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DETERMINATION OF POPULATION DYNAMICS OF *PSEUDOMONAS FLUORESCENS* IN THE SOIL PLANTED WITH TOMATO GROWN ON DE-OILED CAKES OF MAHUA AND KARANJA

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ABSTRACT
The main aim of this study was to determine the effect of de-oiled mahua and karanja cakes on the population dynamics of *Pseudomonas fluorescens*. We also made different combination of these de-oiled cakes and *P. fluorescens* with soil and check their effects on the tomato plant. We made 7 treatments and found that highest recovery of *P. fluorescens* on mixture of mahua and karanja cakes in sterilized soil (230×10^8cfus at 60 DAP) and unsterilized soil (215×10^8cfus at 60 DAP) when this mixture was used for cultivation of tomato plants. It shows the most promising effects on the tomato plant. It has beenalso found during this study that Shelf life of *P. fluorescens* was also high(120 days) in the above said treatment.From the above results it is clear that both the de-oiled cakes have profound effect on the population dynamics and shelf life of *P. fluoresces* which in turn enhance tomato plants root length (70.00%), shoot length (72.28%) and yield (541.65%). Thus we can say that these agro wastes can be used as carrier material for the growth and mass multiplication of *P. fluorescens*.

INTRODUCTION

Pseudomonas fluorescens encompasses a group of common, Gram negative, rod shaped, non-pathogenic saprophytes is mainly present in soil, around/on the root surface of plant and involved in promoting, plant growth and development *via* production and secretion of various growth regulatory substances in the vicinity of rhizosphere.*P. fluorescens* strains are being investigated extensively for control of pathogens in agriculture studied by Ganeshan and Kumar (2006).*P. fluorescens* is well known for its ability to enhance plant growth and yield and to reduce severity of many diseases Wei *et al.* (1996) through the production of secondary metabolites Koche *et al.* (2013).

De-oiled cakes of 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. These cakes remain either unexploited or poorly exploited. These de-oiled cakes are the rich source of carbohydrates, proteins, fatty acids, minerals and biochemical constituents, which may serve as rich source of nutrition for beneficial micro-organisms.Mahua and Karanja cakes for mass multiplication and longevity of *Pseudomonas fluorescens* found to be the best substrate for supporting the population dynamics and longevity of *P. fluorescensin vitro*. Both the cakes, *i.e.*mahua and karanja, supported the population of *P. fluorescens* up to 120 days reported by Maurya *et al.* (2014). These cakes can be exploited as carrier material for the commercial production of *Pseudomonas fluorescens* bio-formulation and can be used as an alternative against chemical fungicides for phytopathogens suppression. The bacterial antagonists have the dual advantage of faster multiplication and higher rhizosphere competence; hence*P. fluorescens*have been successfully used for biological control of several plant pathogens concluded by Ramamoorthy *et al.* (2002).

Mass multiplications of *P. fluorescens*will not only leads to the value added product development from de-oiled cakes of Mahua and Karanja, rather it will prevent huge wastage of these by-products. In addition, application of BCAs grown on de-oiled cakes which are rich in many nutritionally important compounds mayreduce soil-borne diseases by releasing allelo-chemicals generated during product storage or by subsequent microbial decomposition.Tomato plant suffer from a various diseases caused by fungi, bacteria, viruses and nematodes. Thus tomato crop plant was selected to test the population dynamics, shelf life, mass multiplication of *Pseudomonas fluorescens*grown on de-oiled cakes of Mahua and Karanja.

MATERIALS AND METHODS

Present investigations on *P. fluorescens* with special reference to enhancing population dynamics and mass culture, were carried out in the pot and in the laboratory of the Department of Plant Pathology of SardarVallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.).

Sources and Maintenance of culture

*P. fluorescens*culture was isolated from the soil of tomato

rhizosphere, collected from crop research centre (CRC) of SVPUAT Meerut. We take 10 gm of soil sample adhered to roots and rootlets of tomato and dissolved it 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 Petri plate containing king's B Medium (King's *et al.*,1954). The plates were incubated for 2 days at 28 ± 2°C. The individual colonies of pure culture were examined for colony colour, and pigmentation (Buchanon and Gibbson1974).

Population dynamics of *P.fluorescens*grown on Mahua and Karanja cakes in rhizosphere of tomato plant

A pot experiment was conducted with *Pseudomonas fluorescens*grown on Mahua and Karanja cakes, which were incorporated into soil, planted with tomato. For this purpose, 2 days old *P.fluorescens* culture grown on King's B Medium were mixed to the sterilized Mahua cake and Karanja cakes and incubated for 15 days for proper colonization and growth of *P.fluorescens* on substrates (cakes). Now these substrates were added to sterilized and unsterilized soil in clay pots and mixed thoroughly, details of the process are given below.

Experimental details

Biocontrol agent used	*Pseudomonas fluorescens*
Substrates used	De-oiled Mahua and Karanja cakes.
Soil used	sandy loam (sterilized & unsterilized)
No. of pots	21
Pot's size	20x15''
Crop	Tomato
Variety	S-22
Design	Randomized Block Design (RBD)
No. of Treatments	7
No. of Replications	3
Date of sowing	29-10-2012

Treatments details

T1. Tomato plants grown in sterilized soil applied with *Pseudomonas fluorescens* grown on Mahua cake.

T_2. Tomato plants grown in sterilized soil applied with *Pseudomonas fluorescens* grown on Karanja cake.

T_3. Tomato plants grown in sterilized soil applied with *Pseudomonas fluorescens* grown on Mahua cake and Karanja cake.

T_4. Tomato plants grown in unsterilized soil applied with *Pseudomonasfluorescens* grown on Mahua cake.

T_5. Tomato plants grown in unsterilized soil applied with*Pseudomonasfluorescens*grown on Karanja cake.

T_6. Tomato plants grown in unsterilized soil applied with*Pseudomonasfluorescens*grown on Mahua cake and Karanja cakes.

T_7. Tomato plants grown in unsterilized soil without any amendments (Control)

Sterilization of soil

For pot experiments, total 27 kg of soil was sterilized by placing it in poly bags (5 kg.) at 121.6 ºC (1.1 kg/cm^2) for 30 minutes, in an autoclave.

Pot filling and planting with tomato seedlings

After sterilization, soil was left over night for proper cooling. Now 9 pots were filled with sterilized soil at the rate 3 kg per pot and in another set 12 pots were filled with unsterilized soil at the rate 3 kg per pot. In each pot 75g mass culture of *P. fluorescens* grown on de-oiled cakes of Mahua and Karanja were added separately or mixture of these two cakes as per treatment needs, after those pots were planted with 3 seedlings of tomato (S-22) per pots. One set of control was maintained. Three replicates of each treatment were made.

Assessment of CFUs of *Pseudomonas fluorescens*

After 15 days of planting, 1 gram soil sample was taken from each replication of each treatment at 10 cm depth with sterilized disk cutter into test tube. It was put into another test tube containing 10 ml sterilized distilled water and shaken well, and diluted up to 10^{-8} after that 1 ml suspension was spread on petri plate contained with king's B medium. The Petri dishes were incubated for 5 days at 28 ± 2ºC. The CFUs of *P. fluorescens* were counted by plate count method (Johnson and Curl, 1972). Population dynamics of *P. fluorescens* was continuously monitored up to 120 days at each 15 days interval by this procedure.

Assessment of root length, shoot length and fruit weight of tomato

Observations were also recorded to measure root and shoot length of tomato along with weight of total fruits in individual pots. Percent increase in root and shoot length and fruit yield were calculated using following formula.

$$\%\ \text{length increase} = \frac{\text{Length treated pot} - \text{Length in control pots}}{\text{Lentgh in coltrol pots}} \times 100$$

$$\%\ \text{yield increase} = \frac{\text{Yield under protected} - \text{Yield under unprotected}}{\text{Yield under unprotected}} \times 100$$

Statistical Analysis

The data were subjected to analysis of variance, and treatment means were differentiated using Fischer's T test which shows the significant analysis and error variation reduces. The data taken into percentage were first transformed into angular value and then analyzed for test of significance (Gomez, 1996 and Chandel, 2002).

RESULTS

Population of *P. fluorescens* grown on Mahua and Karanja cakes from soil planted with tomato

Data regarding this experiment have been presented in (Table-1). The population of *P. fluorescens* grown on mahua cake in sterilized soil planted with tomato (T_1) recovered were 115×10^8, 165×10^8, 188×10^8, 210×10^8, 165×10^8, 98×10^8, 45×10^8 and 15×10^8 CFUs at 15 DAP (days after planting), 30 DAP, 45 DAP, 60 DAP, 75 DAP, 90 DAP, 105 DAP, 120 DAP respectively. The population of *P. fluorescens* recovered from sterilized soil planted with tomato at each 15

Table1: Grown of CFUs of *P. fluorescens*mahua and karanja cakes in rhizosphere of tomato plants.

Treatment	15DAP	30DAP	45DAP	60DAP	75DAP	90DAP	105DAP	120DAP
T1	115	165	188	210	165	98	45	15
T2	105	153	180	202	152	85	30	12
T3	135	180	212	230	185	115	65	25
T4	102	150	178	200	135	68	28	11
T5	95	145	172	195	130	55	24	9
T6	120	175	193	215	165	105	55	17
T7	0	0	0	0	0	0	0	0

CD @ 5% Cakes =0.5259, Days =0.5622, C×D= 1.488

Table 2: Effect of *P. fluorescens* on root length, shoot length and fruit weight of tomato plants.

Treatment	Root length(c.m.)	Increasing %	Shoot length(c.m.)	Increasing %	Weight of tomato fruit (g.)	Increasing %
T1	26.66	33.30	51.33	46.65	83.33	108.33
T2	28.00	40.00	49.00	40.00	80.00	100.00
T3	34.00	70.00	61.00	72.28	256.66	541.65
T4	29.66	48.30	57.33	63.80	51.66	29.15
T5	28.66	43.30	51.00	45.71	68.33	70.86
T6	32.00	60.00	59.33	69.51	223.33	458.33
T7	20.00	0	35.00	0	40.00	0
CD @ 5%	2.83		5.087		36.010	

days interval were significantly different from each other. The population of *P. fluorescens* grown on grown on karanja cake in sterilized soil planted with tomato (T_2) recovered were 105×10^8, 153×10^8, 180×10^8, 202×10^8,152×10^8, 85×10^8, 30×10^8, 12×10^8 CFUs at 15 DAP, 30 DAP, 45 DAP, 60 DAP, 75 DAP, 90 DAP, 105 DAP, 120 DAP respectively. The population of *P. fluorescens* at each 15 days interval were significantly different from each other. The population of *P. fluorecens* grown mixture of mahua and karanja cakes in sterilized soil planted with tomato (T_3) recovery were 135×10^8, 180×10^8, 212×10^8, 230×10^8, 185×10^8, 115×10^8, 65×10^8 and 25×10^8 CFUs at 15 DAP, 30 DAP, 45 DAP, 60 DAP, 75 DAP, 90 DAP, 105 DAP, 120 DAP respectively. The population of *P. fluorescens* at each 15 days interval were significantly different from each other. The population of *P. fluorescens* grown on mahua cake in unsterilized soil planted with tomato (T_4) recovered were 102×10^8, 150×10^8, 178×10^8, 200×10^8, 135×10^8, 68×10^8, 28×10^8 and 11×10^8 CFUs at 15 DAP, 30 DAP, 45 DAP, 60 DAP, 75 DAP, 90 DAP, 105 DAP, 120 DAP respectively. The population of *P. fluorescens* grown on karanja cake in unsterilized soil planted with tomato (T_5) recovered were 95×10^8, 145×10^8, 172×10^8, 195×10^8, 130×10^8, 55×10^8, 24×10^8 and 9×10^8 CFUs at 15 DAP, 30 DAP, 45 DAP, 60 DAP, 75 DAP, 90 DAP, 105 DAP, 120 DAP respectively. The population of *P. fluorescens* grown on mixture of mahua and karanja cakes in unsterilized soil planted with tomato (T_6) recovered were 120×10^8, 175×10^8, 193×10^8, 215×10^8 165×10^8, 105×10^8, 55×10^8 and 17×10^8 CFUs at 15 DAP, 30 DAP, 45 DAP, 60 DAP, 75 DAP, 90 DAP, 105 DAP, 120 DAP(days after planting) respectively. The population dynamics of each treatment was determined regularly after 15 days interval and it was found different among all the treatments. Control plants (T_7) did not show any recovery of *P. fluorescens*.

Effect of application of *P. fluorescens* on root length, shoot length, fruit weight of tomato plant

The observations were recorded on root length, shoot length, and fruit weight of tomato (Table-2) all treatments were found superior over the control (T_7). Tomato plants grown in sterilized soil applied with *Pseudomonas fluorescens* grown on mahua cake (T_1) showed increased root length (33.30%), shoot length (46.65%) and fruit weight (108.33%). Tomato plants grown in sterilized soil applied with *Pseudomonas fluorescens* grown on karanja cake (T_2) showed increased root length (40.00%), shoot length (40.00%) and fruit weight (100.00%), Application of *P. fluorescens* grown on mixture of mahua cake and karanja cake in sterilized soil planted with tomato (T_3) resulted in maximum root length (70.00%), shoot length (72.28%) and fruit weight (541.65%), followed by application of *P. fluorescens* grown on mixture of mahua cake and karanja cake in unsterilized soil planted with tomato(T_6),resulted increased root length (60.00%), shoot length (69.51%) and fruit weight (458.33%). Tomato plants grown in unsterilized soil applied with *Pseudomonas fluorescens* grown on karanja cake (T_5) resulted in minimum increased root length (43.30%), shoot length (45.71%) and fruit weight(70.86) followed by tomato plants grown in unsterilized soil applied with *Pseudomonas fluorescens* grown on mahua cake (T_4), increased root length (48.30%), shoot length (63.80%) and fruit weight (29.15%). Each treatment were significantly different from each other.

DISCUSSION

Population of *P. fluorescens* grown on Mahua and Karanja cakes from soil planted with tomato

Highest recovery of *P. fluorescens* population were 230×10^8cfus at 60 DAP in treatment (T_3), followed by 215×10^8cfus at 60 DAP in treatment (T_6)and lowest recovery of *P. fluorescens* population were 9×10^8cfus at 120 DAP in treatment (T_5), followed by 11×10^8cfus at 120 DAP in treatment (T_4). Population of *P. fluorescens* tend to increased rapidly up to 60 days whereas after 75 days onward population

was found to be decreasing. Higher population dynamics in the tomato rhizosphere is due to different root exudates along with some other organic matter available in the soil might have helped *Pseudomonas fluorescens* to multiply profusely to maintain comparatively higher level of population dynamics along with longer longevity by providing abundant food sources from mahua and karanja cakes. However it is an unique investigation which is focused on the role of different substrates in supporting the population dynamics of *Pseudomonas fluorescens* and longevity after mixing to the soil, which will also help in making strategy for biological control in a long term perspective Similar finding was reported by Maurya*et al.* (2014) that Mahua and Karanja cakes for mass multiplication and longevity of *Pseudomonas fluorescensin vitro*. Both the cakes, *i.e.* mahua and karanja, supported the population of *P. fluorescens* up to 120 days. Highest population (298.50×10^8cfus) of *P. fluorescens* was noticed on mahua cake after 45 days of inoculation when maintained with 35% moisture. On karanja cake also, highest population (280.00×10^8cfus) of *P. fluorescens* was noticed after 45 days of inoculation when maintained with 35% moisture. A general trend was also noticed in case of. mahua cakes, up to 45 days of inoculation that there was increasing trend in the population of *P. fluorescens,* whereas after 60 days onward 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*.Chaithanya *et al.* (2014) used different carrier inoculants had also significantly affected the population of *P. fluorescens*and showed that significant increase in population of *P. fluoresces* was observed in talc based formulations (86.09×108 cfu/g of carrier). Furthermore, inoculants prepared with FYM(A5), lignite (A3) and SMS(A2) also performed well to support the growth of bacteria *i.e* 81.67×108 cfu/g, 79.75×108 cfu/g and 78.41×108 cfu/g of carrier. Lowest population was recorded in fly ash (75.50cfu/g). Gade *et al.* (2014) evaluated different carriers for shelf life of *Pseudomonas fluorescens* stored at 25 ± 2ºC over a storage period of 6 months. The population dynamics was recorded at monthly intervals. The population of *P. fluorescens* was increased significantly in all carriers *i.e.* Talc, Spent mushroom substrate (SMS) a waste product of mushroom industry, Lignite, Charcoal, Farm yard manure (FYM) and Flyash up to 60 days storage and there was a slow decline in number of viable propagules after 60 days of storage. Spent mushroom substrate (78×108cfu/g) and fly ash (53.67×10^8cfu/g) maintained viable population count at 90 days of storage. This population was very close to the population recorded in carrier's viz., Talc (86.33×10^8cfu/g), Lignite (80×10^8cfu/g). Which may be used for developing commercial formulations of *P. fluorescens* in bio-fertilizer industry.

Effect of application of *P. fluorescens* on root length, shoot length, fruit weight of tomato plant

It was found during the experiment that treatment T_3induced maximum significant increasedon tomato plants root length (70.00%), shoot length (72.28%) and fruit weight (541.65%), followed by treatment T_6increased root length (60.00%), shoot length (69.51%) and fruit weight (458.33%) over other treatments including control. Similar finding was also reportedby Saravanan *et al.* (2013) that inoculation with fluorescent *Pseudomonas* induced a significant increase in root and shoot length (123 and 96%, respectively) of tomato plant over then inoculated control. Khan and Akram (2000) achieved enhancement of plant growth and yield in tomato from nematode (*M. incognita*) and fungus (*F. oxysporum*) infected plants by treating them with *P. fluoresces* in field trials at Aligarh, India.The reason behind this result, seems that sterilization of soil have killed several micro flora which arecompetiting for nutrition and space, which leads to comparatively better root growth than in unsterilized soil. where possibly some harmful microflora might have been present and hence helped poor availability of nutrition in unsterilized soil and this may be main reason behind poor root growth, shoot growth and all these together ultimately manifested in comparatively lower fruit yields.

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