Effect of Lactose on Longevity andSurvival of Trichoderma harzianum

Adesh Kumar*, Ramji Singh, BikeshYadav***,Vipul Kumar*, Vinit P Singh*andAjay Tomar***

*Assistant Professor, Lovely professional University Phagwara Punjab (India)

**Professor &HOD, Dept. of Plant Pathology, S.V.P. University of Agriculture and Technology, Meerut-250110, India

***Technical Assistant, UP Govt., Uttar Pradesh

ABSTRACT

Some naturally occurring antagonistic microorganisms has performed great potential to antagonize plant pathogens, hence, biocontrol involving the use of such beneficial microorganisms for plant protection is being considered as a viable substitute to reduce the use of chemical pesticides [1,2]. These microorganisms need to be multiplied and kept viable often for long period under storage. To ensure this purpose some biodegradable wastes i.e., Neem cake, Jatropha cake, Mahua cake and Karanja cake were evaluated under current study. Neemcake remains best substrate in supporting population of *Trichoderma harzianum* under laboratory. The conidial form of *T. harzianum* could be stored about 4 months when cakes were added with one of the carbon source; Lactose. An increase in number of viable propagules (CFUs/g) was measured from all the substrates when at 15 DAI, CFUs increasesto 42 from 30.67(plane Neem cake) and at 30 DAI, 64.67 from 42 (plane Neem cake) in lactose mixed Neem cake.

Key words- De oiled cakes; Lactose; *Trichoderma harzianum*; self-life; longevity; viability etc.

Abbreviations: CFU=colony forming units, DAI=days after inoculation, BCAs=biocontrol/biological control agents, TBOs=tree borne oilseeds, PDA=potato dextrose agar, CRC=Crop Research Centre, Conc=concentration, Temp=temperature, Plane cakes=cakes not added with Lactose

I. INTRODUCTION

Over the past four decades studies on the use of beneficial microorganisms as biocontrol agents (BCAs) for plant protection has increased greatly. These plant beneficial microorganisms are known to antagonize phytopathogens through competition for niches and or nutrients [3], parasitism that may involve production of hydrolytic enzymes, i.e., chitinase, glucanase, protease and cellulase that can lyse pathogen cell walls inhibition of the pathogens by antimicrobial compounds [4] and induction of systemic resistance in host plants [5,6].

Several kind of substrates like grains of sorghum, corn, rye and millets [7], different organic media like Neem cake, coir pith, farmyard manure, and decomposed coffee pulp [8] and different agricultural wastes i.e., Sugarcane bagasse, vermicompost, paddy straw and talcum powder are used for mass production of *Trichoderma* spp. [9]. Therefore, a study was conducted to culture and enhance the conidial yield of *T. harzianum* onto some de-oiled cakes.

Cow dung, Neem cake, wheat straw, sorghum grains, rice bran and spent compost of mushroom either alone or in certain combinations, with or without additives such as jaggery and wheat flour along with differential moisture levels were evaluated as substrates for mass production of *T. viride* and *T. atroviride.* Jaggery and wheat flour served as nutritional supplements, which enhanced the conidial yield [10].

Bio-formulations are being prepared on many low cost materials as carrier with some limitations of less viability and quick decline in population during storage and transportation. Therefore, it is the need of such materials can be used as substrates for mass multiplication and retain the BCAs population during storage and transportation to a considerable level and it should also provide nutrition on soil applicationrequired for microbial growth as well as plant health.

II. MATERIALS AND METHODS

Collection,isolation and maintenance of antagonist

The spp. of *Trichoderma* was collected from rhizospheric soil of CRC at S.V.P.U.A&T, Meerut. Isolation was done by serial dilution method when 1g of soil was dissolved in 100 ml of sterilized distilled water and made a stock solution of 10^{-2}conc.Stock solution was diluted up to $10^{-6}$and of which 1 ml quantity was spread on TSM [11] containing Petri plates that were put into incubator and incubated for 7 days at 28±2ºC. Fully grown culture initially appears hyaline but turned green as conidia were produced [12]. The fungus was sub-cultured inPDA [13] slants and allowed to grow at 28±2^{0}C temperature. The culture thus obtained was stored in refrigerator at 5^{0}C for further study and was sub cultured periodically [1].

Corresponding author. E-mail: phytopath06@gmail.com. Tel: +91-8699137989.

Screeningofdifferentoilcakesformassmultiplicationof T. harzianum

Cakes of Neem, Jatropha, Mahua and Karanja were collected from local agricultural product-processing units and farms. Materials were cleaned and crushed (in heavy pestle & mortar) to prepare a coarse powder (particles of approximately 1 mm diameter). Cakes were mixed with sterilized water (SDW) to maintain 25% moisture (10: 2.5, w/v) and autoclaved at 1.1 kg/cm^2 for 20 minutes. The flasks were allowed to cool at room temperature prior to inoculation. Flasks containing substrates were inoculated with 3-4 days old actively growing culture of *T. harzianum* (2-3 bits of about 5mm size) under aseptic conditions in laminar air flow. The flasks were shaken thoroughly once a day, and incubated at 28±2^{0}C for 30 days. For each treatment, three replicates of flasks were maintained and arranged in a completely randomized manner (CRD) [1].

Determination of population dynamicsand CFUs counting

Serial dilution plate technique [14] was followed to determine population dynamics of *T. harzianum*and monitored at 15 days intervals. One gram cultured substrate was suspended in 10 ml distilled water to make a suspension of 1:10 (10^{-1}) conc. and was diluted up to 10^{-6}. One ml of last suspension was added to sterile Petri plates replicated thrice in completely randomized mannerand incubated at 28±2^{0}C temp. for 5 days [1,2]. Toknow the density of cells, spores/conidia by plate dilution technique of *T. harzianum* (CFUs) can be measured in laboratory by using below said formula [15]**.**

$$\text{CFUs} = \frac{\text{No. of colonies}}{\text{Amount plated X dilution factor}}$$

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 [1, 16, and 17].

III. RESULTS

Colony forming units after 15 days of cakes inoculation with T. harzianum

The data pertaining to the effect of adding lactose in the substrates have been presented in Table I, Fig I and Plate I. The findings indicated that all the four oil cakes exhibited varying response toward lactose, although some showed similar response also. After 15 days of incubation, Neem cake emerged out to be superior in giving rise to higher population dynamics, which supported 42.00 x10^6 CFUs of *T. harzianum* found at par over other cakes. Jatropha was next supporting 35.00 x10^6 CFUs followed by Mahua cake (32.67 x10^6) and Karanja cake (30.00 x10^6 CFUs).

Colony forming units after 30 & 45 days of cakes inoculation with T. harzianum

Thirty days after incubation, highest mean population was supported by Neem cake (64.67 x10^6) again, which was significantly higher than the mean population supported by Jatropha cake (56.00 x10^6), Mahua cake (50.00 x10^6 CFUs) and Karanja cake (46.67 x10^6) while at 45 DAIs, Neem cake (59.00 x10^6) was greater than the mean population supported by Jatropha, Mahua and Karanja cake i.e., 47.67, 43.33 and 39.33x10^6 CFUs respectively.

Colony forming units after 60 days of cakes inoculation with T. harzianum

A quick declinein population dynamics was recorded at 60 DAIs though highest mean population was supported by Neem cake (44.33 x10^6) that is followed by Jatropha cake (35.33 x10^6). Mahua cake was next to the Jatropha cake, which supported 30.00 x10^6 CFUs of *T. harzianum* and found significantly lower to the Jatropha cake. Lowest population of *T. harzianum* was exhibited by Karanja cake, which supported 25.33 x10^6 CFUs of *T. harzianum*. Karanja cake was found significantly lower among four cakes.

Colony forming units after 75 days of cakes inoculation with T. harzianum

At 75 DAIs, Neem, Jatropha, Mahua and Karanja cakes found supported 34.67, 25.00, 20.67 and 16.67 x10^6 CFUs where no doubt Neem cake was significantly higher than others.

Corresponding author. E-mail: phytopath06@gmail.com. Tel: +91-8699137989.

Colony forming units after 90, 105 and 120 days of cakes inoculation with T. harzianum

When CFUs were counted at 90th day 23.00, 21.00, 17.00 and 15.00 x10^6 CFUs from Neem, Jatropha, Mahua and Karanja cakes respectively found significantly at par with each other while after 105 days, highest population was recorded in Neem cake supporting 18.00 x10^6 CFUs and found significantly higher than Jatropha cake, Mahua and Karanja cakes supporting 13.33, 10.67and 8.33 x10^6 CFUs respectively. Only Neem and Jatropha cakes could support population dynamics of *T. harzianum* i.e., 6.00 and 0.33 x10^6 CFUs with a significant difference while Mahua and Karanja cakes couldn't support the population dynamics of *T. harzianum* after 120 days.

Table I. Effect of Lactose 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	42.00a	64.67a	59.00a	44.33a	34.67a	23.00a	18.00a	8.00a
2.	Jatropha cake	35.00b	56.00b	47.67b	35.33b	25.00b	21.00b	13.33b	0.33b*
3.	Mahua cake	32.67c	50.00c	43.33c	30.00c	20.67c	17.00c	10.67c	0.00b*
4.	Karanja cake	30.00d	46.67d	39.33d	25.33d	16.67d	15.00d	8.33d	0.00b*

***Numbers followed by the same letter are not significantly different (P = 0.05)**

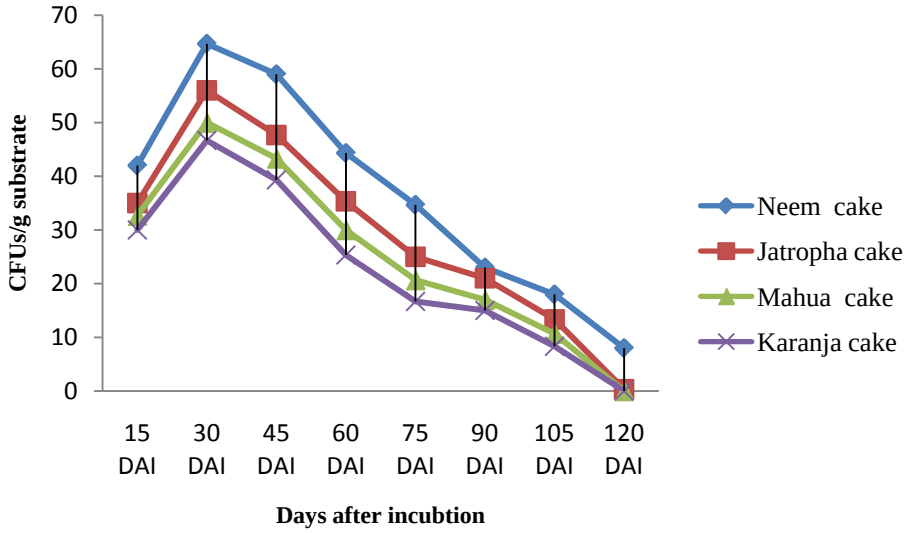

Fig I. Longevity of *Trichoderma harzianum*on Lactose added cakes

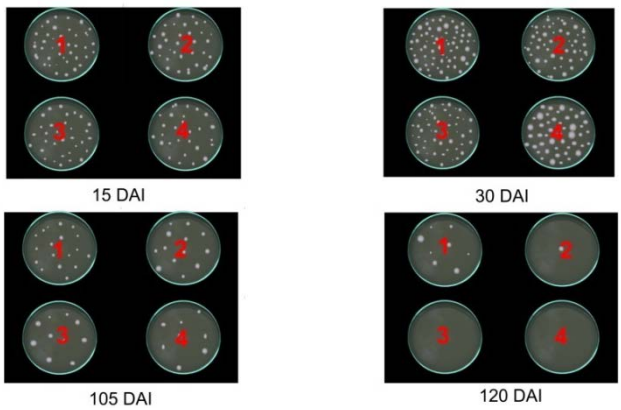

Plate I. CFUs in PDA (L-R) fromNeem cake (1), Jatropha cake (2), Mahua cake (3) and Karanja cake (4)

When capability of all these substrates of supporting population dynamics of *T. harzianum* was compared after addition of lactose, it was noticed that highest CFUs wasrecorded at 30 DAIs that was significantly superior to the values at all other days recorded. A slight reduction in population dynamics of *T. harzianum* was recorded at 45 days and values recovered at 45 DAIs were significantly higher

Corresponding author. E-mail: phytopath06@gmail.com. Tel: +91-8699137989.

than values recorded at 15 and 60 DAIs. A significant reduction in CFUs was recorded after 75 days and values were recorded significantly lower than the values at 60 DAIs but significantly higher to the values at 90 DAIs. However, lowest CFUs were noticed after 120 days when most of the substrates lost their viability except Neem cake.

IV. DISCUSSION

Advancement in mass production procedure with biodegradable products i.e., TBOs of Neem, Jatropha, Mahua and Karanja in enhancing the growth, sporulation and longevity of *T. harzianum* over a comparatively longer period, an experiment was conducted to test the efficacy of sucrose, as amended to four TBOs cakes i.e., Neem, Jatropha, Mahua and Karanja. It was interesting to note that addition of sucrose to these cakes resulted in increasing the survival of *T. harzianum* up to 120 days, whereas without addition survival was up to 105 days.

Carbon sources namely sucrose, molasses or glycerol increased longevity of survival of conidia of *T. harzianum* [18]. They suggested that microencapsulation of conidia with 2% sucrose solution found supporting 7.5 $\times 10^{10}$ CFUs of *T. harzianum*. Sriram*et al.* [19] concluded that addition with glycerol promoted the longevity and viability for 7 months (3% glycerol) and 12 months (6% glycerol) than 4–5 months without addition of glycerol. Same findings regarding addition of sugars were reported by Aamir et al. [20] they confirmed that conidium germination and bioactivity acceleratedand noticeable count was measured even after 6 months of storagewhen dextrose or sucrose were added.Younis et al. [21] observed that best carbon source used was cellobiose*T. harzianum* growth. Syahiddin[22] also found that maximum sporulation of T. harzianum occurred on glucose.Jaggery (a raw form of sucrose) was used by Thangavelu [23] to evaluate performance of substrates used toincrease longevity and survival of *T. harzianum*and it was found effective when conidial yield was recorded for more than 6 months under storage. The results obtained in present study in regards to CFUs enhancing effect of sucrose mixing to the de-oiled cakes are comparable with the previous work of Thangavelu [23] as jaggery which helped in enhancing the population of *T. harzianum*. Bean and Wilcoxson [24] found sucrose as the best growth of *Helminthosporiumsativum*and *H. dictyoides*whileChandwani and Munjal [25]found maltose as best source of carbon followed by sucrose and lactose for *H. sativum*, *H. oryzae*, *H. carbonum*and *H. gramineum*.Different carbon sources viz., Sucrose, Maltose, Dextrose, Glucose, Starch and Cellulose were added to Czapek's Agar (CzA) medium to check the growth of *T. harzianum* and found effective in increasing conidial yield [26].

The results of the present study found comparable with previous studies and shows that amendment of lactose as carbon source significantly enhanced the conidial population of *T. harzianum* in Neem cake, Jatropha cake, Mahua cake and Karanja cake, which without amendment of carbon showed comparatively poor performance.

V. CONCLUSION

Comparative study on population dynamics of *T. harzianum* on different cakes was done where,Neem cake was found superior among all the cakes at the entire DAIs followed by Jatropha cake (120 days). Mahua was next to the Jatropha in supporting the population of *T. harzianum* and the minimum CFUs were supported by Karanja cake upto 105 days after incubation instead of cakes without carbon sources were supporting only up to 90 days during the course of investigation (Fig. II).

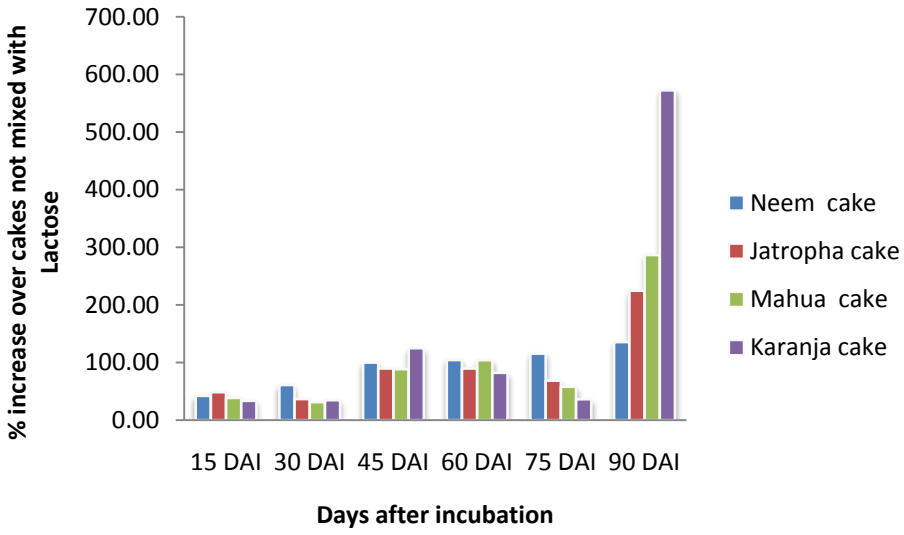

Fig II.Percent increase in CFUs in Lactose added cakes over non added cakes at different intervals (DAIs)

Corresponding author. E-mail: phytopath06@gmail.com. Tel: +91-8699137989.

Once lactose is added a positive effect on CFUs count, longevity and survival of *T. harzianum*has been noticed. Similarly without Lactose JatrophaMahuaand Karanja cakes couldn't support the *T. harzianum* population after 90 days, while with Lactose these cakes could support the population up to 120 days (Karanja, 105 days) (Table I, Fig.I and Plate I).

VI. ACKNOWLEDGMENT

Authors are thankful to the Department of Plant Pathology and University authority for providing facilities to conduct the experiment. Sources of literatures are also dully acknowledged.

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AUTHORS

First Author: Adesh Kumar, Assistant Professor, Lovely professional University (Punjab), phytopath06@gmail.com.
Second Author: Ramji Singh, HOD, Department of plant pathology, S.V.P. University of Agriculture and Technology (Meerut), Singh.ramji@gmail.com.
Third Author: BikeshYadav, Technical Assistant, UP Govt., Uttar Pradesh, bikesh.yadav33@gmail.com.
Fourth Author: Vipul Kumar, Assistant Professor, Lovely professional University (Punjab), vipul.19845@lpu.co.in.
Fifth Author: VinitPratap Singh, Assistant Professor, Lovely professional University (Punjab), vinit.18630@lpu.co.in.
Sixth Author: Ajay Tomar, Assistant Professor, Lovely professional University (Punjab), ajay.19443@lpu.co.in.
Corresponding Author: Adesh Kumar, Dept. of Plant Protection, LPU, Punjab, phytopath06@gmail.com, Tel: +91-8699137989.

Corresponding author. E-mail: phytopath06@gmail.com. Tel: +91-8699137989.