

Population dynamics of *Trichoderma harzianum* on Sodium nitrate added cakes as a source of Nitrogen

Adesh Kumar

¹Department of plant pathology, School of Agriculture, Lovely professional University, Phagwara (Punjab)-144411, India

ABSTRACT

For the mass culture of *Trichoderma harzianum*, four oil cakes were amended with sodium nitrate as a source of nitrogen rather than plain substrates (where no supplement added). This supplement was added to enhance the population dynamics of *T. harzianum*. Of the four cakes wherein sodium nitrate was added Neem cake was found supporting the highest population dynamics of *T. harzianum* followed by Jatropha cake, next best was Mahua cake followed by Karanja cake. Sodium nitrated added Neem cake was found supporting the colony forming units g⁻¹ is 66.67×10^6 that was recorded highest after 30 days of inoculation.

Key words- *Trichoderma harzianum*, oil cakes, carbon sources, nitrogen sources, vitamins, population dynamics

INTRODUCTION

Synthetic chemicals possess negative impact on plant, person and planet since it is evident as pesticides draft greatly potential risk and put bio-life on a great danger [7,12,14] thus it puts undesired load to the nature[1].

High rate and amount application of all the forms of pesticides are contaminating soil biota and killing non-target organisms [8] too and damaging micro-organisms and macro-organisms such as bacteria, fungi, insects, worms lies in the soil as soil biota [13,18] that contributes in soil biomass a component of soil organic matter plays a vital role in soil nutrient cycle [1,5,6]. This is the truth that enhancement in production of agricultural yield is associated with high quantity use of pesticides including insecticides, fungicides, herbicides etc.[2] and it is assumed that by 2050 use of all these category pesticides will increase 2.7 times than it was in 2000 resulting in deleterious health challenges to humans, environment and other life forms [3].

Trichoderma is one amongst the antagonists of plant pathogens being cultivated at big scale and mass multiplied on different organic biodegradables i.e., neem cake, coir pith, farmyard manure, vermicompost, karanja cake and decomposed coffee pulp etc. since long time and proved worthy in various research had held [3,16,17].

In present investigation an effort is made in increasing conidial yield of *T. harzianum* on four de-oiled cakes and in addition added with a carbon source in finding an increase in longevity and survival of conidia under storage conditions.

1.MATERIALS AND METHODS

2.1 Collection, isolation and Maintenance of the culture

Rhizospheric samples were collected from research fields and strain was isolated at PG lab of Plant Pathology department at Sardar Vallab Bhai Patel University of Agriculture & Technology, Meerut, India. Isolation was done by from stock sample and different dilutes were got ready from 10^{-1} to 10^{-6} wherein 1 ml of 10^{-6} were dispensed on Trichoderma Selective Medium (TSM) in sterilized Petri plates [9]. Culture was maintained and incubated in biological oxygen demand incubator for 7 days at $25\pm 2^{\circ}\text{C}$ and visual and microscopic identification is done wherein culture seems initially hyaline but turned green when fully grown as conidia are produced and also confirmed when observed under microscope [15].

2.3 Collection and preparation of cakes for mass culturing of *Trichoderma*

Cakes brought from oil processing units were cleaned and crushed manually in pestle & mortar to achieve a particle size of ± 1 mm diameter with $\pm 25\%$ moisture was maintained upon autoclaving at 1.1 kg/cm^2 [17]. Seven days old culture of *Trichoderma harzianum* then was inoculated in cakes under air laminar flow and providing aseptic conditions. The flasks were kept in incubator and incubated at $25\pm 2^{\circ}\text{C}$ [10,11] and were shaken thoroughly at 2 days interval for 30 days.

2.4 Evaluation of population dynamics and colony forming units

Serial dilution plate technique is used to determine population dynamics where growth of fungus in cakes was measured in the form of colony forming units (CFUs) and monitored at 15 days regular interval for 120+ days under lab condition. To confer the CFUs per gram of cake sample serial dilution technique was performed.

2.4.1 Serial dilution and CFUs counting

One gram of the cake samples were suspended in 10 ml distilled water containing test tubes it gives 1: 10 concentrated suspension called stock solution from which a final 10^{-6} diluted suspension was made and poured onto potato dextrose agar media and incubated for 5 days at $25\pm 2^{\circ}\text{C}$ and colonies were counted as CFUs^{-1} gram soil [3] (Plate1).

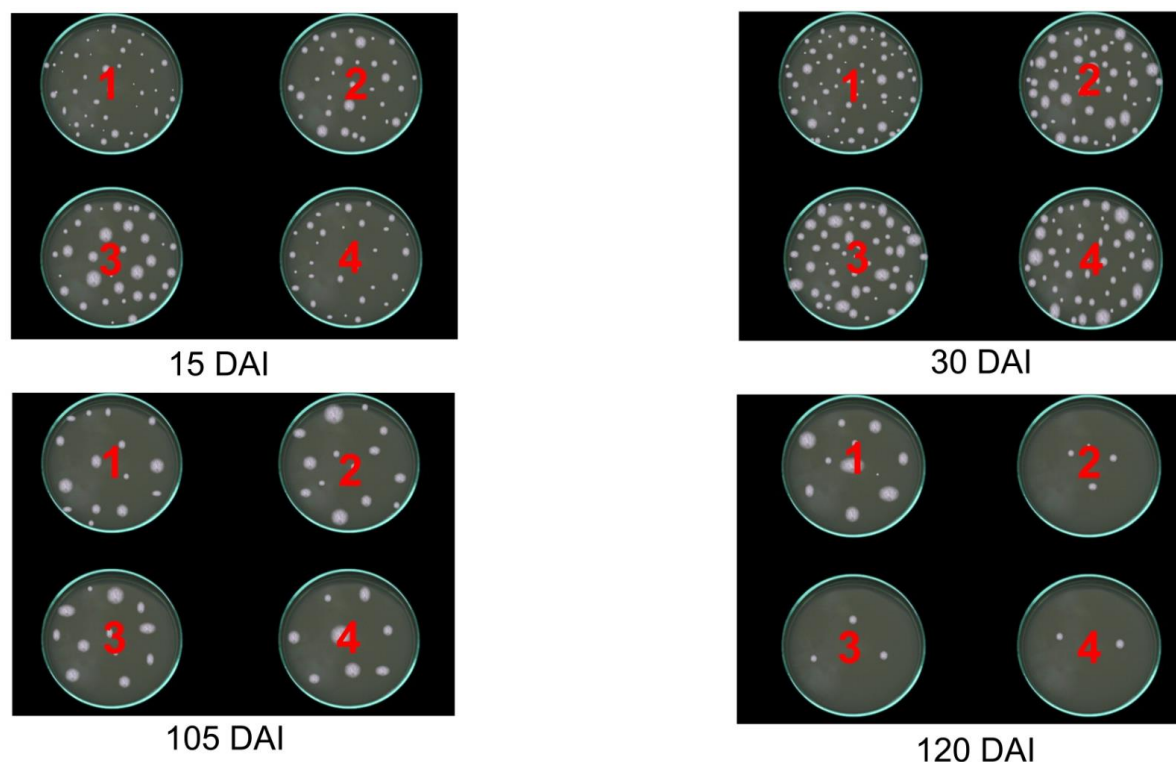


Plate1: CFUs from Neem cake (1), Jatropha cake (2), Mahua cake (3) and Karanja cake (4)

2.6 Statistical analysis

The data's were analyzed using ANOVA and treatment means differentiated using Fischer's completely randomized design (CRD) *in vitro* studies. Statistical analysis was conducted using general linear model procedures of SPSS version 16 [4].

3. RESULTS

3.1 Screening of different oil cakes added with fructose for mass culturing of *T. harzianum*

Fructose was used as a source of carbon in the study and the growth of bioagent was measured in the form of colony forming units (CFUs) at a regular interval of 15 days up to 120 days till the growth of microorganism was found recorded since in the next count no cell was found viable in any of the cakes.

Table-1 Effect of Sodium nitrate as amendment to different cakes on longevity and viability of *T. harzianum*

S.N.	Treatments	CFUs x10 ⁶							
		15 DAI	30 DAI	45 DAI	60 DAI	75 DAI	90 DAI	105 DAI	120 DAI
1.	Neem cake	42.67	66.67	59.67	45.33	35.67	23.33	16.00	10.67
2.	Jatropha cake	36.67	55.67	48.67	34.00	25.00	20.67	14.33	4.00
3.	Mahua cake	33.67	50.00	42.67	30.00	21.67	18.00	11.67	3.33
4.	Karanja cake	32.00	48.33	40.67	26.67	17.33	11.00	7.67	1.67
	CD @ 5%	Substrates=0.96 Days=1.44 Substrates x Days=2.88							

Table-1, Fig.-1 and Plate-1 showed that after 15 days of incubation Neem cake found showing higher population dynamics as CFUs per gram of cake mixed with fructose as a source of carbon and supported 42.67 x10⁶ CFUs of *T. harzianum*. This count was significantly higher than recorded in other three cakes

followed by Jatropha cake as it supported 36.67×10^6 CFUs. Next was Mahua cake (33.67×10^6) followed by Karanja cake (32×10^6) that was at lowest slab in supporting growth of *T. harzianum*.

After 30 days incubation Neem cake found showing higher population dynamics as CFUs per gram of cake mixed with fructose as a source of carbon and supported 66.67×10^6 CFUs of *T. harzianum*. This count was significantly higher than recorded in other three cakes followed by Jatropha cake as it supported 55.67×10^6 CFUs. Next was Mahua cake (50.00) followed by Karanja cake (48.33×10^6) that was at lowest slab in supporting growth of *T. harzianum*.

After 45 days after incubation Neem cake was at top supporting 59.67×10^6 CFUs and was significantly superior than the mean values achieved in Jatropha cake (48.67×10^6), Mahua cake (42.67×10^6 CFUs) while the lowest growth rate was supported by Karanja cake as 40.67×10^6 CFUs of *T. harzianum* was recorded.

At 60th day maximum population of the *T. harzianum* was recorded in Neem cake (45.33×10^6) and is significantly superior to the CFU value in Jatropha cake i.e., 34.00×10^6 . Mahua cake was 3rd in the place and supported 30.00×10^6 CFUs while lowest growth was achieved in Karanja cake which supported 26.67×10^6 CFUs of *T. harzianum*.

Highest CFUs at 75 days after incubation were found in Neem cake (35.67×10^6 CFUs) and it was recorded statistically superior than Jatropha cake (25.00×10^6), Mahua cake (21.67×10^6) and Karanja cake which supported lowest of CFUs i.e., 17.33×10^6 .

When CFUs were recorded after 90 days Neem cake (23.33×10^6) followed by Jatropha cake (20.67×10^6) and Mahua cake (18.00×10^6). Lowest population of *T. harzianum* was supported by Karanja cake @ 11.00×10^6 CFUs and found statistically lesser than other cakes. All these cakes are statistically different to each other.

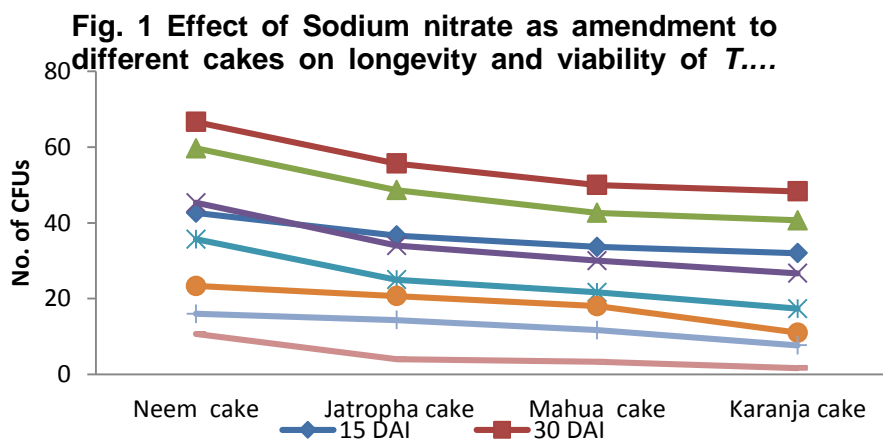
At 105th day, 16.00×10^6 CFUs were supported by Neem cake which was followed by supported by Jatropha cake wherein population dynamics of *T. harzianum* is 14.33×10^6 CFUs per gram of cake. Jatropha was found superior than Mahua cake wherein population dynamics of *T. harzianum* supported is 11.67×10^6 . Lowest CFUs were counted in Karnja cake i.e., 7.67×10^6 .

At 120th day, 10.67×10^6 CFUs were supported by Neem cake which was followed by supported by Jatropha cake wherein population dynamics of *T. harzianum* is 4.00×10^6 CFUs per gram of cake. Jatropha was found superior than Mahua cake wherein population dynamics of *T. harzianum* supported is 3.33×10^6 . Lowest CFUs were counted in Karnja cake i.e., 1.67×10^6 .

Upon comparison between substrates in the see of supporting population of *T. harzianum* after addition of fructose to them, it had been noticed that after 30 days of incubation highest CFUs were exhibited at 30 DAIs that was found statistically superior than the values recorded throughout the period of evaluation. A slight reduction in population of *T. harzianum* in the form of conidia v/v was recorded after 45 days. However, list number was counted at 120th day wherein all substrates except Neem cake lost their viability.

Admixed cakes were evaluated for a period of 120 days wherein it resulted in highest CFUs count at 30th day of incubation. Colony forming units were greater than that of values recorded at 15th and 45th days

of incubation. CFUs were recorded reduced significantly at 75th day and afterward. Almost negligible number of CFUs recorded at 120th and no CFU were achieved from substrate afterward.



DISCUSSION

Discussion with a purpose in finding suitable substrate for mass production of *Trichoderma* that also support longevity and survival four de-oiled cakes were tested. Cakes of Neem, Jatropha, Mahua and Karanja were used as substrates and in order to check impact of an additional source of nitrogen sodium nitrate was mixed in cakes which can enhance growth, sporulation, durability and support longevity of *T. harzianum*. Addition of sodium nitrate to these cakes resulted in increasing the survival of *T. harzianum* beyond 120 days, whereas without addition survival was 105 days.

CONCLUSION

Neem cake from all the cakes was found superior throughout the evaluation period of 120 days and was followed by Jatropha cake. During period of incubation Mahua cake was next to Jatropha cake that was followed by Karanja cake in supporting the population of *T. harzianum*. CFUs were supported up to 105 days after incubation from Jatropha, Mahua and Karanja cakes (Plate 1, Table 1, Fig. 1). Once fructose is added a positive effect in enhancing the CFUs and longevity of survival, has been noticed.

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