Published in the Russian Federation
European Journal of Molecular Biotechnology
Has been issued since 2013.
ISSN: 2310-6255
E-ISSN 2409-1332
Vol. 6, No. 4, pp. 223-228, 2014

DOI: 10.13187/ejmb.2014.6.223
www.ejournal8.com

UDC 616

Determination of Effect of Substrate Concentration and Dilution of Inoculums on Population Dynamics of *Pseudomonas Fluorescens*

[1]* Ajay Tomer
[2] Ramji Singh,
[3] Manoj Kumar Maurya

[1-3] Sardar Vallabhbhi Patel University of Agriculture & Technolody, Meerut, India
250110 (UP)
Department of Plant Pathology
*Corresponding Author's E-mail: ajaytomer1489@gmail.com

Abstract

Deoiled cakes of Neem and Jatropha served as source of diversified nutrition for Pseudomonas flourescens when used as substrate for mass culturing of antagonist. The present investigation were undertaken to test the suitability of neem and Jatropha cakes for liquid Concentration and longevity of Pseudomonas flourescens in vitro. Increasing the dilution of stock of initial inoculums resulted in decrease of total viable counts of Pseudomonas flourescens was comparatively highest population (64.67×10^5) and (201.67×10^5) lesser after 15 days than after 7 days of inoculation on Neem and Jatropha cake concentration respectively.

Keywords: Deoiled Neem cake; Jatropha cake concentration; moisture level; *Pseudomonas flourescens*.

Introduction

Many agro-industrial bioproducts such as deoiled cakes of tree born oils seeds (TBOs) like Neem and Jatropha which are either going waste or being used as a less profitable and usable products since quite long time. The oils extracted from Neem and Jatropha, are either directly used as bio-fuel or as raw material for industrial inputs in various manufacturing industries like cosmetics, agrochemicals and pharmaceuticals (Tiwari, et.al. 2007). Deoiled cakes of these trees remain either unexploited or poorly exploited. These deoiled cakes contains lot of carbohydrates, proteins, fatty acids, minerals and many more biochemical constituents which are served as source of nutrition for beneficial micro-organisms (growth promoting and biocontrol agents) in crop cultivation, Patolia *et al.* (2007), hence might being exploited as substrate for mass multiplication of bacterial bio-control agents such as *Pseudomonas flourescens*. Mass multiplication of *Pseudomonas flourescens* on deoiled cakes of these TBOs may be a boon for popularization of bio-control of plant diseases and thereby for crop cultivation, utilization and popularization of Neem and Jatropha as well. Mass multiplication of *Pseudomonas fluorescens* will not only leads to value added products development from deoiled cakes of Neem and Jatropha; rather it will prevent huge wastage and misuse of these by-products.

Mass culture available in the market generally shows poor efficacy after application in the crop field. This is probably due to long duration taken in transportation from manufacturing unit

to the users (farmers). The mass cultures made at industrial scale are generally talc based, with no nutritional background to support the life of BCAs during storage, transportation and other stress. Deoiled cakes of TBOs may serve as source of diversified nutrition for BCAs when used as substrate for mass culturing of antagonists.

Material and Methods

Collection of soil samples and isolation of biocontrol agent

To isolate the biocontrol agent *i.e.*, *Pseudomonas fluorescens* from tomato crop rhizosphere, soil samples were collected from crop research centre (CRC) of university. For isolation, one gm 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 serially diluting the stock solution (10^{-8}). 1 ml of last serial dilution *i.e.*, 10^{-8} was spread on *Pseudomonas fluorescens* Selective king's B Medium (King's et.al 1954) for isolation of *Pseudomonas fluorescens*. The plates were incubated for 2 days at 37±2°C and after incubation, pure culture was grown; colour of bacterial colony was initially yellow but turned yellow green as pigmentation were produced (Bonds 1957).

Composition of Culture media:
Pseudomonas fluorescens (Selective) King's B Medium (King et, al, 1954)
Composition:

Peptone	20gm
Agar-agar	20gm
Potassium monophosphate (k2Hpo4)	1.5gm
Magnesium sulphate (Mgso4)	1.5gm
Glycerol	10ml
Distilled water	1000 ml

Preparation:

After mixing all the ingredients with distilled water, media was placed into a stainless steel pan and steered with glass rod for proper mixing of all the ingredients. Now the medium was filtered through a muslin cloth by squeezing out whole liquid. 200 ml medium was placed in each 500 ml capacity flasks. Flasks were tightly plugged with non-absorbent cotton plug and wrapped with butter paper and rubber band. Medium was autoclaved at 1.1 kg/cm² pressure for 20 min at 121.6°C and cooled before pouring into Petri plates.

Maintenance of the culture

The Bacteria initially isolated in a pure culture on King's B media and sub cultured on PDA slants were allowed to grow at 28±2°C temperature. The culture thus obtained was stored in refrigerator at 5°C for further studies and was sub cultured periodically.

Determination of effect of substrate concentration and dilution of inoculums on Population dynamics of *Pseudomonas fluorescens*

Initially the *Pseudomonas fluorescens* cultures were grown in King,s B broth medium at 28 ±2°C for 2 days. After 2 days of incubation, a serial dilution of 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} were prepared by the method given at above.

Results and Discussion

Present studies entitled "Longevity and Survival of *Pseudomonas fluorescens* on neem and jatropha cakes" were conducted under laboratory and pot conditions with the objectives to determine the suitability of deoiled cakes of Neem and Jatropha for mass multiplication of *P .fluorescens in vitro* and to determine the longevity of *P .fluorescens* grown on two deoiled cakes in the rhizosphere of tomato at the Department of Plant Pathology, S.V.P. University of Agriculture & Technology, Meerut.

Effect of substrates concentration on population of *Pseudomonas fluorescens:* Population of *Pseudomonas fluorescens* on different concentration of Neem cake extract after 7 days:

The colony forming unit (CFUs) of *Pseudomonas fluorescens* showed variation in different concentration of substrates i.e deoiled Neem cake extract and deoiled Jatropha cake extract after 7 days and 15 days interval as given in table 1, 2, 3 and 4 respectively. It is evident from table-1.1 that with the inoculation of 10^{-5} dilution of *Pseudomonas flourescens* stock of initial inoculums, recovery of *Pseudomonas fluorescens CFUs* were towards increasing with increasing concentration of substrate which were 42.33×10^5, 72.00×10^5, 80.00×10^5, 171.67×10^5, 193.67×10^5, and 201.67×10^5 CFUs of *P .fluorescens* at 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Neeem cake extract after 7 days of inoculation. With the inoculation of 10^{-6}. dilution of *P .fluorescens* cfus stock of initial inoculums in the different concentration of Neem cake extract population of *Pseudomonas fluorescens* recovered after 7 days of inoculatin also showed increasing trends which were 39.00×10^6, 63.00×10^6, 70.33×10^6, 118.00×10^6, 156.00×10^6 and 184.67×10^6 at 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Neem cake extract.

Table 1: Longevity and survivability of *Pseudomonas fluorescens* in Neem cake extract of different concentration at various dilution point with different moisture level for 7 and 15 days

Concentration Dilution	0%	5%	10%	15%	20%	25%	30%
10^5	22.00	42.33	72.00	80.00	171.67	193.67	201.67
10^6	21.00	39.00	63.00	70.33	118.00	156.00	184.67
10^7	20.00	30.67	48.00	54.00	96.33	145.00	175.00
10^8	19.00	23.33	34.33	41.00	76.00	133.67	165.00
CD @ 5% Dilution =1.3523 Concentration= 1.6563 DxC= 3.313							

7 Days

With inoculation of 10^{-7} dilution of *P .fluorescens* CFUs stock into different concentration of Neem cake extract, there was recovery of 30.67×10^7 48.00×10^7 54.00×10^7, 96.33×10^7, 145.00×10^7 and 175.00×10^7 CFUs at 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Neem cake extract after 7 days. With the inoculation at 10^{-8} dilution of *Pseudomonas fluorescens* CFUs stock, recovery of *P. flourescens* population recorded were 23.33×1010^8, 34.33×10^8, 41.00×10^8, 76.00×10^8, 133.67×10^8 and 165.00×10^8 at 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of neem cake extract after 7 days of inoculation. It was observed that the level of CFUs, get increased with increasing in substrates concentration i.e from 5% to 30%. Unless mentioned otherwise level of CFUs recorded with different dilution of initial inoculums of *P.flourescens* i.e 10^{-5} to 10^{-8} and from different concentration of substrate i.e. from 5% to 30% at each 5% interval were significantly different from each other. In plain PDA (Check) no of cfus were quite less (24, 24, 24 and 23) than the PDA added with different concentration of substrate.

Table 2: Population of *Pseudomonas fluorescens* after 15 days:

As given in table 1, it was observed that level of *P.flourescens* population density was comparatively less after 15 days of inoculation than after 7 days. However rest of the trends of population dynamics were same as it was observed after 7days. With the inoculation of 10^{-5} dilution of *Pseudomonas flourescens* stock of initial inoculum, recovery of CFUs, were, 27.00×10^5, 34.67×10^5, 41.00×10^5, 45.67×10^5, 52.23×10^5 and 64.67×10^5 from 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Neem cake extract. With inoculation of 10^6 dilution of *P .fluorescens* stock of initial inoculums, recovery of CFUs, were. 27.00×10^6, 21.67×10^6, 33.67×10^6, 38.00×10^6, 44.00×10^6, and 56.00×10^6 from 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Neem cake extract after 15 days of inoculation.

Concentration Dilution	0%	5%	10%	15%	20%	25%	30%
10^5	20.00	27.00	34.67	41.00	45.67	52.33	64.67
10^6	18.00	27.00	21.67	33.67	38.00	44.00	56.00
10^7	17.00	27.00	17.00	27.00	32.67	38.33	45.67
10^8	16.00	27.00	16.00	23.00	26.00	31.00	42.00
CD @ 5% Dilution = 0.9931 Concentration= 1.2163 DxC= 2.433							

15 days

With the inoculation of 10^{-7} dilution of *P .fluorescens* stock of initial inoculums, recovery of CFUs were, 27.00×10^7, 17.00×10^7, 27.00×10^7, 32.67×10^7, 38.33×10^7 and 45.67×10^7 from 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of neem cake extract after 15 days of inoculation.

With inoculation of 10^{-8} dilution of *P .fluorescens* stock of initial inoculum recovery of CFUs, were 27.00×10^8, 16.00×10^8, 23.00×10^8, 26.00×10^8, 31.00×10^8 and 42.00×10^8 from 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Neem cake extract. Unless mentioned otherwise level of CFUs recorded with different dilution of initial inoculums of *P.flourescens* i.e 10^{-5} to 10^{-8} and from different concentration of substrate i.e. from 5% to 30% at each 5% interval were significantly different from each other except at 5% moisture after where all dilution resulted in similar level of population density. In check plates the cfus of *P.flourescens* were 15,15,14 and 13 at all four dilutions respectively.

Population of *Pseudomonas fluorescens* on different concentration on Jatropha cake extract after 7 days:

Inoculation of 10^{-5} dilution of *Pseudomonas fluorescens* stock of initial inoculum in the different concentration of Jatropha cake extract it resulted in increasing population density of *Pseudomonas fluorescens* with increasing concentration of Jatropha cake extract (Table-3). Population densities recovered were 74.67×10^5, 110.33×10^5, 138.33×10^5, 166.33×10^5, 214.67×10^5 and 245.00×10^5 CFUs of *P .fluorescens* from 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Jatropha cake extract after 7 days of inoculation.

Table 3: Longevity and survivability of *Pseudomonas fluorescens* in Jatropha cake extract of different concentration at various dilution point with different moisture level for 7 and 15 days

Concentration Dilution	0%	5%	10%	15%	20%	25%	30%
10^5	40.00	74.67	110.33	138.33	166.33	214.67	245.00
10^6	38.00	108.67	123.00	116.33	142.67	192.67	213.3
10^7	34.00	63.00	71.00	92.00	127.00	162.33	194.67
10^8	30.00	56.67	66.33	88.67	113.67	144.33	183.00
CD @ 5% Dilution = 4.5911 Concentration= 5.6229 DxC= 11.246							

7 days

With the inoculation of 10^{-6} dilution of *P .fluorescens* stock of initial inoculum into different concentrations of Jatropha cake extract there was recovery of 108.67×10^6, 123.00×10^6, 116.33×10^6,

142.67×10⁶, 192.67×10⁶ and 213.33××10⁶ CFUs of *P .fluorescens* from 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Jatropha cake extract after 7 days of inoculation. With inoculation of 10^{-7} dilution of *P .fluorescens* stock of initial inoculum to different concentration of Jatropha cake there was recovery of 63.00×10⁷, 71.00×10⁷, 92.00×10⁷, 127.00×10⁷, 162.33×10⁷ and 194.67×10⁷ CFUs of *P .fluorescens* from 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Jatropha cake extract after 7 days of inoculation.

With inoculation of 10^{-8} dilution of *P .fluorescens* stock of initial inoculum into different concentration of Jatropha cake extract, there was recovery of 56.67×10⁸, 66.33×10⁸, 88.67×10⁸, 113.67×10⁸, 144.33×10⁸ and 183.00×10⁸ CFUs of *P .fluorescens* from 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Jatropha cake extract after 7 days of inoculation. In this case also the level of population of *P .fluorescens* get increased with the increasing concentration of substrates, whereas increasing the dilution of *P .fluorescens* for inoculation resulted in decreasing level of population of *P .fluorescens*. Unless mentioned otherwise level of CFUs recorded with different dilution of initial inoculums of *P.flourescens* i.e 10^{-5} to 10^{-8} and from different concentration of substrate i.e. from 5% to 30% at each 5% interval, were significantly different from each other.

Table 4: Population of *Pseudomonas fluorescens* after 15 days:

As already observed in case of neem cake extract, here also level of *P.flourescens* population density was comparatively less after 15 days of inoculation than after 7 days (Table 4). After 15days of inoculation of a stock dilution of 10^{-5} of initial inoculums resulted in increasing number of CFUs of *Pseudomonas fluorescens* with increasing concentration of substrates. There was recovery of 31.33×10⁵, 42.67×10⁵, 52.67×10⁵, 59.00×10⁵, 73.33×10⁵ and 78.00×10⁵ number of CFUs of *Pseudomonas fluorescens* from 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Jatropha cake extract after 15 days of inoculation.

Concentration Dilution	0%	5%	10%	15%	20%	25%	30%
10^5	17.00	31.33	42.67	52.67	59.00	73.33	78.00
10^6	16.00	25.67	31.00	39.00	43.33	52.33	56.00
10^7	15.00	14.00	17.00	21.00	24.00	28.00	34.00
10^8	14.00	11.00	14.00	19.33	22.00	25.00	31.00
CD @ 5% Dilution = 1.0600 Concentration= 1.2982 DxC= 2.596							

15 days

With inoculation of 10^{-6} dilution of *P .fluorescens* stock of initial inoculum there was recovery of 25.67×10⁶, 31.00×10⁶, 39.00×10⁶, 43.33×10⁶ 52.33×10⁶ and 56.00×10⁶ number of *P .fluorescens* CFUs from 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Jatropha cake extract after 15 days of inoculation.

With inoculation of 10^{-7} dilution of *P .fluorescens* stock of initial inoculums, there was recovery of 14.00×10⁷, 17.00×10⁷, 21.00 ×10⁷, 24.00×10⁷, 28.00×10⁷, and 34.00×10⁷, number of CFUs of *P .fluorescens* from 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Jatropha cake extract after 15 days of inoculation. With the inoculation 10^{-8} dilution of *P .fluorescens* stock of initial inoculums, there was recovery of 11.00×10⁸, 14.00×10⁸, and 19.33×10⁸, 22.00×10⁸, 25.00×10⁸ and 31.00×10⁸ number of CFUs of *P .fluorescens* from 5%, 10%, 15%, 20%, 25% and 30% concentration respectively of Jatropha cake extract after 15 days of inoculation. Unless mentioned otherwise level of CFUs recorded with different dilution of initial inoculums of *P.flourescens* i.e 10^{-5} to 10^{-8} and from different concentration of substrate i.e. from 5% to 30% at

each 5% interval were significantly different from each other. Level of cfus in plain PDA were 15, 15, 14 and 13 with inoculation of different dilution of inoculums of *P .fluorescens*

Effect of substrates concentration on population of *Pseudomonas fluorescens*

Colony forming units of *Pseudomonas fluorescens* showed variation in different concentration of two substrate i.e. neem cake and Jatropha cake. Total viable count was highest (245.00) for the Jatropha cake at 30% concentration after 7 days of inoculation. At the similar concentration of neem cake the total viable count was comparatively less (201.67) after 7 days .Increasing in substrate concentration was directly proportional to total viable count of *Pseudomonas fluorescens*. Increasing in inoculums dilution was universally proportional to total viable count of *Pseudomonas fluorescens*. Total viable count of *Pseudomonas fluorescens* after 15 days were comparatively quite less at all concentration of two substrates and four dilution of inoculums than total viable counts of *Pseudomonas fluorescens* recovered after 7 days. Abhinav *et al.* (2011) evaluates 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. The substrate concentration and therefore medium viscosity would influence the growth of *Pseudomonas fluorescens* Solomon (1983) has also reported that substrate concentration affects the yield of *Saccharomyces cerevisiae* when grown on an assimilable carbohydrate such as glucose or sucrose. Possibly richness of potassium, protein and carbohydrate content in deoiled cakes of neem and jatrofa may be responsible for enhanced growth of *Pseudomonas fluorescens*. Murugalakshmi and Sudha (2010) concluded that agricultural residues rich in carbohydrates can be utilized in fermentation process to produce microbial protein which in turn can 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 28^{o} C, when supplemented with agricultural wastes in different concentration mixed with agar. The number of colony forming unit were more when compared with nutrient agar. Thus the present findings are well supported by the findings of these workers.

Conclusion

Increasing the dilution of stock of initial inoculums resulted in decrease of total viable counts of *Pseudomonas flourescens*. Population of *Pseudomonas flourescens* was comparatively lesser after 15 days than after 7 days of inoculation.

To achieve comparatively higher population dynamics, of *Pseudomonas fluorescens*, it should be initially grown in king'sB medium and transferred to a basal medium containing 30% concentration of either jatropha cake extract or 30% concentration of neem cake extract.

References

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

2. Augustus, G.D.P.S.; Jayabalan, M. and G.J. Seiler. 2002. Evaluation and bioinduction of energy components of Jatropha curcas. *Biomass and Bioenergy*. 23: 161-164.

3. 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.

4. Solomons, G. L. 1983. Single Cell Protein. In CRC Critical Reviews in Biotechnology 1: 21-58.

5. Murugalakshmi, C.N. and Sudha, S.S., 2010. The Efficacy of agrowaste on cultivation of *Pseudomonas fluorescens*. A potential biocontrol agent *International Journal of Biological Technology,* 1: 32-34.

6. Patolia, J. S., A. Ghosh., J. Chikara., D. R. Chaudharry., D. R. Parmar., and H. M. Bhuva. 2007. "Response of *Jatropha curcas* L. Grown on Wasteland to N and P Fertilization." Paper presented at the FACT Seminar on *Jatropha curcas* L. Agronomy and Genetics, March 26–28, Wageningen. Article No.34.

7. Tiwari, AK.; Kumar, A, and Raheman, H. 2007. Biodiesel production from jatropha oil (Jatropha curcas) with high free fatty acids: an optimized process. Biomass Bioenergy; 31:569–75.