The Bioscan 9(3): 1291-1294, 2014 (Supplement on Plant Pathology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

DETERMINATION OF SUITABILITY OF DEOILED CAKES OF NEEM AND JATROPHA FOR MASS MULTIPLICATION OF *PSEUDOMONAS FLUORESCENS*

AJAY TOMER*, RAMJI SINGH AND MANOJ KUMAR MAURYA
Department of Plant Pathology,
Sardar Vallabhbhai Patel University of Agriculture and Technolody, Meerut - 250 110 (UP)
e-mail: ajaytomer1489@gmail.com

KEYWORDS
Longevity
Survival
Deoiled cakes
Neem
Jatropha

Received on :
08.05.2014

Accepted on :
28.07.2014

***Corresponding author**

ABSTRACT

Jatropha cake was found to be best substrate for supporting the highest population dynamics and longevity of *P. flourescens in vitro*. Both these cakes supported the population of *P. flourescens* up to 120 days at 28±2°C. Highest population (271.00×10x) of *P. flourescens* was noticed on Jatropha cake after 45 days of inoculation and incubation at 28±2°C when maintained with 15% moisture, while on Neem cake, highest population (208.00×10x) of *P. flourescens*was noticed after 60 days of inoculation when maintained with 25% moisture. Jatropha cake was better than Neem cake for supporting population and longer shelf life of *P. flourescens in vitro*. Result of this study may help for using deoiled cakes of different TBOs (tree born oil seeds) in mass multiplication of different bio-agents specially *P. flourescens*.

INTRODUCTION

Various factors act as impediments which are known to affect the commercialization of microbial bio-control agent such as *Pseudomonas fluorescens* (an essential step for improving efficiency of bio-control agents). One of these impediments is the lack of technology for cost-effective mass production of bio-control agents (BCAs) and their insufficient longevity during storage and transportation with a sufficient level of effective population (cfus). It has noticed that *Pseudomonas fluorescens*is often effective in the laboratory, but the level of disease control, achieved in the field is sometimes disappointing and unpredictable. Some of these failures have been attributed to inadequate establishment and survival of this microorganism in soil (Elliot and Lynch, 1995). Since they are well adapted in soil, that's why *P. fluorescens* strains were investigated extensively for use in bio-control of plant pathogens in agriculture (Ganeshan and Kumar, 2006 and Ganesh, 2012). It was observed to enhance plant growth along with yield enhancement and to reduce severity of many diseases (Hoffland *et al.,* 1996; Wei *et al.,* 1996).

Over the past four decades studies on the use of beneficial microorganisms as bio-control agents for plant protection have increased greatly (Sab *et al.*, 2014). Many agro-industrial biproducts such as deoiled cakes of tree born oils seeds (TBOs) like Neem and Jatropha which are either going waste and being used as a less profitable and and less usable products since quite long time. De-oiled cakes of these trees, either remain unexploited and poorly exploited, generally as low value soil amendments and organic mannure. These deoiled cakes contains good amount of N, P, K, Ca, Mg, S, Fe, Mn, Zn and Cu (Patolia *et al.*, 2007). In addition they also contain carbohydrates, proteins, fatty acids,and many more biochemical constituents. With these qualities, these deoiled cakes have been exploited as substrate for mass multiplication of bacterial bio-control agents such as *Pseudomonas flourescens* (Manoj *et al.,* 2014). Mass multiplication of *Pseudomonas fluorescens* will not only result to a value added products development from de-oiled cakes of Neem and Jatropha; rather it will also provide a nutritious substrate for long term survival of bioagents along with voiding huge wastage of these by-products.

The mass cultures made at industrial scale are generally talc based, with no nutritional background to support the life of Bio-agents during storage, transportation and unfavourable environmental conditions. Theses de-oiled cakes of TBOs served as source of diversified nutrition for bio-agents when used as substrate for mass multiplication of antagonists.

MATERIAL AND METHODS

Sources and Maintenance of culture

Pseudomonas fluorescens culture was isolated from tomato rhizospheric soil, collected from crop research centre (CRC) of SVPUAT Meerut. For isolation of microorganism, 10 gm of soil sample, adhered to roots and rootlets of tomato were collected and placed in a 250mL conical flasks containing 100mL of sterilized distilled water (SDW) and mixed thoroughly. Different dilutions of working samples were prepared by serially diluting the stock solution upto10^{-8}. One ml of last dilution point *i. e.*, 10^{-8} was spread on *Pseudomonas fluorescens* selective king's B Medium (King's *et al.*, 1954) for growth and isolation of *Pseudomonas fluorescens*. The plates containing king's B Medium where soil dilution were spread, were incubated for 2 days at 28±2ºC . After a period of two days bacterial colonies were visible which were transferred into culture tube containing PDA slants to maintain pure culture of *Pseudomonas fluorescens*. Conformity of culture was done on the basis of color of bacterial colony which was initially yellow but turned yellow green after pigmentation were produced (Bonds, 1957). Further culture was again reconfirmed by molecular charectarization test at National Beauro of Agriculturally Important Microbes (ICAR) Mau (UP) India. The culture thus obtained were stored in refrigerator at 5ºC for further studies and were sub cultured periodically.

Determining Population Dynamics and Longevity of Pseudomonas fluorescens on Deoiled Cakes of Neem and Jatropha

Deoiled cakes of two tree born oilseeds *i.e.,* Neem and Jatropha were collected from agricultural product-processing units situated in Distt. Mau of Uttar Pradesh (UP) and Raipur in Chhattishgarh. Before using, the cakes were grounded in a metallic pastel and mortar to prepare fine powder. Three different level of moisture *i.e.*, 15%, 25% and 35% (w/v) were maintained by adding required amount of sterilized distilled water to cakes. Before inoculation of *Pseudomonas fluorescens*, cakes containing different level of moisture were placed in 250mL capacity conical flasks (@ 75 gram/flask), plugged tightly with cotton plugs, wrapped with butter paper and autoclaved at 121.6ºC (1.1 kg/cm^2) for 20 minutes. Autoclaved, flasks were allowed to cool overnight at room temperature, prior to inoculation. Autoclaved flasks containing sterilized deoiled cakes with different level of moisture were inoculated with 3-4 days old actively growing culture of *Pseudomonas fluorescens* (2-3 bits of 5mm size from the culture grown on PDA in Petri plates) under aseptic conditions in laminar flow. For each moisture level and each set of duration (Table1 and 2) three replicates were maintained. Flasks inoculated with *Pseudomonas fluorescens* were incubated at 28±2ºC in a BOD incubator and shaken thoroughly once a day for proper mixing of substrates so that bacterium may properly utilize unused substrate.

Monitoring of Population Dynamics InDeoiled Cakes

Population of *Pseudomonas fluorescens* was monitored in the inoculated deoiled cakes of Jatropha and Neem maintained with different level of moisture (15%, 25% and 35% respectively) after each 15 days interval, upto 120 days. Monitoring of *Pseudomonas fluorescens* population was done taking1gm of each cakes where *Pseudomonas flourescens*was inoculated from each flasks maintained for each duration *i.e.*15 to 120 days at every 15 days interval. CFUs were counted using PD Athrough simplified agar plate method for quantifying viable bacteria. (Jett *et al.*, 1997).

RESULTS

Screening of deoiled cakes of Neem and Jatropha for mass multiplication of *Pseudomonas fluorescens*

Deoiled cakes of two Tree Born Oilseeds (TBOs) *i.e,* Neem and Jatropha, Were tested for their suitability to support the population dynamics and longevity of *Pseudomonas fluorescens* at three different level of moisture *i.e.* 15%, 25% and 35%. Results obtained have been presented in Table 1 and 2.

Neem Cakes

Neem cake with 35% moisture resulted in 139.00×10x number of CFUs of *Pseudomonas fluorescens* at 15 days of inoculation. At 30 days of inoculation neem cake with 35% moisture resulted in 168.00×10x CFUs of *P .fluorescens*, whereas at 45 days of inoculation the population of *P. fluorescens* increased to196.67×10x and at 60 days of inoculation, it further goes down to the level of 176.67×10x. At 75 days of inoculation Neem cake with 35% moisture

Showed further reduction in the *Pseudomonas* population and accordingly CFUs decreased to 137.00×10x. At 90 days of inoculation, the Neem cake with 35% moisture resulted in 98.00×10x number of CFUs of *P. fluorescens*. At 105 days

Table 1: CFUs of *Pseudomonas fluorescens* at different moisture level on sterilized Neem cake.

Moisture level	15Days	30Days	45Days	60Days	75Days	90Days	105Days	120Days
15 %	122.00	157.33	187.00	203.00	183.67	125.00	84.67	54.33
25%	129.33	162.00	192.00	208.00	157.67	132.7	48.00	9.33
35%	139.00	168.00	196.67	176.67	130.07	98.00	48.00	10.33
CD @ 5% Moisture % = 7.0655								
Days = 11.5378								
MxD = 19.984								

Table 2: CFUs of *Pseudomonas fluorescens* at different moisture level on sterilized Jatropha cake

Moisture level	15Days	30Days	45Days	60Days	75Days	90Days	105Days	120Days
15%	127.33	188.00	271.00	211.3	141.33	97.67	52.00	16.67
25%	140.00	212.33	262.67	197.00	131.00	92.00	43.00	14.00
35%	148.00	236.67	254.00	214.00	171.00	103.00	46.67	17.67
C.D @ moisture% = 1.2504								
Days = 2.0419								
MxD = 3.537								

the number of CFUs of *P .fluorescens* ,recovered from the cakes were 48.00×10x , whereas at 120 days, the CFUs of *P.fluorescens* recovered, were 10.33×10x . In the case of neemcake the population of *P. fluorescens* observed at each 15 days interval differed significantly from each other while different level of moisture hardly had significant effect on the increase or decrease of population.

Jatropha cake

Jatropha cake containing 35% moisture resulted in 148.00×10x level of CFUs of *Pseudomonas fluorescens* at 15 days of inoculation. At 30 days of inoculation, the number of CFUs increased to the level of 236.67×10x , whereas at 45 days the population further increased to the level of 254.00×10x CFUs of *Pseudomonas fluorescens*. At 60 days onward there was declining trend and population declined to the level of 214.00×10x and at 75 days it was 171.00×10x. At 90 days of inoculation the Jatropha cake containing 35% moisture, resulted in 103.00×10x level of population *of P. fluorescens*.

At 105 days the *P .fluorescens* population decreased to the level of 46.67×10x and at 120 days population further declined to the level of 17.67×10x . Level of CFUs recorded after each 15 days interval and each level of moisture were significantly different from each other.

In the comparison of two deoiled cakes it was found that jatropha cake was quite superior over neem cakes in supporting the population dynamics of *Pseudomonas fluorescens*. In case of neem cake, increasing the level of moisture didn't had any significant effect on population dynamics of *Pseudomonas fluorescens,* whereas in case of jatropha cakes, with increasing in the level of moisture had resulted in significant increase of population dynamics of *Pseudomonas fluorescens*.

DISCUSSION

With a purpose to find out a suitable substrate for mass multiplication and also for a longer shelf life of *Pseudomonas fluorescens,* an experiment was conducted to test the suitability of two de-oiled cakes of Neem and Jatropha with three moisture level *i.e.* 15%, 25% and 35%. Results indicated that Jatropha cake was found to be comparatively better than Neem cake for enhancing population of *Pseudomonas fluorescens* with a highest level after 45 days of inoculation with 15% moisture. It was also noticed that Jatropha and neem cake both could support the population and longevity upto 120 days with ×10x level of population. In case of neem cake it was observed that on Neem cake population of *Pseudomonas fluorescens* was found to be increasing upto 60 days after inoculation with a highest at 60 days after inoculation with 25% moisture, while on Jatropha cake population of *Pseudomonas fluorescens* was found to be increasing upto 45 days only and after that there was a trend of decline in the population of *Pseudomonas fluorescens*.

Reason behind decline of population dynamics after 45/60 days during present investigation observed that, at the initial level there have plenty of nutrition available to utilized for multiplication of *Pseudomonas fluorescens* which later get declined, because they might have been exhausted day by day due to utilization and exploitation by growing *Pseudomonas fluorescens* in the substrate itself and resulted in poor supply after 45/60 days and thereby lower population dynamics with prolonging duration of storage.

Nilkamal *et al.* (2008) assessed Deoiled Jatropha cake as substrate for enzyme production by solid-state fermentation (SSF). Solvent tolerant *Pseudomonas aeruginosa* PseA strain was used for fermentation. The seed cake supported good bacterial growth and enzyme production (protease, 1818 μg/g of substrate and lipase, 625 μg /g of substrate). Maximum protease and lipase production was observed at 50% substrate moisture, at a period of 72 and 120 h, pH of 6.0 and 7.0, respectively. Murugalakshmi *et al.*, 2010 and Jyoti *et al.,* 2012) reported that Agricultural residues rich in carbohydrates can be utilized in fermentation process to produce microbial protein which in turn could be used to determine the factors influencing cell biomass production. *Pseudomonas fluorescens*was cultivated using banana peel out, watermelon skin, and Cane molasses showed that the strain was capable of meeting its components required for growth. The organism was capable of growth at 28ºC, when supplemented with agricultural wastes in different concentration mixed with agar. The number of colony forming units were more when compared with nutrient agar. Abhinav *et al.* (2011) evaluated PGPR strain of *Pseudomonas fluorescens* PS1 to formulate carrier based bioformulations. The viability of *Pseudomonas fluorescens PS1* was monitored at different time intervals during the period of storage at room temperature in different carriers such as soil, charcoal, sawdust and sawdust soil. Sawdust-soil was found to be the most efficient carrier material for *P. fluorescens PS1* followed by other carriers. Sangeetha *et al.* (2012) studied the survival of PGPR isolates by using different carrier materials. The carrier based PGPR consortium with four selected strains *viz., Azospirillumlipoferum*VAZS-18, *Azotobacterc hroococcum* VAZB-6, *Bacillus megaterium*VBA-2, *Pseudomonas fluorescens* VPS-19 was prepared and the shelf life for each inoculants was studied upto six months of storage. The surviving population in the lignite based consortium was 1.64 × 10^8 cfu g^{-1} for *Azospirillumlipoferum* VAZS-18, 1.46 × 10^8 cfu g^{-1} for *Azotobacter chrococcum*, VAZB-6, 1.22 × 10^8 cfu g^{-1} for *Bacillus megaterium* VBA-2 and 2.01 × 10^8 cfu g^{-1} for *Pseudomonas fluorescens* VPS-19 after six month of storage. The surviving population in vermiculite based consortium was 4.32 × 10^8 cfu g^{-1} for *Azospirillum lipoferum* VAZS-18, 1.98 × 10^8 cfu g^{-1} for *Azotobacter chroococcum* VAZB-6, 1.14 × 10^8 cfu g^{-1} for *Bacillus megaterium* VBA-2 and 3.32 × 10^8 cfu g^{-1} for *Pseudomonas fluorescens* VPS-19 after six months of storage. In the pressmud based consortium, the surviving population was 3.25 × 10^8 cfu g^{-1} for *Azospirillum lipoferum* VAZS-18, 3.00 × 10^8 cfu g^{-1} for *Azotobacter chroococcum* VAZB-6, 2.14 × 10^8 cfu g^{-1} for *Bacillus megaterium* VBA-2 and 3.42 × 10^8 cfu g^{-1} for *Pseudomonas fluorescens* VPS-19 after six months of storage. In the alginate bead based consortium the surviving population was 64.61 × 10^8 cfu g^{-1} for *Azospirillum lipoferum* VAZS-18, 56.81 × 10^8 cfu g^{-1} for *Azotobacter chroococcum* VAZB-6, 47.83 × 10^8 cfu g^{-1} for *Bacillus megaterium* VBA-2 and 63.89 × 10^8 cfu g^{-1} for *Pseudomonas fluorescens* VPS-19 after six months of storage. Although scanty literatures are available regarding use of deoiled cakes for mass multiplication of

Pseudomonas fluorescens but after thorough scanning of literature it is clear that the carriers rich in organic substances and carbohydrate are highly supportive of multiplication of *Pseudomonas fluorescens* thus the findings of present studies are well supported by previous findings as mentioned above.

Based on the findings reported by all the groups mentioned above it hasbeen cleared that these materials rich in protein, carbohydrates and vitamins have been found to be comparatively better carrier for mass multiplication of *Pseudomonas fluorescens* as compared to those substrates which nutrient less like agar based or having less nutrient. Thus the findings of these workers are in conformity with the present findings.

ACKNOWLEDGMENTS

Help and support received from SVPUAT Meerut and NOVOD Board during the course of investigation is dully acknowledged.

REFERENCES

Abhinav, A., Dubey, R. C., Maheshwari, D. K., Pandey, P., Bajpai, K., V. and Kang Sun, C. 2011. PGPR strain *Pseudomonas fluorescens* PS1 was evaluated to formulate carrier based bioformulations. *European J. Plant Pathology* **l:** 81-93.

Ambardar, V. K. and Sood, A. K. 2010. Suitability of different growth substrate for mass multiplication of bacterial antagonists. *Indian Phytopathology*. **63:** 4

Bonds, G. J., Jensen, C. E. and Thamasen, J. 1957. A water soluble fluorescing bacterial pigment which deplomerizehylorenic acid. *Acta Pharmocol, Toxicol.* **13:** 184-193.

Elliot, L. F. and Lynch, J. M. 1995. The International Workshop on the Establishment of Microbial Inocula in soils. Cooperative research project on biological resource management of the Organisation for Economic Cooperation and Development (OECD). *Am. J. Altern. Agric.* **10:** 50-73.

Ganeshan, G. and Kumar, M. A. 2006. *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. *J. Plant Interactions*. **1(3):** 123-134.

Ganesh, S., Kotgire, S. T. Patel. And vijay, K. V. G. 2012. Management of grain infecting fungi of of sorghum through spray on earhead by chemical and non-chemical means. *The Bioscan*. **7:** 597-599.

Hoffland, E., Halilinen, J. and Van Pelt, J. A. 1996. Comparison of systemic resistance induced by avirulent and non pathogenic *Pseudomonas* sp. *Phytopathology*. **86:** 757-762.

http://en.wikipedia.org/wiki/Agar_plate

Jyoti, N. and Umamahe, S. R. V. 2013. Production of protease and urease by kerosene utilizing *Fluorescent Pseudomon ads* isolated from local red latrite soil. *The Bioscan*. **8:** 353-357.

Jett, B. D., K. L. Hatter, M., Huycke, M. and Gilmore, M. S. 1997. Simplified agar plate method for quantifying viable bacteria. *Bio.Techniques*. **23:** 648-650.

King, E. O., Ward, M. K. and Raney, D. E. 1954. Two simple media for the demonstration of Pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine*. **36:** 100-102.

Manoj, K. M., Singh, R. and Tomer, A. 2014. Determination of suitability of deoiled cakes of Karanj and Mahua for mass multiplication of *Pseudomonas fluorescens.The Bioscan*. **9:** 371-374.

Murugalakshmi, C. N. and Sudha, S. S. 2010. The Efficacy of agrowaste on cultivation of *Pseudomonas fluorescens*. A potential biocontrol agent*International J. Bio. Tech.* **1(3):** 32-34.

Neelkamal, M., Gaur, R., Gupta, A. and Khare, S. K. 2008. Purification and characterization of lipase from solvent tolerant *Pseudomonas aeruginosa* Pse A, *Biological Sciences*. **43:** 1040-1046.

Patolia, *et al.* 2007. Preparing Status Reports on Themes Related to Technical and Scientific Aspects of Biofuels Utilization by *Winrock International India* (WII), New Delhi India.

Sab, J., Nagaraja, A. and Nagamma, G. 2014. Efficacy of biopesticides against *Sclerotium rolfsi* Sacc. causing collar rot of chickpea (*Cicer arietinum* L.) *The Bioscan*. **9:** 335-339.

Sangeetha, D. 2012. Survival of plant growth promoting bacterial inoculants in different carrier materials, *International J. Pharmaceutical and Biological Archive*. **3:** 221-243.