Population dynamics of Trichoderma harzianum on Fructose added cakes as a source of Carbon

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ABSTRACT: Under storage a challenge has happened population decreases drastically very next it gets its peak. This seems an opportunistic challenge to bring of such substrates where certain microorganisms might be replicated and stored for long time to be used by farmers. Many substrates from many researches came into record and has been used for mass production of antagonistic microorganisms (BCAs) known for biological control of other group of microorganisms called plant pathogens. Therefore, few tree borne oilseeds (TBOs) known for their medicinal and nutritional properties been used under current investigation. There were four cakes of Neem, Jatropha, Mahua and Karanja evaluated and found supporting population dynamics of Trichoderma harzianum when used as substrate. It was revealed that Neem cake was the best substrate in vitro supported Trichoderma growth for four months while Jatropha cake was found supporting population dynamics in vivo. The antagonist could be stored for more than 4 months when mixed with the sources of carbon and nitrogen. Among all substrates Neem cake found maintaining high population at any level when counted as colony forming units (CFUs) per gram of substrate.

Key words- Tree borne oilseeds (TBOs); substrates; sucrose; Trichoderma harzianum; self-life; longevity; viability etc.

1. INTRODUCTION

Application of organic materials in multiplication of bio-fungicides being used in integrated plant disease management requires patented skills for its implementation in real time than a calendric application of conventional inputs. Conventionally used chemicals have direct negatively impact on plant and soil stature wherein it directly affect human health influencing surrounding environment, food and feed[19-49]. There are evidences that many of the pesticides poses potential risk and are hazardous to humans [12,14] and other forms of life [7] by putting unwanted load to the environment[1,7].
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.

2. MATERIALS AND METHODS

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±2ºC 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].

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 ±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 ± 2^0C [10,11] and were shaken thoroughly at 2 days interval for 30 days.

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[27-45].

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 ± 2^0C and colonies were counted as CFUs^{-1} gram soil [3] (Plate1).

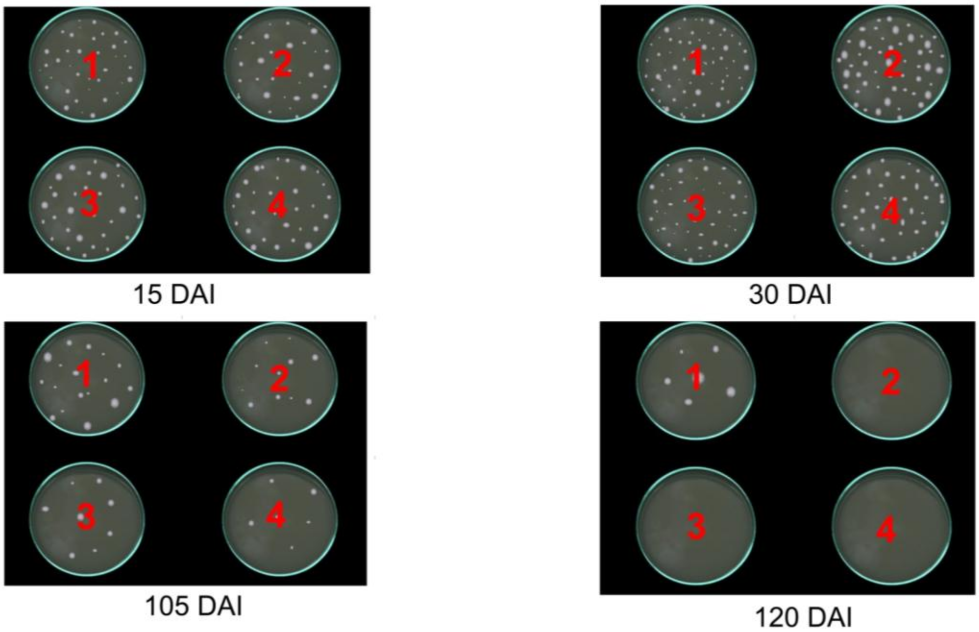


Plate1: CFUs from Neem cake (1), Jatropha cake (2), Mahua cake (3) and Karanja cake (4)

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

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 upto 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 Fructose 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	39.67	63.00	56.67	42.33	32.67	20.33	17.00	6.00
2.	Jatropha cake	33.00	53.67	46.00	33.33	23.00	17.67	11.33	0.00
3.	Mahua cake	30.00	47.33	40.67	28.00	18.67	15.33	8.67	0.00
4.	Karanja cake	29.00	45.33	38.00	23.33	14.67	11.67	6.33	0.00
	CD at 5%	Substrates=1.18 Days=1.77 Substrates x Days=3.55							

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 39.67 x10^6 CFUs of *T. harzianum*. This count was significantly higher than recorded in other three cakes followed by Jatropha cake as it supported 33.00 x10^6 CFUs. Next was Mahua cake (30.00 x10^6) followed by Karanja cake (29.00 x10^6) 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 63.00 x10^6 CFUs of *T. harzianum*. This count was significantly higher than recorded in other three cakes followed by Jatropha cake as it supported 53.67 x10^6 CFUs. Next was Mahua cake (47.33) followed by Karanja cake (45.33 x10^6) that was at lowest slab in supporting growth of *T. harzianum*.

After 45 days after incubation Neem cake was at top supporting 56.67 x10^6 CFUs and was significantly superior than the mean values achieved in Jatropha cake (46.00 x10^6) , Mahua cake (40.67 x10^6 CFUs) while the lowest growth rate was supported by Karanja cake as 38.00 x10^6 CFUs of *T. harzianum* was recorded.

At 60th day maximum population of the *T. harzianum* was recorded in Neem cake (42.33 x10^6) and is significantly superior to the CFU value in Jatropha cake i.e., 33.33 x10^6. Mahua cake was 3rd in the place and supported 28.00 x10^6 CFUs while lowest growth was achieved in Karanja cake which supported 23.33 x10^6 CFUs of *T. harzianum.*

Highest CFUs at 75 days after incubation were found in Neem cake (32.67 x10^6 CFUs) and it was recorded statistically superior than Jatropha cake (23.00 x10^6), Mahua cake (18.67 x10^6) and Karanja cake which supported lowest of CFUs i.e., 14.67 x10^6.

When CFUs were recorded after 90 days Neem cake (20.33 x10^6) followed by Jatropha cake (17.67 x10^6) and Mahua cake (15.67 x10^6). Lowest population of *T. harzianum* was supported by Karanja cake @11.67 x10^6 CFUs and found statistically lesser than other cakes. All these cakes are statistically different to each other.

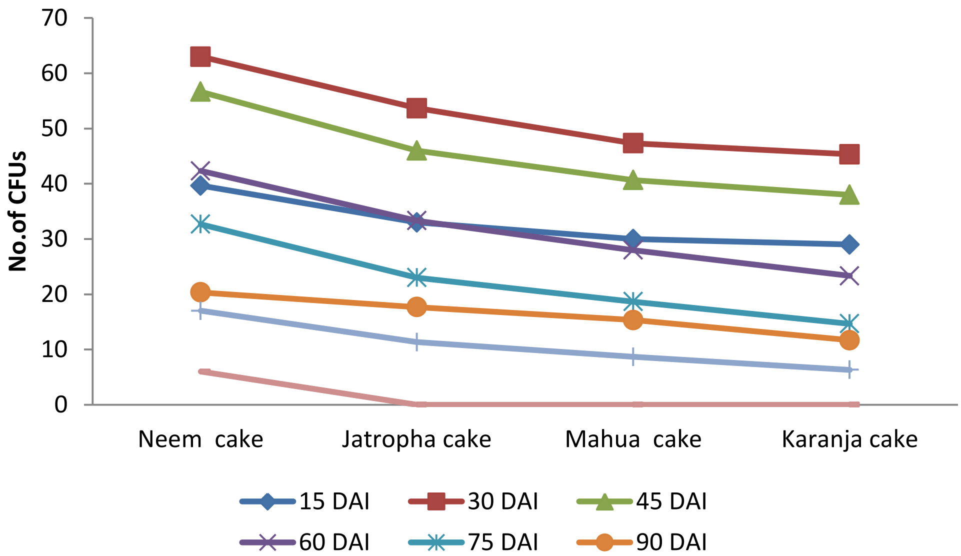
At 105th day, 17.00 x10^6 CFUs were supported by Neem cake which was followed by supported by Jatropha cake wherein population dynamics of *T. harzianum* is 11.33 x10^6 CFUs per gram of cake. Jatropha was found superior than Mahua cake wherein population dynamics of *T. harzianum* supported is 8.67 x10^6. Lowest CFUs were counted in Karnja cake i.e., 6.33 x10^6.

After 120 days of inoculation of cakes only Neem cake was recorded supporting 6.00 x10^6 CFUs of *T. harzianum.*

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.

When four cakes added with fructose were compared up to 120 days, it was noticed that highest CFUs were exhibited at 30 DAI that was significantly superior to the values at 15 and 45 DAIs, although values of Jatropha cake at 15 and 60 DAIs were found to be statistically at par with each other. A significant reduction in CFUs was recorded after 75 days and values were recorded significantly lower than the values at 60 DAI but significantly higher to the values at 90 DAI. However, lowest CFUs were noticed after 120 days when most of the substrates lost their ability except Neem cake in supporting the viability of *T. harzianum*.

Fig.1 Effect of fructose as amendment to different cakes on longevity and viability of *T. harzianum*



4. 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 carbon mixed in cakes which can enhance growth, sporulation, durability and support longevity of *T. harzianum* addition of fructose to these cakes was made which resulted in increasing the survival of *T. harzianum* beyond 120 days, whereas without addition survival was 105 days.

5. 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.

REFERENCES

[1] Aktar, W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary toxicology*, *2*(1), 1-12.

[2] Azam, F., Farooq, S., & Lodhi, A. (2003). Microbial biomass in agricultural soils-determination, synthesis, dynamics and role in plant nutrition. *Pakistan Journal of Biological Sciences*, *6*(7), 629-639.

[3] Chakrabarty, R., Acharya, G. C., & Sarma, T. C. (2014). Evaluation of substrates for multiplication of bio-agent, Trichoderma viride. *Afric. J. Agric. Res*, *9*(25), 1938-1940.

[4] Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research*. John Wiley & Sons.

[5] Jenkinson, D. S., & Powlson, D. S. (1976). The effects of biocidal treatments on metabolism in soil—I. Fumigation with chloroform. *Soil Biology and Biochemistry*, *8*(3), 167-177.

[6] Joko, T., Anggoro, S., Sunoko, H. R., & Rachmawati, S. (2017). Pesticides usage in the soil quality degradation potential in wanasari subdistrict, Brebes, Indonesia. *Applied and Environmental Soil Science*, *2017*.

[7] Kale, S. P., Raghu, K., Dierig, D. A., Thompson, A. E., & Ray, D. T. (1989). 2367301. Relationship between microbial numbers and other microbial indices in soil. *Bulletin of environmental contamination and toxicology*, *43*(6), 941-945.

[8] Kearney, P. C., & Kellogg, S. T. (1985). Microbial adaptation to pesticides. *Pure and Applied Chemistry*, *57*(2), 389-403.

[9] Kumar, A., Singh, R., Yadav, B., & Kumar, V. (2016). Longevity of Survival of *Trichoderma harzianum* on Sucrose added Cakes, *Journal of Agriculture and Veterinary Science*, 9 (8), 66-70.

[10] Kumar, V., Kumar, A., Singh, V. P., & Tomar, A. (2017). Effectiveness Measurement of Bio-agents and Botanicals against *Pyricularia oryzae*. *Journal of Pure and Applied Microbiology*, *11*(1), 585-592.

[11] Kumar, V., Kumar, A., Srivastava, M., Pandey, S., Shahid, M., Srivastava, Y. K., & Trivedi, S. (2016). *Trichoderma harzianum* (Th. azad) as a mycoparasite of *Fusarium* and growth enhancer of tomato in glasshouse conditions. *J. Pure Applied Microbiol*, *10*, 1463-1468.

[12] McGill, A. E. J., & Robinson, J. (1968). Organochlorine insecticide residues in complete prepared meals: a 12-month survey in SE England. *Food and cosmetics toxicology*, *6*(1), 45-57.

[13] Meena, R. S., Kumar, S., Datta, R., Lal, R., Vijayakumar, V., Brtnicky, M. & Pathan, S. I. (2020). Impact of agrochemicals on soil microbiota and management: A review. *Land*, *9*(2), 34.

[14] Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., & Hens, L. (2016). Chemical pesticides and human health: the urgent need for a new concept in agriculture. *Frontiers in public health*, *4*, 148.

[15] Rifai, M. A. (1969). A revision of the genus Trichoderma. *Mycological papers*, *116*, 1-56.

[16] Sharma, S. N., & Chandel, S. S. (2003). Screening of biocontrol agents In vitro against *Fusarium oxysporum* f. sp. *gladioli* and their mass-multiplication on different organic substrates. *Plant Disease Research*, *18*(2), 135-138.

[17] Singh, Ramji, Adesh Kumar, and Ajay Tomer. "De-oiled cakes of Neem, Jatropha, Mahua and Karanja: A New Substrate for mass multiplication of *T. harzianum*." *J Plant Pathol Microb* 6.288 (2015): 2.

[18] Vallaeys, T. (1997). Pesticides: microbial degradation and effects on microorganisms. *Modern soil microbiology*.

[19] ChitraMani, P. K. (2020). Evaluation of antimony induced biochemical shift in mustard. Plant Archives, 20(2), 3493-3498.

[20] Sharma, M., & Kumar, P. (2020). Biochemical alteration of mustard grown under tin contaminated soil. Plant Archives, 20(2), 3487-3492.

[21] Chand, J., & Kumar, P. (2020). Yield attribute shift of mustard grown under cadmium contaminated soil. Plant Archives, 20(2), 3518-3523.
[22] Naik, M., & Kumar, P. (2020). Role of growth regulators and microbes for metal detoxification in plants and soil. Plant Archives, 20(2), 2820-2824.
[23] Kumar, P., & Naik, M. (2020). Biotic symbiosis and plant growth regulators as a strategy against cadmium and lead stress in chickpea. Plant Archives, 20(2), 2495-2500.
[24] Kumar, P., & Dwivedi, P. (2020). Lignin estimation in sorghum leaves grown under hazardous waste site. Plant Archives, 20(2), 2558-2561.
[25] Devi, P., & Kumar, P. (2020). Concept and Application of Phytoremediation in the Fight of Heavy Metal Toxicity. Journal of Pharmaceutical Sciences and Research, 12(6), 795-804.
[26] Kumari, P., & Kumar, P. (2020). Trichoderma fungus in mitigation of rhizosphere arsenic: with special reference to biochemical changes. Plant Archives, 20(2), 3512-3517.
[27] Kaur, S., & Kumar, P. (2020). Ameliorative effect of trichoderma, rhizobium and mycorrhiza on internodal length, leaf area and total soluble protein in mung bean (Vigna radiata [L.] R. Wilazek) under drought stress. Journal of Pharmacognosy and Phytochemistry, 9(4), 971-977.
[28] Devi, P., & Kumar, P. (2020). Effect of bioremediation on internodal length and leaf area of maize plant cultivated in contaminated soil with chromium metal. Journal of Pharmacognosy and Phytochemistry, 9(4), 1408-1413.
[29] Sharma, K., & Kumar, P. (2020). Mitigating the effect of biofertilizers on morphological and biochemical level in pearl millet grown under mercury toxicity. Journal of Pharmacognosy and Phytochemistry, 9(4), 955-961.
[30] Kumar, S. B. P. (2020). Salinity stress, its physiological response and mitigating effects of microbial bio inoculants and organic compounds. Journal of Pharmacognosy and Phytochemistry, 9(4), 1397-1303.
[31] Devi, P., & Kumar, P. (2020). Enhancement effect of biofertilizers on germination percentage and plant height in maize grown under chromium toxic soil. Journal of Pharmacognosy and Phytochemistry, 9(4), 702-707.
[32] Chand, J., & Kumar, P. (2020). Biochemical shift of mustard grown under cadmium contaminated soil. Journal of Pharmacognosy and Phytochemistry, 9(3), 178-183.
[33] Kumar, P. (2019). Evaluation Of Internodal Length And Node Number Of Pea Treated With Heavy Metal, Polyamines And Glomus. Journal of the Gujarat Research Society, 21(10s), 518-523.
[34] Kumar, D., Rameshwar, S. D., & Kumar, P. (2019). Effect Of Intergated Application Of Inorganic And Organic Fertilizers On The Roots Of Chickpea. Plant Archives, 19(1), 857-860.
[35] Dey, S. R., & Kumar, P. (2019). Analysis of Available Nitrogen of Wheat Cultivated Soil Treated with Organic and Inorganic Source of Fertilizers. Int. J. Curr. Microbiol. App. Sci, 8(8), 2986-2990.
[36] Kumar, P., Siddique, A., Thakur, V., & Singh, M. (2019). Effect of putrescine and glomus on total reducing sugar in cadmium treated sorghum crop. Journal of Pharmacognosy and Phytochemistry, 8(2), 313-316.
[37] Dey, S. R., & Kumar, P. (2019). Cadmium induced biochemical shift in maize. Journal of Pharmacognosy and Phytochemistry, 8(1), 2038-2045.
[38] Kumar, P., & Pathak, S. (2018). Short-Term Response of Plants Grown under Heavy Metal Toxicity. Heavy Metals, 69.

[39] Kumar, P., & Dwivedi, P. (2018). Plant lectins, agricultural advancements and mammalian toxicity. Molecular Physiology of Abiotic Stresses in Plant Productivity, 360.
[40] Kumar, P., & Pathak, S. (2018). Nitric oxide: a key driver of signaling in plants. MOJ Eco Environ Sci, 3(3), 145-148.
[41] Kumar, P., Pathak, S., Amarnath, K. S., Teja, P. V. B., Dileep, B., Kumar, K., ... & Siddique, A. (2018). Effect of growth regulator on morpho-physiological attributes of chilli: a case study. Plant Archives, 18(2), 1771-1776.
[42] Kumar, P., & Hemantaranjan, A. (2017). Iodine: a unique element with special reference to soil-plant-air system. Advances in Plant Physiology (Vol. 17), 314.
[43] Dwivedi, P., & Prasann, K. (2016). Objective plant physiology. Objective plant physiology., (Ed. 2).
[44] Kumar, P. (2014). Significance of soil-root system and aquaporins for water homeostasis in plant-a review. Advances in Plant Physiology (Vol. 15), 15, 324.
[45] Kumar, P. (2013). Food Security and Nutritional Safety: A Challenge Ahead. Journal of Functional and Environmental Botany, 3(1), 12-19.
[46] Prasann, K., Biswapati, M., & Padmanabh, D. (2013). Combating heavy metal toxicity from hazardous waste sites by harnessing scavenging activity of some vegetable plants. Vegetos, 26(2), 416-425.
[47] Prasann, K. (2012). Feeding the future: crop protection today. Acta Chimica and Pharmaceutica Indica, 2(4), 231-236.
[48] Kumar, P., & Dwivedi, P. (2011). Future Habitat Loss: Greatest Threat to the Soil Microbial Biodiversity. Journal of Functional And Environmental Botany, 1(2), 82-90.
[49] Kumar, P., Singh, B. N., & Dwivedi, P. Plant Growth Regulators, Plant Adaptability And Plant Productivity: Areview On Abscisic Acid (Aba) Signaling In Plants Under Emerging Environmental Stresses. Sustaining Future Food Security In Changing Environments, 81.