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Longevity of Survival of *Trichodermaharzianum*on Sucrose added Cakes

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Abstract: *Tree borne oilseeds (TBOs) namely Neem cake, Jatropha cake, Mahua cake and Karanja cake were evaluated for mass culturing of Trichodermaharzianum showed that Neem cake is the best substrate in vitro while Jatropha cake in vivo. The antagonist could be stored for more than 3 months but an increase in number of viable propagules up to 4 months was measured from all the substrates except Karanja (up to 105 days) when mixed with Sucrose; a kind of sugar. Among all substrates Neem cake found maintaining high population at 30 days of inoculation with T. harzianumin the form of colony forming units (CFUs) per gram of substrate.*
Keywords:*Tree born oilseeds (TBOs); sucrose; Trichodermaharzianum; self-life; longevity; viability etc.*

I. Introduction

Since antagonists are present as low population in native soils, further augmentation of their density to reach a higher stability through artificial application is necessary. Various agricultural wastes and by-products for multiplication of bio-control agents have been used by several workers [1], [2], [3] and [4].

*Trichoderma*as an effective antagonist against a range of plant pathogens won considerable scientific attention [5] and [6]. Different organic media like Neem cake, coir pith, farmyard manure, and decomposed coffee pulp also have been suggested for its multiplication [7]. Therefore, a study was conducted to enhance the conidial yield of *T. harzianum* on these substrates using nutritional supplements.

II. Materials And Methods

2.1 Sourcesandmaintenanceofculture

2.1.1Collection of soil samples and isolation of bio-control agent

To isolate the bio-control agent i.e., *Trichodermaharzianum* from rhizospheric soil, samples were collected from fields of Crop Research Centre (CRC) of university. For isolation, 1g of soil sample was placed in a 250 ml conical flask containing 100 ml of sterilized distilled water (SDW) and mixed thoroughly. Different dilutions of working samples were prepared by diluting the stock solution. 1 ml of last serial dilution i.e., 10^{-6} was spread on Trichoderma Selective Medium (TSM) for isolation of *T. harzianum*[8]. The plates were incubated for 7 days at 28±2ºC and after incubation, pure culture was grown; it was initially hyaline but turned green as conidia produced [9].

2.2 Maintenance of the culture

The fungus was sub-cultured in the PDA slants and allowed to grow at 28 ± 2^0C temperature. The culture thus obtained was stored in refrigerator at 5^0C for further study and was sub cultured periodically.

2.3 Screeningofdifferentoilcakesformassmultiplicationof T. harzianum

2.3.1 Collection of oil cakes and other substrates

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 bitsof about 5mm size) under aseptic conditions in laminar flow. The flasks were shaken thoroughly once a day, and incubated at 28 ± 2^0C for 30 days. For each treatment, three replicates of flasks were maintained and arranged in a completely randomized manner (CRD).

2.4 Determination of population dynamics

Population dynamics were determined by serial dilution plate technique. Growth and development of *T. harzianum* were monitored after each 15 days interval up to 120 days under laboratory conditions. 1g substrate of each cake was taken from each flask for colony forming units (CFUs) count, using PDA medium.

2.5 Colony Forming Units (CFUs)

2.5.1 Serial dilution and CFUs counting

One gram of sample (substrate where *Trichoderma* was being grown) was suspended in 10 ml distilled water to make microbial suspension diluted 10 times, it will give 1: 10 conc. or 10^{-1} dilution of original sample, i.e. the original sample has been diluted to 1/10th. Similarly it was prepared up to 10^{-6} on dilution of the original sample. Finally 1ml of microbial suspension from last serial dilution i.e., 10^{-6} was added to sterile Petri dishes (triplicate in completely randomized manner) and incubated for 5 days at 28 ± 2^0C. The density of cells, spores/conidia of *T. harzianum* can be measured in laboratory by Plate Dilution technique [10].

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

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 [11] and [12].

III. Results

3.1 Screeningofdifferentoilcakesadded with sucrose formassculturingof T. harzianum

3.1.1 AT 15 DAI

Four oil cakes exhibited varying response toward sucrose, after 15 days of incubation. Neem cake (43.67 $x10^6$ CFUs) amended with sucrose emerged out to be significantly superior in giving rise to higher population dynamics followed by Jatropha cake (40.67 $x10^6$ CFUs), Mahua cake (37.00 $x10^6$). Karanja cake was found significantly lower than Mahua cake, which supported only 33.00 $x10^6$ CFUs of *T. harzianum*(Table 2, Fig. 2).

3.1.2AT 30 DAI

At 30 DAI, the highest mean population of the *T. harzianum* was supported by Neem cake (67.67 $x10^6$ CFUs), which was significantly higher than the mean population supported by Jatropha cake (57.00 $x10^6$). Mahua cake was next best formulation, which supported 51.67 $x10^6$ CFUs of *T. harzianum* was significantly lower than Jatropha cake and followed by Karanja cake (49.00 $x10^6$), which was found significantly lower to the Mahua cake (Table 2, Fig. 2).

3.1.3AT 45 DAI

A slight reduction in CFUs was recorded after 45 days when Neem cake supported 60.67 $x10^6$ CFUs whereas, mean population supported by Jatropha cake is 50.00 $x10^6$ followed by Mahua cake, which supported 44.67 $x10^6$ CFUs, while lowest population was supported by Karanja cake i.e. 42.00 $x10^6$ CFUs of *T. harzianum*and it was found significantly lower than Mahua cake (Table 2, Fig. 2).

3.1.4AT 60 DAI

After 60 days, highest mean population of the *T. harzianum* was supported by Neem cake (46.33 $x10^6$), which was significantly higher than Jatropha cake (37.33 $x10^6$), Mahua cake (32.00 $x10^6$ CFUs) and Karanja cake which supported 27.33 $x10^6$ CFUs of *T. harzianum.* Karanja cake was found significantly lower than Mahua cake (Table 2, Fig. 2).

3.1.5AT 75 & 90 DAI

Neem cake found significant in supporting CFUs of *T. harzianum*, which supported 36.67 $x10^6$ and was significantly higher than Jatropha cake, Mahua cake and Karanja cake which supported 27.00 $x10^6$, 22.67 $x10^6$ and 18.67 $x10^6$ CFUs, while after 90 days of inoculation maximum no. of CFUs were recorded again in Neem cake (24.33 $x10^6$) followed by Jatropha cake (21.67 $x10^6$), Mahua cake (19.33 $x10^6$). Least performing cake was Karanja cake with 15.67 $x10^6$ CFUs of *T. harzianum*and was found significantly lower than Mahua cake (Table 2, Fig. 2).

3.1.1 AT 105 &120 DAI

Even after 105 and 120 days of inoculation highest population dynamics of *T. harzianum* was exhibited by Neem cake (20.00 &7.33x10^6) found significantly higher than Jatropha cake (15.33 &2.33x10^6) while Mahua cake with supporting 12.67 &1.00x10^6 CFUs and found significantly lower than Neem and Jatropha cake. Least population of *T. harzianum* was supported by Karanja cake (10.33 x10^6)found significantly lower than others and didn't support any CFUs after 120 days (Table 2, Fig. 2).

IV. Discussion

In order to find out an additional source of carbon which can support 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.

Thangavelu [13] reported that addition of jaggery (10% w/v) to the substrate i.e., dried banana leaves increased the multiplication of *T. harzianum* which survived up to more than 6 months on the stored substrate. 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 [13] as jaggery is a raw form of sucrose which helped in enhancing the population of *T. harzianum*.

Bean and Wilcoxson [14] found the best growth of *Helminthosporiumsativum*and *H. dictyoides* on sucrose. Whereas, Chandwani and Munjal [15] reported that maltose was the best source of carbon followed by sucrose and lactose for *H. sativum, H. oryzae, H. carbonum*and *H. gramineum* that supports the finding of present investigation.

Microencapsulation with sugars, such as sucrose, molasses or glycerol, significantly ($P < 0.05$) increased the survival percentages of conidia after drying. Xixuan and Custis [16] suggested that microencapsulation of conidia with 2% sucrose solution resulted in the highest survival percentage of *T. harzianum* (7.5×10^{10} CFUs) supports current findings of research performed. Result shown in present investigation found comparable with work of Srirame*t al.* [17] and they declared that the addition of glycerol at 3 and 6% extended the shelf-life (with viability of $>2 \times 10^6$ CFU g^{-1}) for 7 and 12 months, respectively compared to 4–5 months shelf-life in formulations derived without the addition of glycerol.

Aamir et al. [18] also carried a study to assess the effects of temperatures (20°C or 30°C) and sugars (dextrose or sucrose) on conidium germination and bioactivity as fresh conidia, or after 6 months of storage supported the findings of present study.

V. Conclusion

Neem cake was found to be superior among all the cakes at the entire DAIs followed by Jatropha cake. Mahua was next to the Jatropha in supporting the population of *T. harzianum* and the minimum CFUs were supported by Karanja cake up to 105 days after incubation without addition of Sucrose during the course of investigation (Table 1, Fig. 1). Once sucrose is added a positive effect in enhancing the CFUs and longevity of survival, has been noticed. Similarly without sucrose Jatropha and Mahua cakes couldn't support the *T. harzianum* population after 105 days, while with sucrose even these two cakes could support the population up to 120 days (Table 2, Fig. 2).

Sucrose is main constituent of several semi-synthetic or synthetic culture media for fungal isolation and further sub-culturing, thus, its role is well justified for fungal growth promotion. Sucrose being the disaccharide might have supplied comparatively little more amount of carbon than either mono-saccharides could have helped in increasing the longevity of *T. harzianum* in Jatropha and Mahua cakes from 105 to 120 days and also in increasing the population dynamics.

Table 1 Screening of different oil cakes up to 105 days after incubation (DAI) without added sugar

S.N.	Treatments	CFUs x10^6						
		15 DAI	30 DAI	45 DAI	60 DAI	75 DAI	90 DAI	105 DAI
1	Neem cake	30.67^{a}	42^{a}	30.33^{a}	22.67^{a}	17^{a}	10.33^{a}	0.67^{a*}
2	Jatropha cake	27.33^{b*}	41.67^{b}	26.33^{b}	19.67^{b}	16^{b}	6.67^{b}	0^{a*}
3	Mahua cake	26.67^{b*}	39.33^{c}	23.67^{c}	15.67^{c*}	14.33^{c*}	5.0^{c}	0^{a*}
4	Karanja cake	24.67^{c}	36.33^{d}	18.67^{d}	15^{c*}	13.67^{c*}	2.33^{d}	0^{a*}

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

Table 2 Screening of different oil cakes up to 120 days after incubation (DAI) added with Sucrose

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	43.67^a	67.67^a	60.67^a	46.33^a	36.67^a	24.33^a	20.00^a	7.33^a
2.	Jatropha cake	40.67^b	57.00^b	50.00^b	37.33^b	27.00^b	21.67^b	15.33^b	2.33^b
3.	Mahua cake	37.00^c	51.67^c	44.67^c	32.00^c	22.67^c	19.33^c	12.67^c	1.00^{c*}
4.	Karanja cake	33.00^d	49.00^d	42.00^d	27.33^d	18.67^d	15.67^d	10.33^d	0.00^{c*}

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

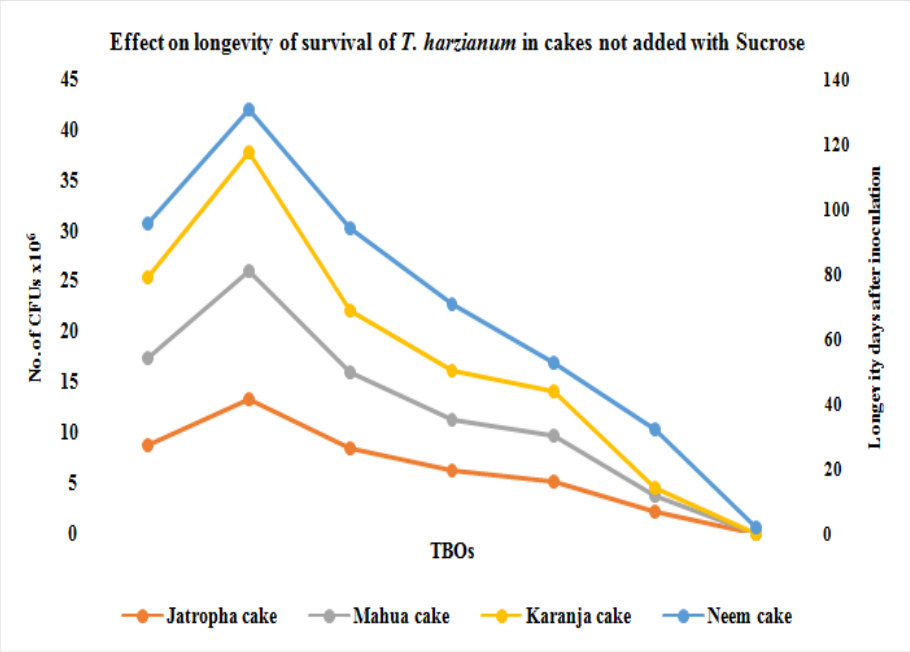

Fig. 1 Screening up to 105 days of incubation into different TBOsnot added with Sucrose

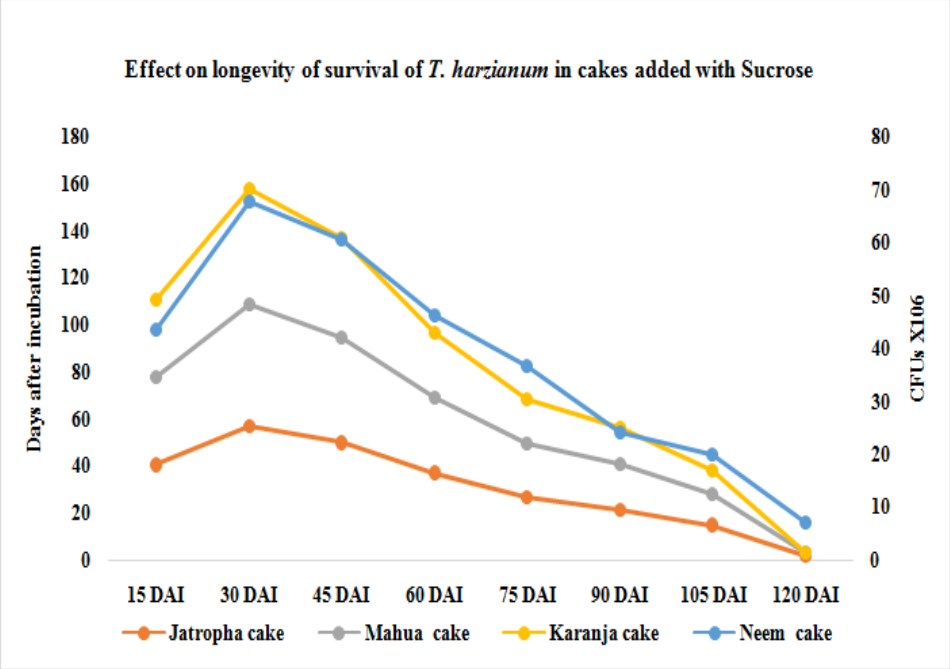

Fig. 2 Screeningup to 120 days of incubation into different TBOs added with Sucrose

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