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Growth Rate of Pseudomonas fluorescens and Trichoderma in Indian Climate

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Abstract

The study was aimed for the economical development by improving various formulations using whey liquid and molasses inoculated by plant growth promoting organisms; to reduce environmental pollution by whey water from dairy industries; to apply biofertilizers in the soil through drip irrigation tubes without making any blockage in dripper's holes; to reduce the high cost of biofertilizers and save agriculture. Totally 102 PGPR bacterial and 22 PGPR fungal strains were isolated based on morphological techniques, biochemical studies and cultural characteristics from various rhizosphere soil samples of different crops in various locations of Gobichettipalayam, Erode. From these isolates 35 bacterial and 7 fungal PGPR isolates were screened based on physical and nutritional parameters such as pH, temperature, glucose and NaCl and identified as Pseudomonas fluorescens and Trichoderma viride. The cell viability was observed qualitatively for Pseudomonas fluorescens and Trichoderma viride in whey liquid and molasses. The optimum temperature and pH was checked for both P. fluorescens and T. viride. The gradual growth rate was observed for both P. fluorescens and T. viride in whey liquid and molasses media. The different combinations of liquid media using whey liquid (W) and molasses (M) - (W : M (ml) were prepared and inoculated by PGPR to find maximum growth rate of P. fluorescens and T. viride as individual and co-culture. Finally the highest growth results were observed in 6:4, 10:0 (whey liquid) and 5:5 combinations (for pending patent, Patent App. No 201741023234) of whey liquid and molasses for P. fluorescens, T. viride and Co-culture of P. fluorescens and T. viride respectively. From this part of the research work, the present findings suggest that the whey liquid can be used as a new liquid carrier material for the production of biofertilizers.

Key words: 1. Cell viability, 2.Formulations, 3.Molasses, 4.P. fluorescens, 5.T. viride, 6.Whey liquid.

Introduction

Bio-fertilizers are the biological decomposition of organic materials; also known as the cultures of microorganisms like bacteria, fungi and blue green algae, which directly give nutrition to crop plants; packed with a carrier material. These organisms are added to the rhizosphere of the plant to enhance their activity in the soil to get high yield. Hence, it is necessary to look for alternative disease management practices, which include the use of eco-friendly biological control agents (BCAs) and pathogen-resistant crop cultivars. The term "Biofertilizer" or more appropriately a "Microbial inoculants" can generally be defined as preparation containing live or latent cells of efficient strains of Nitrogen fixing, Phosphate solublising or cellulolytic microorganisms used for application to seeds, soil or composting areas with the objective of increasing the number of such microorganisms and accelerate those microbial process which augment the availability of nutrients that can be easily assimilated by plants. Biofertilizer can provide an economically viable support to small and marginal farmers for realizing the ultimate goal of increasing productivity. Biofertilizers are low cost, effective and renewable source of plant nutrients instead of chemical supplements.

Fluorescent Pseudomonads are considered to be the most promising group of plant growth-promoting rhizobacteria involved in biocontrol of plant diseases (Moeinzadeh et al., 2010). They produce secondary metabolites such as antibiotics, phytohormones (Keel et al., 1992), volatile

compound Hydrogen cyanide (HCN) and siderophores. Plant growth-promoting ability of these bacteria is mainly because of the production of indole-3-acetic acid (Patten and Glick 2002), siderophores and antibiotics.

Trichoderma spp. has evolved numerous mechanisms that are involved in attacking other fungi. These mechanisms include competition for space and nutrients (Elad et al., 1999), mycoparasitism, production of inhibitory compounds (Sivasithamparam and Ghisalberti, 1998), inactivation of the pathogen's enzymes (Roco and Perez, 2001) and induced resistance. Today, more than 50 different Trichoderma-based agriculture products can be found as registered in many countries; are sold and applied to protect plants and soil and for improving yield of vegetables, ornamentals and fruit trees. Trichoderma is completely safe and in 55 years of research there has never been a recorded adverse reaction on humans and livestock (Anonymous, 2005).

Among different BCAs, Pseudomonas spp and Trichoderma spp have proved effective and selective enough against most of the various fungal and bacterial diseases. The various currently used biological approaches, use of microbial inoculants at the seedling stage could prove as a promising approach. Several symbionts like arbuscularmyc-orrhizal (AM) fungi, Trichodermaspp, a known biocontrol agent, and phosphorus solubilising bacteria (PSB) like Pseudomonas fluorescens can be implemented for green gram cultivation. There are several reports of Trichoderma and Pseudomonas mediated growth promotion and development of seedlings of several vegetable crops, namely tomato, green gram, lettuce, cabbage, and chilly with selective growth factors like molasses and whey protein.

Since the late 1950s, drip irrigation technology has been developed in many parts of the world to meet. One of the methods of drip irrigation technology involves applying water directly to the root zone of plants and keeping the soil wet. However, it has been observed that while applying the solid carrier based biofertilizer through the pipeline of dripper, the water flow gets disturbed and the dripper holes get blocked. Further, the carrier material (eg., Talcum powder) within the biofertilizers is not fully dissolved by water.

There is another issue about the disposal of whey water remains the greatest waste problems for dairy industries, because of acidic properties and high biological oxygen demand (BOD). The one third of whey protein production is directly utilized in human foods and animal feeds. The remaining two thirds of the whey is disposed of as truck transfer, landfill by pipeline and most of the whey waste is still being transported directly into streams and sewer systems. This causes for environmental pollution of natural water resources (O'Leary et al., 1977a). But the areas with vegetation and abundant rainfall some fertilization benefits can be obtained (Jones et al., 1993). However, the whey contains high levels of nitrogen, phosphorus and minerals can kill vegetation, so the spread has to be managed carefully (Zall, 1979).

The whey products are usually coming from sick cows. Because to extend the cow's milking cycle, the recombinant bovine growth hormone (rBGH) has been giving to the cows and the engineered hormone Prolisac is injected into commercial cows that causes for mastitis. In research trials, the milk producers have begun adding newspaper, cardboard, sawdust, industrial sewage and oils into feed for fatten animals and more quickly cattle feed programs and to reduce costs. US Department of Agriculture has said that cement dust may become an attractive feed supplement in future, because of producing 30% faster weight gain of cattle than on its regular feed. But presently whey is available as whey protein powder in market. Though, it has health benefits such as losing weight, anti-cancer properties, high cholesterol, asthma, anti-thyroid, lowering blood pressure, reducing risk of cardiovascular disease, the consumption of severely high doses can cause side

effects like stomach pains, cramps, reduced appetite, nausea, headache, fatigue, kidney stones and heart failure etc.

To overcome the existing problems, whey water can be used as a liquid carrier material for the production of biofertilizers, because it has rich source of lactose, nitrogenous substances and vitamin for the growth of certain bacteria and fungi. Instead of using respective media for culturing microorganisms in the production of biofertilizers, we can use solid waste, molasses and whey water as food supplements for the growth of microorganisms, because it has high ratio of carbon and nitrogen sources. Moreover, this will reduce many management problems in many industrial wastes.

The best way to reduce substrate cost for microbiology at present is to use wastes with the right balance of carbohydrates and lipids to support optimum bacterial growth and which are either free or carry a cost credit for environmental benefit. As is known, millions tons of hazardous and non-hazardous wastes are generated each year throughout the world. There is a great need for better management of these wastes via the concept: reduce, reuse, and recycle. So far, several renewable substrates include various agricultural and industrial by-products and waste materials have been intensively studied for microorganism cultivation at a laboratory scale, for example: olive oil mill effluent, waste frying oil, oil refinery wastes, soap stock, molasses, whey, starch wastes, cassava flour processing effluent and distillery waste (Makkar and Cameotra, 2002). All these problems made to find out new liquid carrier materials.

Here we have chosen whey as substrate for microbial growth and which is obtained as byproduct of cheese production process after the separation fat and casein from milk. The whey contains high value of chemical oxygen demand (COD) in the range of 50000 – 70000 g/l. Therefore, the disposal of whey is a major pollution problem. Though we have liquid biofertilizers in market, they are manufactured using selective broth media which is high cost and nowadays commercially molasses is used as a liquid carrier material for the production of biofertilizers. So, in this study molasses has also used to compare with whey liquid and find the liquid carrier material which has the high growth rate of PGPR organisms. Molasses or black treacle is a viscous by-product of the refining of sugarcane or sugar beets into sugar. Apart from improving nutritional status and growth of various vegetable crops, there are several reports of their use as biocontrol agent against a wide range of soil borne fungal pathogens of crucifers (El-Mohamedy, 2012). The present study was conducted to find out the new liquid carrier material using whey liquid which is the waste product of dairy industries and causes for environmental pollution for the production of liquid biofertilizer.

Materials and methods

Soil sampling procedure

Forty Rhizosphere soil samples were collected from the rhizosphere of different crops in various locations of Gobichettipalayam, Erode. Soil samples from soil surface (0-5 cm) and at a depth of approximately 20 cm (around the plant roots) were collected in fresh container and finally theses samples (Rhizosphere and subsoil samples) were mixed together to obtain the final sample (composite sample). The composite samples were sieved (mesh size < 4 mm) and stored at 4°C for no more than one week until the isolation of PGPR (Plant Growth Promoting Rhizobacteria**)** organisms.

Identification of Plant Growth Promoting Rhizosphere (PGPR) microorganisms

Two techniques, i) Micro-morphological in slide culture techniques and biochemical studies ii) visual observation on culture plates were adopted for identification of Pseudomonas fluorescens and Trichoderma viride.

Isolation of Pseudomonas and Trichoderma sp.

Isolation of Pseudomonas sp. were performed by making serial dilution of taken samples and the dilution used for studies were 10^{-4} to 10^{-7}. The King's B media was weighed out and prepared according to the manufacture's specification, with respect to the given instructions and directions by using spread plate technique. The plates were incubated at 30°C for 48 hrs. Each individual colony was selected based on the colour of colonies from the culture plates and sub-cultured. Distinct morphological characteristics were observed for identification (Battu Prasanna Reddy et al., 2009).

For isolation of Trichodermasp, a serial dilution technique was followed and 10^{-2} to 10^{-5}dilutions of each sample was prepared. 0.1 ml of each solution was pipetted onto a Potato Dextrose Agar (PDA) plate and incubated at 28°C for 4 days. The culture plates were examined daily and each colony that appeared was considered to be one colony forming unit (CFU). After enumeration of cfu, individual colonies were sub-cultured and from the same plates each uncommon colony was re-isolated and sub-cultured onto a fresh Potato Dextrose Agar (PDA) plate. Distinct morphological characteristics were observed for identification (Ahsanur Rahman et al., 2011).

Screening of Pseudomonas and Trichoderma sp.

Among these strains, to isolate stable strains of Pseudomonas fluorescence and Trichoderma viride were screened qualitatively based on physical and nutritional parameters (pH, temperature, glucose and NaCl) with good growth on selective media for further analysis as described by Płaza et al., 2006. The screening of bacterial and fungal isolates were done by estimating the growth of organisms at various pH ranges such as 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0; the temperatures were 4°C, 15°C, 20°C, 25°C, 28°C, 30°C, 35°C, 37°C, 40°C and 45°C and for carbon and nitrogen sources the various ranges were 0.5, 1.0, 1.5 and 2.0 % of glucose and Nacl done individually. The experiments were carried out using King's B and PDA medium for P. fluorescens and T. viride respectively.

Identification of P. fluorescens and T. viride

The pure cultures of bacterial isolates were subjected to micro-morphological such as Gram's stain and motility; biochemical characterization tests such as Indole production, Methyl red, Vogesproskauer, Citrate utilization, Hydrogen sulfide production, Catalase, Oxidase, Nitrate reduction and Gelatin liquefaction to identity Pseudomonas fluorescens with reference to Bergey's Manual of Determinative Bacteriology. For visual observation, the isolates were grown on King's B agar for 2 days. After incubation the color of colony (greenish fluorescence), colony morphology and changes of medium color for each isolate were examined every day.

For micro-morphological studies, a slide culture technique was used; fungal isolates were identified by morphological structures observed by lacto phenol cotton blue staining under 100X lens. For visual observation, the fungal isolates were grown on PDA agar for 4 days. The mode of mycelia growth, colour, odour and changes of medium colour for each isolates were examined every day.

Ultra violet light observation

After two days of incubation period of Pseudomonas isolate plate which had developed greenish yellow fluorescent pigment which is visible to us was undergone ultra violet light observation (wave length = 320 nm) to observe the color difference in fluorescence.

Phosphate solubilization

The Pikovskya's agar plates were inoculated with isolated Pseudomonas culture and incubated at 30°C for 2 days. After incubation the plates were examined and results were noted.

Compatibility Test

A sterile nutrient agar plates were prepared. Matt inoculation was done in a half of the plate by Pseudomonas fluorescens and other half was Trichoderma viride in aseptic condition and control plates were maintained. The plates were incubated at room temperature for two to three days. Then the results were observed for compatibility.

Collection of whey water

Whey liquid has been chosen as cultivating media for the selected PGPR microorganisms. The bulk amount of whey liquid (waste product from dairy industries) was collected from Milky Mist Dairy Industry Pvt. Lmt., located in Chithode, Erode.

Cell viability in whey liquid and molasses

In a two sets of conical flasks, a set of flasks (two flasks) containing 100 ml of sterile whey liquid were inoculated with P. fluorescens and T. viride respectively and another set of flasks containing 100 ml of sterile molasses were also inoculated with the same PGPR organisms. The bacterial and fungal inoculated flasks were then incubated at 30 and 28°C respectively to observe greenish yellow color in bacterial inoculated flasks and greenish mold powdery appearance in fungal flasks. During incubation the viability was checked qualitatively at different time intervals.

Gradual growth rate

To find out the gradual growth rate, P. fluorescens and T. viride were inoculated in different ranges of diluted whey liquid and molasses. The two sets (each sets contain 5 tubes) of tubes containing 2ml, 4ml, 6ml, 8ml and 10ml of whey liquid and two sets of tubes containing 2ml, 4ml, 6ml, 8ml and 10ml of molasses were made up to 10ml with distilled water. The set of diluted whey liquid and molasses tubes were inoculated by P. fluorescens and other set of diluted whey liquid and molasses tubes were inoculated by T. viride and then incubated at 28 and 30°C for 2 and 4 days respectively. Following incubation, the absorbance of organisms was checked by Spectrophotometer at 600 nm.

Optimization of whey liquid

The tubes containing whey liquid were optimized for the good growth of isolated P. fluorescens and T. viride at various pH levels such as 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0; at various temperature levels such as 4°C, 15°C, 20°C, 25°C, 28°C, 30°C, 35°C, 37°C, 40°C and 45°C; at various levels of carbon and nitrogen (glucose and sodium chloride) sources at a concentrations of 0.5, 1.0, 1.5 and 2.0 %. The un-inoculated whey liquid tubes were used as controls. Finally the growth of organisms was checked by spectrophotometer at 600 nm.

Growth rate in selective media, whey liquid and molasses

To compare the growth rate of PGPR microorganisms in selective broth media (King's B, Potato dextrose), whey liquid and molasses were inoculated by P. fluorescens and T. viride.

The PGPR isolate of P. fluorescens inoculated in 10ml of sterilized King's B, whey liquid and molasses medium. After inoculation the growth rate of bacterial isolate was monitored by spectrophotometer at 600nm. In meanwhile to compare the growth rate of PGPR isolate of T. viride inoculated in 10ml of sterilized Potato dextrose broth, whey liquid and molasses medium. Following

incubation, the absorbance of organisms was checked by Spectrophotometer at 600 nm. Un-inoculated king's B, PD broth, whey liquid and molasses containing tubes were maintained as control. Again two set of tubes containing selective broth (King's B / PD broth), liquid whey and molasses, the inoculation was repeated two times in order to reduce error and the mean values were calculated.

Growth rate in different combinations of liquid whey and molasses (as individual and co-culture)

Three sets of different combinations of whey liquid (W) and molasses (M) (W : M (ml) - 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0) tubes were inoculated with P. fluorescens, T. viride and Co-culture of P. fluorescens and T. viride respectively. After incubation, the growth rate of PGPR organisms was checked by spectrophotometer at 600nm. Again two sets of tube- inoculation were repeated to reduce error and the mean values were calculated. Un-inoculated tubes were maintained as control.

Results and Discussion

Isolation, Screening and Identification of Plant Growth Promoting Rhizosphere (PGPR) microorganisms

From serially diluted rhizosphere soil samples, 112 positive resulted Pseudomonas strains and 58 Trichoderma strains were isolated based on colony morphology (on King's B agar and PDA media) and cell morphology (Table 1). Among these strains, five bacterial and two fungal isolates were screened based on physical and nutritional parameters such as pH, temperature, glucose and NaCl on selective media and identified by morphological and biochemical characteristics.

Table 1: The percentage of Pseudomonas and Trichoderma strains in collected rhizosphere soil samples

Source	Number of samples	Selected colonies	Positive resulted colonies	Percentage (Presence of PGPR organisms)
Rhizosphere soil	130	120	112	86.15
		60	58	44.61
Total	130	180	170	65.38

Identification of Pseudomonas fluorescens

Pseudomonas spp are effective root colonizers and biocontrol agents, by production of antibiotics and other antifungal metabolites including antibiotics, hydrogen cyanide and siderophores (Table 2) (O'Sullivan and O'Gara, 1992).

Table 2: Morphological and Biochemical characterization for Pseudomonas fluorescens

Characteristics	Tests performed	Results
Colony and cell morphology	Colony Morphology on King's B	Greenish yellow colony, smooth edges, convex surface
	Fluorescence on King's B	+
	Gram's Staining	Straight rod-shaped Gram negative bacterium
	Motility	Motile
Biochemical characteristics	Indole production	-
	Methyl Red	+
	VogesProskaeur	+
	Citrate Utilization	-
	H_2S Production	+
	Catalase	+
	Oxidase	+
	Nitrate Reduction	-
	Gelatin Liquefaction	+
Organism Tentatively Identified as Pseudomonas fluorescence		

Ultra violet light observation

For the speciation of Pseudomonas fluorescens, the isolate was undergone ultraviolet light. The P. fluorescence had ability to develop a greenish yellow fluorescent pigment which is visible to us and this Fig. 1 shows that there was no color difference in fluorescence under ultraviolet light.

The fluorescence occurs because pyoverdine (or pyoverdin) are fluorescent siderophores produced by Pseudomonas fluorescens and absorbs the UV light and re-radiates light back to our eyes. This light has a greenish-yellow glow to it. A siderophores is a compound secreted by bacteria which binds with iron in the environment. This could be taken as an indicator for the presence of Pseudomonas fluorescens. Normally, this glowing will be visible from 1 to 3 days and 2 to 8 days after the commencement of the clouded growth. The production of pyoverdine was investigated in King B medium (King et al., 1954) prepared in liquid form, and in synthetic media (KClO-25%, NaClO.25%, K_2HP0, 0.1 %, MgSO, 0.05%, mannitolO.25% and amino acid 0.5%) with various amino acids as the only source of nitrogen.

Phosphate solubilization

Pseudomonas isolate was successfully conformed that it can solubilize phosphate by forming hallow in the center of the plate (Fig. 2).

Phosphorous is an important plant nutrient next to nitrogen and is referred as ''Master key element'' to crop production. Its vital function is nucleus formation, cell division, fat and albumin formation, cell organization and stimulation of root growth. Soil microorganisms are involved in many activities in the soil such as solubilization of bound phosphates, mineralization of organic phosphate compound by which organic phosphorous is converted to inorganic forms and the immobilization of phosphorous by which inorganic ions are incorporated into the microbial cell material. The insoluble forms of P such as tricalcium phosphate (Ca_3PO_4)2, aluminium phosphate (Al_3PO_4), iron phosphate (Fe_3PO_4), etc. may be converted to soluble P by P-solubilizing organisms inhabiting different soil ecosystems (Gupta et al., 2007; Song et al., 2008; Khan et al., 2013; Sharma et al., 2013).

Identification of Trichoderma viride

Trichoderma spp. are effective in control of soil/seedborne fungal diseases in several crop plants (Table 3) (Kubicek et al., 2001), including groundnut (Podile and Kishore, 2002). Major mechanisms involved in the biocontrol activity of Trichoderma spp. were competition for space and nutrients, production of diffusible and/or volatile antibiotics, and hydrolytic enzymes like chitinase and β-1,3-glucanase. These hydrolytic enzymes partially degrade the pathogen cell wall and leads to its parasitization (Kubicek et al., 2001).

Total microbial pathogens in contaminated soil were more than that in non- contaminated soil. This result could be regarded as destabilization of the soil ecological balance arising from contamination. Environmental stresses brought by the contamination could be adduced for the reduction in microbial population and diversity. In our investigation, the most dominant genera of bacteria isolated from soil were P. fluorescence and T. viride. This conclusion could attribute to the extensive use of domestic fertilizers in the soil irrigated with canal water (Randy et al., 2009).

Compatibility test

In dual culture method, there was no zone of clearance was observed between Pseudomonas fluorescens and Trichoderma viride isolates (Fg. 3).

Application of P. fluorescens and T. viride in combination with chitin induced accumulation of phenols and activities of PR proteins in coconut palm as compared to single-microbe-treated and control plants (Karthikeyan et al., 2006).

Cell viability in whey liquid and molasses combinations

After two days, in whey liquid and molasses the growth of P. fluorescence was observed as greenish yellow coloue and powdery appearance on bottom of the conical flask respectively (Fig. 4 & 5). The bacterial cell growth and viability was checked at different time intervals by qualitatively.

After four days of incubation, in whey liquid and molasses the growth of T. viride was observed for greenish mold powdery appearance in the conical flasks. The fungal cell growth and viability was checked at different time intervals by qualitatively.

Gradual growth rate

The growth rate of P. fluorescens and T. viride was gradually increased in whey liquid and molasses tubes. It shows that diluted whey liquid and molasses did not increase the growth rate of bacterial and fungal cells than undiluted whey liquid and molasses.

Optimization of whey liquid

The optimum temperature was observed as 30 and 28°C and the pH was observed at 6.5 and 6 for both P. fluorescens and T. viride respectively in whey liquid (Table 4). Normally the maximum

growth rate of fungi is occurred in low pH. But according to the T. viride in whey liquid, not much difference was observed between the growth rate in low pH-3.5 and pH at 6. Among the carbon and nitrogen supplements (glucose and NaCl), the most suitable concentration for P. fluorescence was 0.5% of glucose which showed the greenish fluorescent color and maximum growth rate of bacterial cells in whey liquid medium than compared with other concentrations. In T. viride (glucose and NaCl) has been attained the most abundant sporulation without add any nutrient supplements in whey liquid.

pH is one of the environmental factors with the greatest relevance to the growth of microorganisms. As a general principal bacterial growth decreases at more acidic pH values so that in our research the pH of the medium was adjusted to 6.5 & 6 to avoid negative effects on growth of PGPR organisms. Microbes prevail in certain ranges of pH that favor the nutrition, reproduction and survival. pH is responsible for a higher degree mortality, especially when the change is sudden. Temperature is a critical parameter that has to be controlled and it varies from organism to organism. Temperature influences secretion of extra cellular enzymes by changing the physical properties of the cell membrane. Carbon sources have major roles in enzymatic activity of microorganisms (Costa et al., 2001).

The Graph 2 shows, after the incubation period (2 and 4 days) of PGPR isolates, the highest growth rate of Pseudomonas fluorescens and Trichoderma viride were observed on King's B and Potato dextrose broth respectively at the absorbance of 600 nm to compare the growth rate with whey liquid, molasses and different combinations of whey liquid and molasses.

Growth rate in whey liquid and molasses

Graph 3 shows that in whey medium, the growth rate of Pseudomonas fluorescens and Trichoderma viride was higher than on King's B, PD broth and molasses. But the growth rate of T. viride cannot be conformed that its growth rate is higher than the growth rate of P. fluorescens, because of its different kingdom. The lowest growth rate was observed in molasses medium. So this confirms whey liquid can be used as a liquid carrier material for the production of biofertilizers. But to find out good liquid carrier material than whey liquid, P. fluorescens and T. viride were inoculated in different combinations of whey liquid and molasses.

Growth rate in different combinations of whey liquid and molasses (as individual)

The Graph 4 shows that among all the eleven different combinations, 6:4 combination of whey liquid and molasses showed the highest growth rate of Pseudomonas fluorescens. This confirms the liquid carrier material prepared with 6:4 (in percentage) combination of whey liquid and molasses is one of the good support medium for the growth of Pseudomonas fluorescens than whey liquid and 10:0 combination of whey liquid and molasses showed the highest growth rate of T. viride. This shows that the whey liquid supports the maximum growth of Trichoderma viride than the liquid carrier materials prepared by using whey liquid and molasses.

Growth rate in different combinations of whey liquid and molasses (as co-culture)

Though P. fluorescens and T. viride are in different kingdom, due to the mutual relationship between them, they were inoculated as co-culture in different combinations of liquid whey and molasses. As co-culture inoculated in different combinations of whey liquid and molasses, the highest growth rate was observed (Graph 5) in 10:0 combinations on fourth day. But due to the different incubation period of P. fluorescens and T. viride and slow growth rate of organisms in molasses which cannot be conformed that there will be an equal growth though there is a mutual relationship between them. So the incubation period was increased up to a particular period of time to confirm

the highest growth rate of co-culture. Finally 5:5 combinations showed the best result of Co-culture on 16th day. This confirms 5:5 combination of whey liquid and molasses act as a good support liquid carrier material for the growth of co-culture of P. fluorescens and T. viride than other liquid carrier materials.

Conclusion

Bioconversion of dairy industrial waste (whey water) into useful product (used as feed for PGPR organisms) being a source of new liquid carrier material for the production of liquid biofertilizer as well as reduce environmental pollution. Moreover, this will reduce many management problems of processing industrial waste. By this research work, the isolated PGPR organisms were also confirmed as P. fluorescens and T. viride based on morphological, biochemical and cultural characteristics. The ultimate aim of this work was done by conforming the proportions of 6:4 (W:M) to enable optimal growth of Pseudomonas fluorescens, the only whey liquid carrier material (proportions of 1:0 - W:M) for optimal growth of Trichoderma viride and finally the combination having equal proportions (5:5 - W:M) shows the best result of Co-culture of P. fluorescens and T. viride. Therefore, the proportions of whey liquid and molasses act as a good support liquid carrier material and organic media for the growth of P. fluorescens and T. viride.

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