The Bioscan 9(1): 371-374, 2014 (Supplement on Plant Pathology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

DETERMINATION OF SUITABILITY OF DEOILED CAKES OF MAHUA AND KARANJA FOR MASS MULTIPLICATION AND LONGER SHELF LIFE OF *PSEUDOMONAS FLUORESCENS*

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KEYWORDS
Cfus
Deoiled cake
Karanja
Mahua
Pseudomonas flourescens.

Received on :
27.01.2014

Accepted on :
24.02.2014

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ABSTRACT
The present investigations were under taken to determine suitability of Mahua and Karanja cakes for mass multiplication and longevity of *Pseudomonas flourescens in vitro*. Mahua cake was found to be best substrate for supporting the population dynamics and longevity of *P. flourescens in vitro*. Both the cakes, *i*.e. mahua and karanja, supported the population of *P. flourescens* up to 120 days. Highest population (298.50×10x) of *P. flourescens* was noticed on mahua cake after 45 days of inoculation when maintained with 35% moisture. On karanja cake also, highest population (280.00×10x) of *P. flourescens* was noticed after 45 days of inoculation when maintained with 35% moisture. Mahua cake was better than karanja cake for supporting population and longer shelf life of *P. flourescens in vitro.* A general trend was noticed in case of. mahua cakes, up to 45 days of inoculation ,there was increasing trend in the population of *P .fluorescens,* whereas after 60 days on ward population showed decreasing trend, while in case of karanja cake increasing trend was noticed upto 60 days. Enhancement in the level of moisture resulted in increased population of *P .fluorescens.*

INTRODUCTION

In nature wide range of organic substrates are utilized as source of nutrients by several micro flora possessing antagonistic capacity, which could also be used for the solid-state fermentation for mass multiplication of biocontrol agents. Solid - state fermentation media consisting of inert carriers with food bases have been used for mass production of biocontrol agents (Lewis, 1991). Solid substrates include straws, wheat bran, sawdust, moistened bagasse, sorghum grains, paddy chaff,decomposed coir pith, farmyard manure and other substrates rich in cellulose for mass production Meena, *et al.* (2013). Kloepper and Schroth (1981) demonstrated the potentiality of talc to be used as a inert carrier for formulating rhizobacteria.

Many agro-industrial by-products, such as deoiled cakes of tree borne oil seeds (TBOs) like Mahua and Karanja, are either going waste or being used as a less valuable and usable products, since quite long time. The oils extracted from Mahua and Karanja, are either directly used as bio-fuel or as raw material for industrial inputs in various manufacturing industries like cosmetics, agrochemicals and pharmaceuticals. Deoiled cakes of these trees remain either unexploited or poorly exploited. These deoiled cakes contain good amount of carbohydrates, proteins, fatty acids, minerals and many more biochemical constituents, which may serve as rich source of nutrition for beneficial micro-organisms (growth promoting and biocontrol agents) in crop production. These characteristics of deoiled cakes of TBOs make them an ideal source of nutrition for microorganisms, hence may be 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 Mahua and Karanja cakes as well. Mass multiplication of *Pseudomonas fluorescens* will not only leads to value added products development from deoiled cakes of Mahua and Karanja, rather it will prevent huge wastage of these by-products. It is worthwhile to mention that these cakes themselves too posses antifungal properties (Anjali *et al.,* 2013).

Commercial formulation of *Pseudomonas* spp. which are available in the market, generally show poor efficacy after application in the crop field. This is probably due to quite long duration, taken in transportation from manufacturing unit to the users (farmers). The formulations prepared at industrial scale are generally talc based, with no nutritional background to support the life of BCAs during storage, transportation and other unavoidable stress situation. Deoiled cakes of TBOs may serve as source of diversified nutrition for BCAs when used as substrate for mass multiplication of antagonists.

In addition, application of BCAs grown on deoiled cakes which are rich in many nutritionally important compounds, may

reduce soil-borne diseases by releasing allelo-chemicals generated during product storage or by subsequent microbial decomposition Mina *et al*. (2013**)**.

MATERIALS AND METHODS

Sources and Maintenance of culture

Pseudomonas fluorescens culture was isolated from the soil of tomato rhizosphere, collected from crop research centre (CRC) of SVPUAT Meerut. For isolation of microorganism, 10gm of soil sample adhered to roots and rootlets of tomato were collected and placed in a 250mL conical flask 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 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 28 ± 2ºC and after two days of incubation, pure culture was maintained in PDA slants. Conformity of culture was done on the basis of color of bacterial colony which was initially yellow but turned yellow green as pigmentation were produced (Bonds,1957). Further culture was again reconfirmed by molecular conformity test at National Beauro of Agriculturally Important Microbes (ICAR) Mau (UP) India. The culture thus obtained was stored in refrigerator at 5ºC for further studies and was sub cultured periodically.

Determination of population dynamics of *Pseudomonas fluorescens* on deoiled cakes of mahua and karanja

Deoiled cakes of mahua were collected from Distt. Ambedkar nagar (U.P.) and karanja from Raipur (Chattisgarh) respectively. Before using, the cakes were grinded in a metallic pastel and mortar to prepare fine powder and three different level of moisture *i.e.,* 15%, 25% and 35% (w/v) were maintained by adding required amount of sterilized distilled water. Before inoculation of *Pseudomonas fluorescens*, cakes containing different level of moisture were placed in conical flasks of 250mL capacity, plugged tightly with cotton plugs, wrapped with butter paper and autoclaved at 121.6ºC (1.1 kg/cm^2) for 20 minutes. The flasks were allowed to cool overnight at room temperature prior to inoculation. Flasks containing substrates (Sterilized cakes) 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 (Longevity) three replicates were maintained. Flasks inoculated with *Pseudomonas fluorescens* were incubated at 28 ± 2ºC and shaken thoroughly once a day.

Monitoring population dynamics in de-oiled cakes

Population of *Pseudomonas fluorescens* was monitored from the deoiled cakes of Mahua and Karanja maintained with different level of moisture (15%, 25% and 35% respectively) after each 15 days interval upto 120 days. For this purpose, 1gm of each cakes inoculated with *Pseudomonas flourescens,* were taken from each flasks maintained for different duration *i.e.* 15 to 120 days and cfus were counted using PDA through dilution plate technique as given bellow.

Dilution plate technique

The colony forming units (CFUs) of *Pseudomonas fluorescens were* counted by serial dilution method by agar plate technique (http://en.wikipedia.org/wiki/Agar_plate). For this purpose 1g of each cakes, where *P. fluorescens* was being grown, was suspended in 10 ml distilled water to make 10 times dilution of this stock (microbial suspension), thus it will give 1: 10 conc. or 10^{-1} dilution of original sample, *i.e.* the original sample has been diluted to 1/10th. Again 1mL of suspension from first tube get into second tube contained 9 ml of sterile water; this suspension was used to make microbial concentration as 1:100 (10^{-2}). Similarly it was prepared as 1: 1000(10^{-3}), 1: 10,000 (10^{-4}), 1: 100,000 (10^{-5}) up to 10^{-8} by dilution of the original sample. Finally 1mL of microbial suspension from last serial dilution *i.e.*, 10^{-8} was added to sterile Petri dishes (triplicate in completely randomized manner) containing 20 mL of sterilized King,s B medium and incubated for 5 days at 28 ± 2ºC. After five days of incubation cfus were counted Colony Counter (http://en.wikipedia.org/wiki/Colony_counter).

RESULTS

Population dynamics of *Pseudomonas fluorescens* on Mahua cake

It is evident from Table 1 that Mahua cake with 15% moisture, resulted in 135.00×10x level of CFUs of *P. fluorescens* after 15 days of inoculation. After 30 days of inoculation, there was 195.00×10x CFUs. At 45 days of inoculation the mahua cake with 15% moisture resulted in 285.00×10x CFUs. At 60 days of inoculation, mahua cake with 15%moisture resulted in 250.00×10x CFUs. At 75 days of inoculation mahua cake with 15% moisture resulted in 175×10x CFUs, whereas at 90 days, same cake resulted in 115.00×10x CFUs of *Pseudomonas fluorescens*. At 105 days of inoculation mahua cake with15%of moisture resulted in 65.00×10x CFUs, whereas after 120 days CFUs reduced to 22.00×10x ? The population of *P .fluorescens* at each 15 days interval starting from 15-120 days were significantly different from each other.

Mahua cake with 25% moisture, resulted in 148.00×10x CFUs of *Pseudomonas fluorescens,* whereas at 30 days CFUs were 225.00×10x and at 45 days 295.00×10x CFUs were recovered. At 60 days there was 255.00×10x CFUs of *Pseudomonas fluorescens* . At 75 days of inoculation, the mahua cake with 25% moisture resulted in 195.00×10x CFUs. The CFUs were little less *i.e* 130.00×10x at 90 days of inoculation. At 105 days of inoculation, mahua cake with 25% moisture resulted in 75.00×10x CFUs. At 120 days, the population of *P .fluorescens* declined to 30.00×10x . However, population of *Pseudomonas fluorescens* at each 15 days interval differed significantly from each other.

Mahua cake with 35% moisture, resulted in 150.00×10x number of CFUs of *Pseudomonas fluorescens* at 15 days of inoculation. At 30 days of inoculation, mahua cake with 35% moisture, resulted in 245.00×10x CFUs , whereas at 45 days of inoculation, the population of *P .fluorescens* increased to 298.50×10x and at 60 days of inoculation, it further declined to 272×10x . At 75 days of inoculation, mahua cake with 35% moisture, showed further reduction in the *Pseudomonas*

population and CFUs declined to 202.00×10x . At 90 days of inoculation it resulted in 145.00×10x CFUs of *P .fluorescens*. At 105 days,population of *P .fluorescens* recovered were 82.00×10x , whereas at 120 days, the CFUs declined to 32.00×10x . In this case also population of *P.fluorescens* observed at each 15 days interval, differed significantly from each other.

Population dynamics of *Pseudomonas fluorescens* on Karanja cake

It is evident from table 2 that karanja cake with 15% moisture, resulted in 125.00×10x CFUs of *Pseudomonas fluorescens*. At 30 days of inoculation, the karanja cake with 15% moisture, resulted in178.00×10x CFUs. At 45 days of inoculation, karanja cake containing 15% moisture, exhibited 210.00×10x CFUs ,whereas at 60 days of inoculation, it resulted in 224.00×10x CFUs. At 75 days of inoculation, the karanja cake containing 15% moisture resulted in 195.00×10x CFUs and after 90 days it resulted in 145.00×10x CFUs . At 105 days of inoculation the karanja cake containing 15% moisture resulted in 92.20×10x level of CFUs, whereas at 120 days of inoculation it resulted in 42.00×10x CFUs of *P .fluorescens*

Karanja cake containing 25% moisture resulted in 140.00×10x CFUs of *P .fluorescens*, after 15 days of inoculation, whereas, at 30 days, the same cake exhibited 190.00×10x and at 45 days 219.00×10x number of CFUs of *P .fluorescens*. At 60 days, Karanja cake containing 25% moisture, yielded 238.00×10x CFUs, whereas, at 75 days population of *Pseudomonas* goes down to198.00×10x . At 90 days it was 155.00×10x and at 105 days it was 105.00 ×10x and at 120 days the population further declined to 36.00×10x cfus of *P.fluorescens*.

Karanja cake containing 35% moisture resulted in 146.00×10x of CFUs of *Pseudomonas fluorescens* at 15 days of inoculation. At 30 days the number of CFUs increased to 229.00×10x , whereas at 45 days the population further increased to 280.00×10x . At 60 days onward there was declining trend and population declined to the level of 250.00×10x ,which further declined to 210.00×10x , 160.00×10x ,115.00×10x and 44.00×10x after 75 ,90,105 and 120 days of inoculation respectively. Level of CFUs recorded after each 15 days interval and each level of moisture were significantly different from each other.

A general trend was noticed in both the cakes *i.e.* mahua and karanja, that upto 45 days of inoculation ,there was increasing trend in the population of *P .fluorescens,* whereas after 60 days on ward population showed decreasing trend. Enhancement in the level of moisture resulted in increased population of *P .fluorescens*.

DISCUSSION

Results indicated that mahua cake was found to be comparatively better than karanja cake for enhancing population of *Pseudomonas fluorescens* with a highest level of cfus after 45 days of inoculation with 15% moisture. It was also noticed that mahua and karanja cake both could support the population and longevity upto 120 days with ×10x level of population. In case of karanja cake it was observed that, population of *Pseudomonas fluorescens* was found to be increasing upto 60 days after inoculation with 15% and 25% moisture, while on mahua cake population of *Pseudomonas fluorescens* was found to be increasing upto 45 days only and after that there was a decline in the population. In case of karanja cake, upto 105 days, increase in the moisture level resulted in increase of population but at 120 days such trend was not noticed. In case of mahua cake, upto 120 days, increase in the moisture, level resulted in increasing in population.

Reason behind higher population dynamics of *Pseudomonas fluorescens* on the de-oiled cakes of two tree born oilseeds (TBO's) may be because of their richness in different type of nutrients, minerals and other constituents which are required and may be supportive for multiplication of *Pseudomonas fluorescens* . Reason behind decline of population dynamics after 45/60 days during present investigation may be that, at the initial level there may be plenty of nutrition available for utilization by *Pseudomonas fluorescens* which later get declined, because they might have been exhausted day by day due to utilization 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.

Kloepper and Schroth (1981) demonstrated the potentiality of talc to be used as a carrier for formulating rhizobacteria. The fluorescent Pseudomonads did not decline in talc mixture with 20% xanthum gum after storage for two months at 4ºC. *Pseudomonas fluorescens* isolate Pf1 survived up to 240 days

Table 1: CFUs of *Pseudomonas fluorescens* at different moisture level on sterilized mahua cake at different time interval

DaysMoisture Level	15Days	30Days	45Days	60Days	75Days	90Days	105Days	120Days
15%	135.00	195.00	285.00	250.00	175.00	115.00	65.00	22.00
25%	148.00	225.00	295.00	255.00	195.00	130.00	75.00	30.00
35%	150.00	245.00	298.00	272.00	202.00	145.00	82.00	32.00

CD @ 5% Moisture % =1.1054, Days =1.8050 M×D = 3.126

Table 2: CFUs of *Pseudomonas fluorescens* at different moisture level on sterilized karanja cake at different time interval

DaysMoisture Level	15Days	30Days	45Days	60Days	75Days	90Days	105Days	120Days
15%	125.00	178.00	210.00	224.00	195.00	145.00	92.20	42.00
25%	140.00	190.00	219.00	238.00	198.00	155.00	105.00	36.00
35%	146.00	229.00	280.00	250.00	210.00	160.00	115.00	44.00

CD @ 5% Moisture % =0.5806, Days =0.9480 M×D = 1.642

in storage. The initial population of Pf1 in talc-based formulation was 37.5 x 10^7cfu/g and declined to 1.3 x 10^7cfu/g after 8 months of storage.

Nilkamal *et al*. (2008) and Jyothi *et al*. (2013) assessed, Deoiled Jatropha seed cake for its suitability 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) as evident by its chemical composition. Maximum protease and lipase production was observed at 50% substrate moisture, a growth period of 72 and 120 h, and a substrate pH of 6.0 and 7.0, respectively was maintained.Although jatrofa cakes are not used in the present investigation but overall these findings of Nilkamal *et al*. (2008) and Jyothi *et al*. (2013) can be used as supportive for the present findings

Abhinav *et al*. (2011) evaluateds 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. this finding partially support our findings in the way that saw dusts are cellulosic materials while deoiled cakes may be rich in protein and cellulosic contents hence supported excellent growth.

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*., *Azospirillum lipoferum* VAZS-18, *Azotobacter chroococcum* 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. 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.

CONCLUSION

Among two de-oiled cakes, Mahua cake was found to be best substrate in supporting the population dynamics of *Pseudomonas flourescens in vitro*. Karanja cake was next in order to support the population of *Pseudomonas flourescens* rather closely followed the Mahua cake in supporting the population of *Pseudomonas flourescens in vitro*. Both these cakes (Mahua and Karanja) supported the population of *Pseudomonas flourescens* up to 120 days with a conciderable level of viable counts of *Pseudomonas flourescens*.

Highest population of *Pseudomonas flourescens* was noticed on Mahua cake after 45 days of inoculation when maintained with 35% moisture, and on Karanja cake highest population of *Pseudomonas flourescens* was noticed after 60 days of inoculation when maintained with 35% moisture. Mahua cake was better than Karanja cake for supporting population and longer shelf life of *Pseudomonas fluorescens*.

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