



EFFECTS OF BIOPESTICIDE (NEEMAZAL-T/S) AND CHEMICAL PESTICIDE (OMITE) ON THE GROWTH OF THE PLANT *Lycopersicon esculentum* (PHYSICO-CHEMICAL ANALYSIS) AND THEIR EFFECTS ON THE SOIL MICROFLORA

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ABSTRACT

Farmers use either chemical pesticide or biopesticide to protect the crops from pests. This study was undertaken to investigate the effects of biopesticide (Neemazal-T/S) and chemical pesticide (Omite) on the growth and the physicochemical analysis of the plant *Lycopersicon esculentum* and their effects on the soil microflora. Three tomato plants were grown as control, for biopesticide and chemical pesticide apply. Evaluation of three plant soil under field conditions was for soil parameters and microflora. Evaluation of three types of plant under field conditions was for shoot, root and leaf length, number of branches and physiochemical analysis of the leaf both qualitatively and quantitatively. Chemical pesticide affected plant growth as well as the soil microflora. While biopesticides induce the plant growth and does not affect the soil microflora in the field condition. Control plant remains.

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INTRODUCTION

Agriculture has been facing the destructive activities of numerous pests like fungi, weeds and insects from time immemorial, leading to radical decrease in yields. (Salma Mazid *et al.*, 2011). Pests are one of the serious challenges facing crop production today. (Okrikata E & Oruonye E D, 2012).

The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematocides, plant growth regulators and others. (Md.Wasim Aktar, *et al.*, 2009). The use of pesticides is as old as human civilization in fact when human being started the agricultural activity for the sustainable life. In ancient times, the first intentional use of a pesticides dates back to 2500 BC when the Sumerians rubbed foul smelling sulfur compounds on their bodies to control insects and mites. (Talat Parween *et al.*, 2015)

Pesticides of chemical origin are highly effective against the target organism. However, the chemical pesticides have toxic effects on several non-target species. (Gayathri P K, *et al.*, 2016)

According to the guidelines for the approval of pesticides, effects of pesticides on soil microorganisms and soil fertility should be determined. The use of pesticides to protect crops

may alter the soil biological activity either by direct or indirect action, but the knowledge of soil microbial ability to degrade pesticides and the influence of pesticides on microbial diversity in soil are still limited. Certain pesticides stimulate the growth of microorganisms but other pesticides have depressive effects or no effects on microorganisms when applied at normal rates. (Chi-Chu Lo, 2010)

Biopesticides are natural materials derived from animals, plants and bacteria, as well as certain minerals that are used for pest control. (Christos A Damalas and Spyridon D Koutroubas, 2018).Neem based pesticides have great potential in pest management due to their mode of action, specificity and cost efficiency. (Arutselvi R *et al.*, 2012)

Tomato (*Lycopersicon esculentum*) is a herbaceous sprawling plant growing to 1-3m in height with weak woody stem. Tomato is attacked by number of pests including Tomato fruit worm (*Helicoverpa armigera*). Like other vegetables tomato is prone to the insect pest and disease attack because of its tenderness and softness. (Syed Arif Hussain Rizvi and Saleem Jaffar, 2015)

MATERIALS AND METHODS

Sample Preparation

The tomato seeds were sown in three pots. It was allowed to grow. The tomato plants were grown in three pots. In those three plants, one was taken as control, the other was sprayed by biopesticide which contains Azadirachtin and the last one was sprayed by chemical pesticide which contains Propargite.

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The soil sample was collected from these three pots for following procedures.

Serial Dilution Technique

Seven test tubes with 9ml distilled water were taken. They were marked as 10^{-1} to 10^{-7} . 1g of soil sample was inoculated into the first test tube. It was mixed and 1ml of 10^{-1} dilution factor was transferred to 10^{-2} . It occurs simultaneously upto 10^{-7} . 1ml from 10^{-7} was poured out. For isolation of bacteria, 10^{-3} , 10^{-4} and 10^{-5} are taken.

Plating Technique

Nutrient agar medium was prepared and poured onto the petridish. It was allowed to solidify. 0.1ml of 10^{-3} , 10^{-4} and 10^{-5} dilution factors are inoculated on the agar plate. It was spread by using L-rod or glass spreader. It was kept for incubation at 37°C for 24hrs. The colonies which were undergo following observations. The colonies were observed for microscopic examination, motility, gram's staining, biochemical tests such as indole, MR, VP, citrate utilization, TSI, catalase, urease, starch hydrolysis, lipid hydrolysis, nitrate reduction, H_2S production and specificity tests for identifying the genus.

Soil Parameter Analysis

The soil parameters such as pH, calcium, magnesium, sulphur and phosphorous were analysed in three soil samples.

Physical Analysis of Plant

Plants were plucked from the soil and the leaf, root and stem measurements were taken by using scale. The number of branches was also measured.

Phytochemical Analysis

Sample Preparation

Plant leaves were dried in sunlight for 2 days. After dried it was crushed into powder. The powdered pack material was air dried and then used for following determination. The dried plant leaves were undergo qualitative tests such as alkaloids, saponins, tannins, flavanoids and reducing sugar. They undergo quantitative analysis for determination of alkaloids, phenolics and flavanoids.

RESULT

The aim of present study focus on comparative effects of biopesticide (Azadirachtin 1% EC) and chemical pesticide (Propargite) (Fig 1.2) on tomato plants.

The tomato seeds were sown on three different pots on 17.10.2018 shown in fig 1.1. Germination was observed on 22.10.2018 shown in fig 2.1. After certain growth of the plants, they were undergo sprayed by pesticides. First pot was taken as control which is no pesticide sprayed; the second pot is sprayed by biopesticide (Azadirachtin 1% EC) while the third one was sprayed by chemical pesticide (Propargite) on 03.01.2019.

The soil from three pots were collected and calculated for microbial counts. In those, the control soil sample showed 500 colonies on 10^{-3} dilution, 390 colonies on 10^{-4} dilution and 300 colonies on 10^{-5} dilution. The biopesticide soil sample showed 490 colonies on 10^{-3} dilution, 390 colonies on 10^{-4} dilution and 370 colonies on 10^{-5} dilution. While the chemical pesticide soil

sample showed 300 colonies on 10^{-3} dilution, 210 colonies on 10^{-4} dilution and 160 colonies on 10^{-5} dilution. (Table 1.1)

The number of colonies converted into colony forming units; shortly referred as cfu in one mg of soil sample. In those, control soil sample has showed 5×10^5 cfu/mg in 10^{-3} dilution, 3.9×10^6 cfu/mg in 10^{-4} dilution and 3×10^7 cfu/mg in 10^{-5} dilution. The biopesticide soil sample has showed 4.9×10^5 cfu/mg in 10^{-3} dilution, 4.2×10^6 cfu/mg in 10^{-4} dilution and 3.7×10^7 cfu/mg in 10^{-5} dilution. While the chemical pesticide soil sample has showed 3×10^5 cfu/mg in 10^{-3} dilution, 2.1×10^6 cfu/mg in 10^{-4} dilution and 1.6×10^7 cfu/mg in 10^{-5} dilution. (Table 1.2)

I have isolated bacteria from three soil samples. First the bacteria isolated from the control soil sample which does not sprayed by any type of pesticide. They are *Proteus* sp., *Azotobacter* sp., *Pseudomonas* sp. and *Staphylococcus* sp. Second, the bacteria isolated from biopesticide soil sample are *Proteus* sp., *Azotobacter* sp., *Pseudomonas* sp. and *Staphylococcus* sp. At last, the bacteria isolated from chemical pesticide soil sample are *Proteus* sp., *Pseudomonas* sp. and *Staphylococcus* sp. (Table 2.1)

The soil parameters of three soil samples were analysed by using different estimation methods. The parameters which have analysed are pH, Calcium Magnesium, Sulphur and Phosphorous in control, biopesticide and chemical pesticide soil samples. (Table 3)

The plant physical growth is necessary to be analysed for checking the growth condition. The three tomato plants physical growth was measured on 30.01.2019 by using measuring scale. The physical growth measurement such as the length of shoot, root, leaf and number of branches of the plants. (Table 4)

Usually the plant consists of number of phytochemicals needed for their growth and metabolism. Hence in this study, the phytochemicals of the tomato plant leaves were analysed both qualitatively and quantitatively.

The phytochemicals which were analysed qualitatively are alkaloids, flavanoids, saponins, reducing sugar and tannins. In those. The phytochemicals which are present in the leaves are alkaloids, flavanoids, saponins and tannins. The phytochemical reducing sugar is not present in any of the three plant leaves. (Table 5.1)

The phytochemicals which were analysed quantitatively are phenol, flavanoid and alkaloid in three tomato plants. The control plants have 0.07mg of phenol, 11mg of flavanoid and 4g of alkaloid. While the biopesticide applied plant contains 0.05mg of phenol, 10mg of flavanoid and 4g of alkaloid. Finally the chemical pesticide applied plant contains 0.02mg of phenol, 8mg of flavanoid and 2g of alkaloid. (Table 5.2)

DISCUSSION

The total bacterial counts of Parks and Garden soil, Biological soil and Hall of residences soil were counted range from 9.5×10^7 cfu/g, 8.0×10^5 cfu/g respectively. (Ogunmwonyi I *et al.*, 2008). In this investigation, the obtained colony forming units differ according to the pesticides used.

The qualitative and quantitative analysis of phytochemicals of tomato pulp were analysed by using various extracts. In that study, the methanolic extracts has showed existence of all kind

of phytochemicals and has high amount of carbohydrate in it. (Murali Krishna *et al.*, 2013). But in this study, only the aqueous extract of the tomato leaves were used and quantitative measurements showed significant differences in the three plants.

CONCLUSION

Pests are the unwanted or destructive insects or other animals which affect the food or crops. The compounds which are used to kill the pests called pesticides. There are two types of pesticides depend upon the compounds which we use-biopesticide and chemical pesticide. Chemical pesticides are the pesticides first used by farmers. They are prepared from inorganic compounds which are toxic to pests and also affect plant metabolism and contaminate the environment. On the other hand, biopesticides are prepared from the microbes or another plant enzyme to kill the pests. They are specific in nature. Since it is from natural products, it does not affect the environment.

By comparing chemical and biopesticides, it has been shown that biopesticides does not affect the normal microbial population as well as the parameters present in the soil. The physicochemical parameters of plants are somewhat coincide with the control plant where no pesticide sprayed. In contrary, chemical pesticide does affect the microbial population and the soil parameters. Hence, the plant growth was also affected.

From my study, I conclude that the biopesticides are far more convenient than the chemical pesticides to kill the pests without affecting the crops and environment. They even does not affect the microbial population in soil which needed for plant growth. The plant which was sprayed by biopesticide showed better growth than the normal plant. The plant which was sprayed by chemical pesticide showed stunted growth than the normal plant and it also affect the soil microflora which is needed for plant growth.

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Table 1.1 Colonies estimation from three soil samples on different dilution factors

Sample	Dilution factors		
	10 ⁻³	10 ⁻⁴	10 ⁻⁵
Control soil sample	500	390	300
Biopesticide soil sample	490	390	370
Chemical pesticide soil sample	300	210	160

Table 1.2 Estimation of microorganisms from three soil samples on different dilution factors

Sample	Dilution factors		
	10 ⁻³ (cfu/mg)	10 ⁻⁴ (cfu/mg)	10 ⁻⁵ (cfu/mg)
Control soil sample	5×10 ⁵	3.9×10 ⁶	3×10 ⁷
Biopesticide soil sample	4.9×10 ⁵	4.2×10 ⁶	3.7×10 ⁷
Chemical pesticide soil sample	3×10 ⁵	2.1×10 ⁶	1.6×10 ⁷

Table 2.1 Isolation of bacteria from three soil samples

Soil sample	Bacteria identified
Control soil sample	<i>Proteus sp.</i> , <i>Pseudomonas sp.</i> , <i>Staphylococcus sp.</i> , <i>Azotobacter sp.</i>

Biopesticide soil sample	<i>Azotobacter sp.</i> , <i>Pseudomonas sp.</i> , <i>Staphylococcus sp.</i> , <i>Proteus sp.</i>
Chemical pesticide soil sample	<i>Proteus sp.</i> , <i>Pseudomonas sp.</i> , <i>Proteus sp.</i>

Table 2.2 Characteristics of Isolated Bacteria from the soil sample

Characteristics	<i>Proteus sp.</i>	<i>Azotobacter sp.</i>	<i>Pseudomonas sp.</i>	<i>Staphylococcus sp.</i>
Shape	Rod	Oval	Rod	Spherical
Gram's staining	G -ve	G +ve	G -ve	G +ve
Motility	Motile	Motile	Motile	Non-motile
Indole	+	+	-	-
MR	+	+	-	+
VP	-	+	-	+
TSI test	K/A, Gas	K/A, Gas	K/K	K/A
Citrate utilization	-	+	+	+
Catalase	+	+	+	+
Urease	+	+	-	+
N ₂ reduction	+	+	+	+
H ₂ S production	+	-	-	-
Lipid hydrolysis	+	+	+	+
Starch hydrolysis	+	+	-	+
Carbohydrate fermentation	+	+	-	+

Where K/A- Alkaline slant, Acid butt; K/K- Alkaline slant, Alkaline butt;
Gas-Gas formation

Table 3 Analysis of different soil parameters in three soil samples

Parameter	Unit	Control soil sample	Biopesticide soil sample	Chemical pesticide soil sample
pH	log H	7	8	8
Calcium	(me/l)	1.2	1.04	0.88
Magnesium	(me/l)	0.288	0.366	0.192
Sulphur	(mg/kg)	212.5	214.5	207.5
Phosphorous	(mg/kg)	16	16	12

Table 4 Measurement of Physical growth of tomato plants in three pots

Plant physical parameters	Unit	Control plant	Biopesticide plant	Chemical pesticide plant
Length of Shoot	cm	45	44	37
Length of Root	cm	6.5	4.5	4
Length of Leaf	cm	5.5	6	4.5
Number of Branches	Nil	6	7	8

Table 5.1 Qualitative measurement of phytochemicals of three sample plants

Phytochemicals	Control plant	Biopesticide plant	Chemical pesticide plant
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Reducing sugar	-	-	-
Tannins	+	+	+

Table 5.2 Quantitative measurement of phytochemicals of three sample plants

Phytochemicals	Unit	Control plant	Biopesticide plant	Chemical pesticide plant
Phenol	mg	0.07	0.05	0.02
Flavonoid	mg	11	10	8
Alkaloid	g	4	4	2



Fig 1.1 Tomato Seed Sowing



Fig 1.2 pesticide

Figure 2 Plants Growth On 15 Days Duration



Fig 2.1 Germination on 22.10.2018



Fig 2.2 (15 DAYS)



Fig 2.3 (30 DAYS)



Fig 2.4 (45 DAYS)



Fig 2.5 (60 DAYS)



Fig 2.6 (75 DAYS)

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