



EFFECT OF SOIL APPLICATION OF NEEM CAKE AMENDED WITH *TRICHODERMA* AND *PSEUDOMONAS FLUORESCENS* ON SOIL HEALTH AND YIELD OF TOMATO CROP

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Abstract

Chemical pesticide usage was increased and it may lead to the deposit of these pesticide residues on agricultural crops. Organic farming promotes eco-friendly agricultural practices without making use of synthetic inputs and majorly relies upon the use of organic wastes to raise crops. Neem cake which is a form of organic manure used in organic farming for the enhancement of crop yield. This experiment was designed to carry out the synergistic effect of neem cake amended with *Trichoderma viridi* and *Pseudomonas fluorescens* species. Neem cake along with 0.1% of microbial load did not affect the physical nature and chemical constituents of the cakes. We observed that the *Trichoderma* population was reached up to 1×10^7 cfu/gm and the *Pseudomonas* population was estimated as 1×10^8 cfu/ml.

Keywords- Neem cake, organic manure, microbial load, *Trichoderma viridi*, *Pseudomonas fluorescens*.

I. INTRODUCTION

Pesticides are used in agriculture to protect crops against destructive pests both in the field and during storage. They are also used in public health and other areas for the eradication of disease vectors and other pests. The steady growth of the world's population necessitates increased food production, which in its turn places the responsibility upon the international chemical industry to find new compounds to protect crops against pests and other biological threats. The increases in crop yields from pesticide use are considerable, resulting in their environmental accumulation and their consequent entrance to the human food chain, both directly, and via domesticated livestock. Public concern about food quality has intensified in recent years. Against this background, demand for organically grown food has been growing rapidly. Organic farming is an alternative to regular farming. It makes use of compost, manure, green manure, bone meal rather than using fertilizers and pesticides (AmadouBinta and Barbier, 2015). Microbes are used as bio-fertilizers to increase production without polluting the environment (Bagchi *et al.*, 2015).

Seed cake also contains nitrogen (2 - 5%), phosphorus (0.5 - 1.0%), potassium (1 - 2%), calcium (0.5 - 3%) and magnesium (0.3 -1%). Neemcake also reduces alkalinity in soil, as it produces organic acids on decomposition. The use of neem cake, which is a form of organic manure, has become essential. Neem (*Azadirachta indica*) seed cake (residue of neem seed after oil extraction), when added to the soil, not only improves the soil with organic matter, but also lowers nitrogen losses by inhibiting nitrification. The material left after oil is squeezed out from seeds and is popularly known as the seed cake; it acts as a bio fertilizer and helps in providing the required nutrients to plants. It is widely used to ensure a high yield of crops. Neem is used as a fertilizer both for food crops and cash crops, particularly rice and sugarcane crop (Subbalakshmi *et al.*, 2012).

Neem seed cake performs the dual function of both fertilizer and pesticide, acts as a soil enricher, reduces the growth of soil pest and bacteria, provides macro nutrients essential for all plant

growth, helps to increase the yield of plants in the long run, bio degradable and Eco friendly and excellent soil conditioner (Subbalakshmi et.al, 2012). It increases the yield of crops on the long term, an excellent soil conditioner and has no negative effect on the environment. Neem seed cake, as a form of organic manure on decomposition, promotes an increase in soil microbial communities and this in turn will affect the growth and yield of crops. It is bio degradable and eco friendly, nourishes the soil and plants by providing all the macro and micro-nutrients, helps to eliminate bacteria responsible for denitrifying the soil, ideal for cash crops and food crops, increases the yield of crops, helps to reduce the usage of fertilizer, thus reducing the cost of growing plants, antifeedant properties that help to reduce the number and growth of insects and pests.

Soil contains a variety of microorganisms included bacteria that can be found in any natural ecosystem. Microorganisms play an important role on nutritional chains that are an important part of the biological balance in the life in our planet. Where, bacteria are essential for the closing of nutrient and geochemical cycles such as the carbon, nitrogen, sulfur and phosphorous cycle (Chitra et al., 2014). The use of biological control agents as an alternative to fungicides is increasing rapidly in the present day agriculture due to the deleterious effects of chemical pesticides (Meera and Balabaskar, 2012). Members of the genus *Pseudomonas* and *Trichoderma* have long been known for their potential to reduce the plant disease caused by fungal pathogens and they have gained considerable importance as potential antagonistic microorganisms (Pant and Mukhopadhyay, 2001). More recent research indicated that certain strains of *Trichoderma* can induce systemic and localized resistance to several plant pathogens. Plants treated with *Trichoderma* in the root zone can produce higher levels of peroxidase, chitinase activity, deposition of callose-enriched wall appositions on the inner surface of cell walls and pathogenesis-related proteins. Moreover, some strains may enhance plant growth and development (Tran, 2010). Among these the bacterial antagonists have the twin advantage of faster multiplication and higher rhizosphere competence hence, *P. fluorescens* have been successfully used for biological control of several plant pathogens (Ramamoorthy et al., 2002) and biological control using PGPR strains especially from the genus *Pseudomonas* is an effective substitute for chemical pesticides to suppress plant diseases (Compant et al., 2005). The present study was aimed at screening of rhizosphere isolates of *P. fluorescens* and *Trichoderma* amended with neem cake. The field study was carried out in tomato.

II. MATERIALS AND METHODS

Neem cake were amended by *P. fluorescens* and *Trichoderma* by different percentage to avoid the contamination and obtained good growth. *P. fluorescens* observations on the number of colony forming units (CFU) per gram were recorded at 7th, 14th, 21st, 28th and 35 days after inoculation by serial dilution agar plate technique. Population dynamics was examined by mixing 1 g of formulations aseptically with 10 ml sterile distilled water for 20 min in a rotary shaker. Serial dilutions were prepared and 0.1 ml aliquot from 10⁻⁵ to 10⁻⁸ dilutions were spread on KMB plates (Chakravarty et al., 2011). After incubating the plates at 28 ± 1°C for 48 h, the cfu/g formulations were counted out of *Pseudomonas fluorescens* on by using following formula (Aneja, 2003). Then, the colonies were transferred on Potato Dextrose Agar (PDA) medium plates and incubated at (28 ± 1°C), and observed regularly to monitor fungi growth for 5-6 days.

Polythene bags were (12 x 8 cm²) sterilized in autoclave or pressure cooker. After sterilization, polythene bag were cooled and the 48 h KMB slant culture of *P. fluorescens* and *Trichoderma* cell suspension of concentration of 10⁸ cfu/ml was pipetted separately into the each bag thoroughly mixed with neem cake concentration of 0.01, 0.05 and 0.1% with the help of sterilized spoon and plugged by using cotton and nylon strings. Four replications for each treatment were maintained, these bags were incubated at room temperature (35 to 40± 1°C) and cooler temperature (20 to 25± 1°C). Observations on the number of colony forming units (CFU) per gram were recorded at 7th, 14th, 21st, 28th and 35 days after inoculation by dilution plate technique. Population dynamics was examined by mixing 1 g of formulations aseptically with 10 ml sterile

distilled water for 20 min in a rotary shaker. Serial dilutions were prepared and 0.1 ml aliquot from 10^{-5} to 10^{-8} dilutions were spread on KMB plates (Chakravarty *et al.*, 2011). After incubating the plates at $28 \pm 1^\circ\text{C}$ for 48 h, the cfu/g formulations were counted.

III. FIELD STUDY

Field study was conducted during January to April, 2018 in farmer's field at Attur, Salem District, Tamil Nadu. The soil samples were collected from the study area before and after cropping. The experimental design was a randomized block design, which consisted of two treatments, Neem Cake and untreated control. Neem cake is applied with the dosage of 100 kgs/acre.

IV. METHOD OF APPLICATION

Neem cake was given as soil application and the soil is thoroughly mixed. Neem cake is applied before 3 - 4 weeks of sowing or transplanting. Since Neem cake acts as a slow release, water is applied to the soil after application of Neem cake in experimental site. Biometric parameters such as Plant height (Cms), number of fruits / plot, Single fruit weight (Gms), Yield / plot (Kgs) were recorded. Percent yield increase was calculated based on the yield/hectare by comparing the treated and untreated plots.

V. RESULTS

One gram of substrate from each bag was used for assessment of difference in growth of *P. fluorescens* and *Trichoderma*. The observations were recorded on 7, 14, 21, 28, and 35 DAI and data regarding number of colony forming units (cfu). Neem cake along with 0.1% of microbial load did not affect the physical nature and chemical constituents of the cakes. We observed that the *Trichoderma* population was reached up to 1×10^7 cfu/gm and the *Pseudomonas* population was estimated as 1×10^8 cfu/ml. The population trend was similar for *P. fluorescens* and *Trichoderma* population when from 7 to 35 DAI.

Trichoderma and *Pseudomonas* cultures were inoculated into 10 grams of sterile neem cake. Appropriate control group was also included in the study. The findings revealed that the physical state of neem cake moderately changed its colour and state. The treated samples were shown in figure 1 and 2. These indicate both the organisms utilized the nutrients present in the neem cake and also due to the interactions between the metabolites of organisms and the ingredients of neem cake. *Trichoderma* and *Pseudomonas* cultures were inoculated into 10 grams of sterile neem cake supplemented with 1% glucose. The treated samples were shown in figure 3 and 4. The findings revealed that the physical state of substrate had no visual change even after three weeks. These indicate both the organisms utilized the glucose as alternate substrate for their survival and growth. Thus physical state of neem cake remained unaltered. Both the organisms were grown in neem cake with or without glucose. Unfortunately, and even a moderate colour change in the commercial product is not acceptable and moreover the nutritional content of the product may also be reduced due to the consumption of microbes over a period of time as the packed product has its self-life. Hence an alternative substrate such as glucose can be incorporated in to the neem product before packing, and the desired plant-beneficial microbe can also be inoculated. Therefore, the original state of neem cake will have no alteration as well as it will retain its nutritional value.

The soil properties was analysed and the results are given in Table 1. The soil of the experimental site was slightly acidic before cropping season and at the end of the experiment it was noted that there was improvement in soil pH, which may be attributed to the Neem cake application. The organic matter content was low at the onset of the experiment, but at the end of the cropping season it was moderate. Similar trend of increase in soil attributes was noted in N, P and K also.

Table 1. Soil properties of the experimental site.

Time	pH	Percent (%)		Ppm		
		OC	OM	N	P	K
Before cropping	6.8	0.59	0.91	0.04	4.6	0.29
After cropping	7.2	1.05	1.79	0.14	5.7	0.38



Figure 1. Trichoderma amended with neem cake (0.1%) Figure 2. Pseudomonas amended with neem cake 0.1%

Biometric parameters of tomato treated with neem cake is given in Table 2. Neem cake was applied as soil application before 3 – 4 weeks of sowing/transplanting. Biometric parameters like plant height, number of fruits/plot, single fruit weight, yield/plot, yield / Hec were recorded and percent yield increase was calculated. The biometric data revealed that the neem cake treated plot shows good results in both vegetative characters and also in the productivity of the crop. Neem cake treated tomato crop recorded 33.04 % of yield increase compared to untreated control plot (Figure 5). Biometric parameters of tomato treated with neem cake are given in Table 2. Neem cake was applied as soil application before 3 – 4 weeks of sowing/transplanting. Biometric parameters like plant height, number of fruits/plot, single fruit weight, yield/plot, yield / Hec were recorded and percent yield increase was calculated. The biometric data revealed that the neem cake treated plot shows good results in both vegetative characters and also in the productivity of the crop. Neem cake + *Trichoderma* + glucose and Neem cake + *P. fluorescens* + glucose treated tomato crop recorded about 22 % of yield increase compared to untreated control plot. The other treatments like Neem cake, Neem cake + *Trichoderma viridi* and Neem cake + *Trichoderma* + glucose (Figure 6,7,8,9) recorded percent yield increase of 18.76, 17.99 and 19.47 % respectively compared to untreated control.

Table 2. Biometric parameters and yield data of tomato treated with neem Cake.

S. No.	Treatments	Plant height (Cm)	No. of fruits/plot	Single fruit weight (Gms)	Yield/plot (Kgs)	Yield /Hec (Kgs)	Yield increase (%)
1.	Neem Cake	82.14	204.43	73.31	97.24	5235.99	18.76
2.	Neem cake + <i>Trichoderma viridi</i>	84.32	178.33	77.81	96.32	5186.45	17.99
3.	Neem cake + <i>P. fluorescens</i>	86.65	238.31	72.67	98.10	5282.29	19.47

4.	Neem cake + <i>Trichoderma</i> + glucose	87.91	231.23	79.79	102.38	5512.75	22.84
5.	Neem cake + <i>P. fluorescens</i> + glucose	88.52	257.83	79.54	101.32	5455.68	22.03
6.	Untreated control	74.39	111.66	59.8	79.85	4253.34	-

Table 3. *Trichoderma* and *P. fluorescens* population cfu/ml.

S. No.	Treatments	<i>Trichoderma</i>			<i>P. fluorescens</i>		
		T1- 0.01%	T2- 0.05%	T3- 0.1%	T1- 0.01%	T2- 0.05%	T3- 0.1%
1.	Neem Cake	-	-	-	-	-	-
2.	Untreated control	-	-	-	-	-	-
3.	Neem cake+ <i>Trichoderma</i>	10 ²	10 ⁴	10 ⁷	-	-	-
4.	Neem cake+ <i>P. fluorescens</i>	-	-	-	10 ²	10 ⁴	10 ⁸
5.	Neem cake+ <i>Trichoderma</i> +glucose	10 ⁴	10 ⁶	10 ⁷	-	-	-
6.	Neem cake+ <i>P.fluorescens</i> +glucose	-	-	-	10 ⁴	10 ⁵	10 ⁸

The results of the present study clearly indicate the inoculation of the microbes were highly beneficial for enhancing the yield besides effecting a reduction in the cost of inorganic fertilizers and also significantly increases the soil fertility, soil beneficial microbes, and decreases the rate of diseases incidence (Garikapati and Sivasakthivelan, 2013).



Figure 3. *Trichoderma* + neem cake and glucose



Figure 4. *Pseudomonas* + neem cake and glucose



Figure 5. Neem cake



Figure 6. Neem cake + *Trichoderma viridi*



Figure 7. Neem cake + *P. fluorescens*



Figure 8. Neem cake + *Trichoderma* + glucose



Figure 9. Neem cake + *P. fluorescens* + glucose



Figure 10. Untreated control

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