that pesticides are still being used in agriculture areas is concerning. Insecticides, fumigants, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators, and other substances fall under the category of pesticides. The four main

categories of synthetic insecticides still in use are as follows. They are the carbamate, pyrethroid, organochlorine, and organophosphate groups. These are sophisticated chemical substances that may have stimulatory, inhibitory, or neutral effects depending on their ingredients, concentration, contact duration, and treatment frequency (Maly and Ruber, 1983).[6-7]

PESTICIDES AND THEIR DANGERS

Pesticides have expanded economic potential via greater food and fiber production and reduced vector-borne illness, but at a high cost to human and environmental health. At this point, there is incontrovertible proof that some of these compounds may be harmful to people and other organisms, in addition to having unintended consequences for the ecosystem. There is no safe level of pesticide exposure, and the people of poor nations and high-risk groups within each country have a disproportionate share of the responsibility for the potentially devastating health impacts of pesticide exposure (WHO, 1990). About 1 million people a year suffer from chronic illnesses and untimely deaths as a result of pesticide contamination (Environews Forum, 1999).[8]

Workers in manufacturing, formulation, spraying, mixing, loading, and agriculture are at a heightened risk of exposure to pesticides. Manufacturing and formulation both entail non-zero risk factors. Pesticides, raw materials, poisonous solvents, and inert carriers are just some of the hazardous substances handled by employees in industrial environments.[9]

Air, lakes, seas, fish, and birds that eat fish might all be contaminated by OC compounds due to the widespread dispersion of these substances throughout the environment. According to the National Academy of Sciences of the United States, exposure to DDT and its metabolite, DDE, causes eggshell thinning, which is a major factor in the fall of the bald eagle population in the USA (Liroff, 2000). Long-term, low-dose exposure to environmental chemicals, such as pesticides, is increasingly linked to negative human health outcomes like immune suppression, reproductive problems, diminished intelligence, reproductive abnormalities, and cancer due to the chemicals' ability to mimic or antagonize the body's natural hormones, which is how they are known to elicit their adverse effects.[10-11]

Workers (N=356) at four units producing HCH in India had neurological symptoms (21%), which were correlated with exposure levels (Nigam et al., 1993). The level of the toxicity risk associated in the spraying of methomyl, a carbamate insecticide, under field circumstances was examined by NIOH. (Saiyed et al., 1992). Electrocardiogram (ECG) alterations, increases in blood lactate dehydrogenase (LDH) levels, and decreases in cholinesterase (ChE) activities were all seen in the spraymen, showing cardiotoxic effects of methomyl. In male formulators working in the

unorganized sector to create dust and liquid formulations of different pesticides, limited health surveillance observations showed a high incidence of generalized symptoms in addition to psychological, neurological, cardiorespiratory, and gastrointestinal symptoms, and low plasma ChE activity (Gupta et al., 1984).[12]

One thousand and ten couples were studied for their exposure to pesticides (organochlorine, organophosphate, and carbamates) during the male's work in cotton fields and its potential effects on reproduction.[13-14]

Malaria sprayers (N=216) were recruited for a research to examine the effects of a brief exposure to HCH in field circumstances over the course of 16 weeks. The sole impact proven with confidence as a result of dioxin creation in a study of persons afflicted by the Seveso disaster of 1976 in Italy during the manufacturing of 2,4,5-T, a herbicide, was chloracne (almost 200 instances with a clear exposure dependency) (Pier et al., 1998). There were conflicting findings from the first health studies looking at things like liver and immunological function, neurological damage, and reproductive impacts. It was discovered that there was an increase in mortality from respiratory and cardiovascular disorders; this may be attributable to both the chemical exposure and the psychological fallout from the disaster. Diabetes was also identified in higher than normal numbers.[15]

A higher rate of cancer in the digestive tract, lymph nodes, and blood was found in the follow-up study of cancer incidence & death. Several factors, including a lack of individual-level exposure data, a very brief latency period, and a relatively small population numbers for specific cancer types, mean that the results cannot be considered definitive. All-cause and cancer-related death rates were stable in 2001, according to a research conducted that year. The findings, however, provide credence to the theory that dioxin is carcinogenic to humans also confirm the hypothesis linking it to effects on the cardiovascular system and the endocrine system. The United States military used roughly 19 million litres of herbicide on around 3.6 million acres of land in Vietnam and Laos to clear vegetation from the perimeters of US bases and to eliminate forest cover and damage crops. [16]

Operation Ranch Hand was implemented between the years of 1962 and 1971. Different formulations of herbicides were employed, with most including a combination of the phenoxy herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) or 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (2,4,5-T). During the Vietnam War, almost 3 million Americans fought in the military services there. They were among the many who were exposed to defoliant mixes including Agent Orange, including Vietnamese fighters and civilians as well as personnel of the

military forces of other countries. There existed data on the cancer risk of Vietnamese people, Vietnam War veterans, and employees exposed to herbicides and dioxins on the job. [17-18]

MATERIAL AND METHOD

Material for Planting Seeds,

Greengram (*Vigna radiata* (L.) Wilczek.) variety CO4 seeds were sourced from the Regional pulses Research Institute's Regional Research Station in the Cudalore District of Tamil Nadu, India.[19]

Fertilizers

We got our hands on several biofertilizers and chemical pesticides from the Government Authorized Agrocentre in Chidambaram, Cuddalore District of Tamil Nadu. These included *Bradyrhizobium japonicum*, *Bacillus megaterium*, and *Bacillus mucilaginosus*. [20]

Obtaining Samples:

Ten distinct areas had random soil samples taken from the surface (0-15cm). A wide variety of locations (including Nellikkuppam, Panruti, Chidambaram, Kurinjipadi, Neyveli, Virudhachalam, Kattumannarkoil, Thirunavalur, and Parangipettai) are used to compile the samples. To ensure their integrity during transit to the lab, all samples were sealed in plastic bags and maintained at 4 degrees Celsius until analysis. Prior to the chemical tests, these materials were air-dried and crushed till they fit through a 2 mm filter. pH, soil chemistry, and texture measurements were taken.[21-22]

RESULT

Soil from a field in Cuddalore, India, was used to isolate ammonifying and nitrogen-fixing bacteria.

Only dirt from one location was found to have bacteria like *Pseudomonas fluorescens*. Ten soil samples were tested for the presence of nitrogen-fixing *Bradyrhizobium* sp. one hundred percent accounted for. When compared to *Azotobacter* sp. were the signals S1, S8, and S9 captured. On S2, *Micrococcus bovis* was only found at one of the locations. *Bacillus megaterium*, *B.subtilis*, *Nitrobacter* sp., *Nitrococcus* sp., and *Streptococcus* spp. The 10 soil samples showed alternating detection of *Nitromonas* sp, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Only three bacteria were consistently isolated and identified from the 10 soil samples.[23]

Table1: Sampling soil for ammonifying and nitrogen-fixing bacteria

S. No	Name of the bacteria	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀
1	<i>Pseudomonas fluorescens</i>	+	-	-	-	-	-	-	-	-	-
2	<i>Bradyrhizobium</i> sp	+	+	+	+	+	+	+	+	+	+
3	<i>Azotobacter</i> sp	+	-	-	-	-	-	+	-	+	-
4	<i>Micrococcus bovis</i>	-	+	-	-	-	-	-	-	-	-
5	<i>Bacillus cereus</i>	-	-	+	-	-	-	-	-	-	-
6	<i>Nitrobacter</i> sp	-	-	-	+	-	-	-	-	-	-
7	<i>Nitrococcus</i> sp	-	-	-	+	-	-	-	-	-	-
8	<i>Streptococcus</i> sp	-	-	-	-	+	-	+	-	-	+
9	<i>Nitrosomonas</i>	-	-	-	-	+	-	-	-	-	+
10	<i>B. subtilis</i>	-	-	-	-	+	-	-	-	-	-
11	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	+	-	-	-	-
12	<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	-	-	+	-

Pesticide Evaluation

Nellikkuppam, Panruti, ethidambaram, Kurinjipadi, Neyveli, Virudhachalam, Kattumannarkovil, Thirunavalur, Parangipettai, and Vadalur were analyzed for pesticide residue using a colorimeter, with respective results of 0.30, 0.29, 0.27, 1.32, 0.52, 0.42, 1.12, 0.41, 0.61, and The soil in Kurinjipadi showed the highest concentration of pesticide residue out of the 10 testing areas, while the soil in Chidambaram showed the lowest concentration. [24-25]

Table 2: Pesticide Residue Colorimetric Estimates from Multiple Locations

S. No	Name of the places	Pesticide residue(mg/Kg)
S ₁	Nellikkuppam	0.30
S ₂	Panruti	0.29
S ₃	Chidambaram	0.27
S ₄	Kurinjipadi	1.23
S ₅	Neyveli	0.52
S ₆	Virudhachalam	0.42
S ₇	Kattumannarkoil	1.12
S ₈	Thirunavalur	0.41
S ₉	Parangipettai	0.61
S ₁₀	Vadalur	0.98

The highest retention times of Nellikkuppam, Chidambaram, Kurinjipadi, and Kattumannarkovil were used in an HPLC study to estimate the amount of pesticide residue in the soil at 10 different locations. We measured the shortest retention duration ever in Virudhuchalam soil. [26]

Table 3: Pesticide-contaminated soil samples analyzed using high-performance liquid chromatography in a variety of locations

S. No	Name of the places	Retention time
S ₁	Nellikuppam	4.209,6.113,12.056
S ₂	Panruti	3.805,7.889
S ₃	Chidambaram	5.550,6.134,6.704
S ₄	Kurinjipadi	4.281,8.008,10.208
S ₅	Neyveli	4.616,9.585
S ₆	Virudhachalam	3.649
S ₇	Kattumannarkoil	5.831,22.247,24.813
S ₈	Thirunavalur	3.535,5.300
S ₉	Parangipettai	4.561,10.567
S ₁₀	Vadalur	3.557,5.613

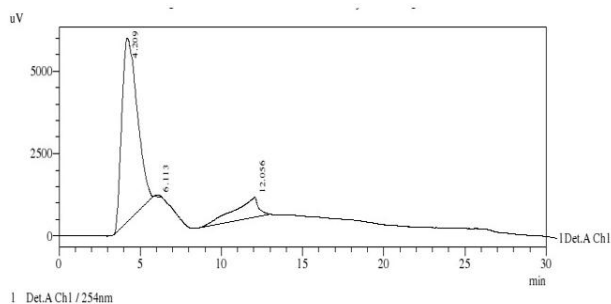


Fig 1: Using high-performance liquid chromatography, we analyzed a soil sample from Nellikuppam that had been contaminated with pesticides.

PeakTable					
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.209	365361	5550	84.548	89.365
2	6.113	870	47	0.201	0.757
3	12.056	65906	613	15.251	9.878
Total		432137	6210	100.000	100.000

CONCLUSION

The study's findings suggest that the pesticides' effects on the development of bacteria that fix nitrogen varied depending on the location. There is evidence that the pesticides used in field conditions may have had a negative impact on the population of these bacteria because of their high toxic nature. Biochemical values were estimated, including total chlorophyll, carbohydrate, and protein concentrations. Seedlings were evaluated for their biofertilizer and pesticide exposure, as well as their catalase and peroxidase activity. Green gram seedlings were cultivated with and without the use of several pesticides and biofertilizers, and their mineral content, including nitrogen, phosphate, potassium, calcium, mg, zinc, copper, iron, and manganese, was evaluated. Green gram seedlings cultivated with a synergistic application of biofertilizers had more biochemical, enzyme activity, and mineral content than those grown with fungicide, insecticide, and pesticide treatments.

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