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RESEARCH ARTICLE

ISOLATION, CHARACTERIZATION & DEVELOPMENT OF LIQUID FORMULATIONS OF POTASSIUM SOLUBILIZING FUNGI.

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Abstract

90% of Potassium in the soil exists in the form of insoluble rocks and silicate minerals and the concentrations of soluble Potassium in the soil is very low but it is one of the major essential macronutrient required for the growth and development of plant. Few microorganisms have ability to dissolve Potassium from insoluble K-bearing minerals. The aim of the present investigation was to isolate, characterize and develop potential Potassium solubilizing fungi from Sugarcane rhizosphere from different sites in & around Nagpur region (Maharashtra). The isolates were screened on Aleksandrov medium containing mica powder on the basis of zone of solublisation. Nine isolates showed zone of Potassium solublisation. Quantitative estimation of Potassium solubilization by these isolates was found in the range of 34.2 µg/mL to 51.2 µg/mL. Morphological and molecular characterization of KSF 05 indicated it to be *Aspergillus niger* .Furthr Liquid formulations were made and survival of KSB 05 was seen uptil 180 days of incubation, advocating its use as potash biofertilizer.

Introduction:-

The microbial flora of soil has been destroyed by indiscriminate use of synthetic fertilizers for nourishment of plant and have become the main culprit for the contamination of water and soil, hence using biofertilizers is an eco-friendly approach for nutrient nourishment to the plants (Pettigrew,2008). Presently used biofertilizers are solid based biofertilizers which have drawbacks such as low shelf life, less cell count and reduced efficiency *(Shanware et al.,2014).* Hence there is need to develop biofertilizers with liquid carriers having longer shelf and higher efficiency. Potassium (K), the third primary macronutrient required by plants plays vital role in numerous biochemical and physiological processes in plants like stomatal regulation ,activation of enzymes, transportation of water and nutrients, improving shelf life of crops and providing disease resistance (Usherwood, 1985).

The soluble and insoluble K form in Indian soils is in range of 2% and 98% respectively.
Few microorganisms have ability to solubilize the Potassium and make it available to the plants. In order to achieve optimum plant growth, there is need to exploit more and more microorganisms that have ability to solubilize Potassium. Potassium solubilizing microorganisms solubilize Potassium by producing microbial organic acids and

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lowering pH of soil .The organisms with Potassium solubilizing ability not only increase the availability of soluble potash but also enhance plant growth by production of plant growth promoting regulators (Vessey, 2003*).* Many microorganisms have ability to grow on muscovite, biotite, orthoclase microclase and mica *in vitro which are minerals of Potassium [6]*.like *Paenibacillus glucanolyticus, Bacillus mucilaginosus, Aspergillus niger, Aspergillus terreus, Burkholderia* sp., *Pseudomonas* sp., *Bacillus megaterium are known* to posses Potassium solublising ability (*Shanware et al.,2014)*

Solubilization of this soil mineral, by fungi and bacteria are well established, which reflects their use as competent biofertilizers (Prajapati, 2013) .Present investigation aimed to isolate, characterize and develop liquid formulation of Potassium solubilizing fungi from rhizosphere soil of *Saccharum officinarum* (Sugarcane) in and around Nagpur, (Maharashtra).

Materials and methods:-

Collection of Sample:-

The rhizospheric soil samples were collected from depth of 15 cm from the *Saccharum officinarum* (Sugarcane) plant from different sites of adjoining areas of Nagpur (Maharashtra). Three samples were collected from each site and pooled together to make the composite sample. The serial dilutions of the soil samples were made and plated on Aleksandrov medium plates. The plates were incubated at 28±2°C for 72-96 h, pH - 6.5. The colonies forming halo zone of clearance on Aleksandrov medium around them were counted as Potassium solubilizers (Sugumaran and Janarthanam, 2007).

Screening of Potential Potassium Solubilizing Fungi (KSF):-

Colonies that showed clear zone around them on Aleksandrov medium were selected as Potassium solubilizers. Further screening of isolates was done by finding Khandeparkar's selection ratio (Archana *et al,* 2013).The fungal isolates were grown on 100 ml Aleksandrov broth, incubated at at 28±2°C on 120 rpm for 7 days by using mica as insoluble source of Potassium. The Potassium released was determined by Rajawat, *et.al.* (2014) using Sodium cobaltinitrite. The amount of Potassium solubilised by the isolates was calculated from the standard curve prepared by using various concentrations of 10 ppm KCl .The pH value of the broth was also measured after each 24 hours.

Characterization of Fungi:-

Screened fungal strains were grown on Potato Dextrose Agar (PDA) medium and their colony characteristics were studied, the cell morphologies of the isolates was studied under compound microscope using lactophenol cotton blue as stain. Molecular characterization of the potential fungi was done with help of 18S rRNA sequence analysis The fungi were identified by extracting the DNA from the pure culture. The rDNA was amplified by using universal primers. The crude sequence was aligned with help of tools available in the National Center for Biotechnology Information (NCBI) database (White, 1990).

Formulation of Liquid bioinoculant:-

For the formulation of liquid bioinoculant, the fungal culture was grown in Potato Dextrose broth amended with an additive Glycerol in different concentrations (1%, 2%, 5%). The survival rate of the fungal spores was seen periodically.

Results and Discussion:-

Isolation & Screening of Potassium solubilizing fungi:-

09 fungal colonies were obtained which showed clear zone around their colonies on Aleksandrovs agar (Fig.1). Among them, KSF 05 showed highest Potassium solubilization The amount of Potassium released in the Aleksandrovs broth by each of the isolates was quantitatively measured using Sodium cobalt nitrite method.

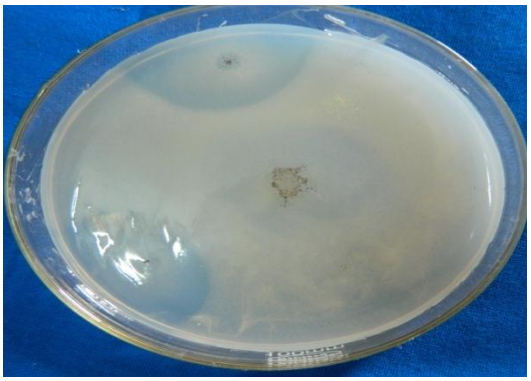

Fig.1:- Different fungal colonies showing zone of solubilization obtained on Aleksandrovs medium.

In quantitative estimation, Potassium solubilization was found between 34.2 µg/mL to 51.2 µg/mL (Table1). Measurement of the pH was also carried out every day. After a week, pH was decreased from 6.5 to 4.1 indicating pH decreases with increases with amount Potassium solubilization (Fig.2).

Table 1:- Potassium solubilization values of fungal isolates by Khandeparkar's selection ratio.

Isolate Code	Diameter of Zone(D) in mm	Diameter of Colony (d) in mm	D/d ratio	Quantitative K Solubilization (in µg/mL)
KSF 01	10	08	1.25	37.3
KSF 02	09	08	1.12	34.2
KSF 03	13	10	1.3	36.5
KSF 04	10	07	1.42	43.2
KSF 05	15	06	2.5	51.12
KSF 06	12	09	1.33	39.4
KSF 07	08	05	1.6	41.2
KSF 08	13	09	1.44	42.4
KSF 09	09	05	1.8	46.7

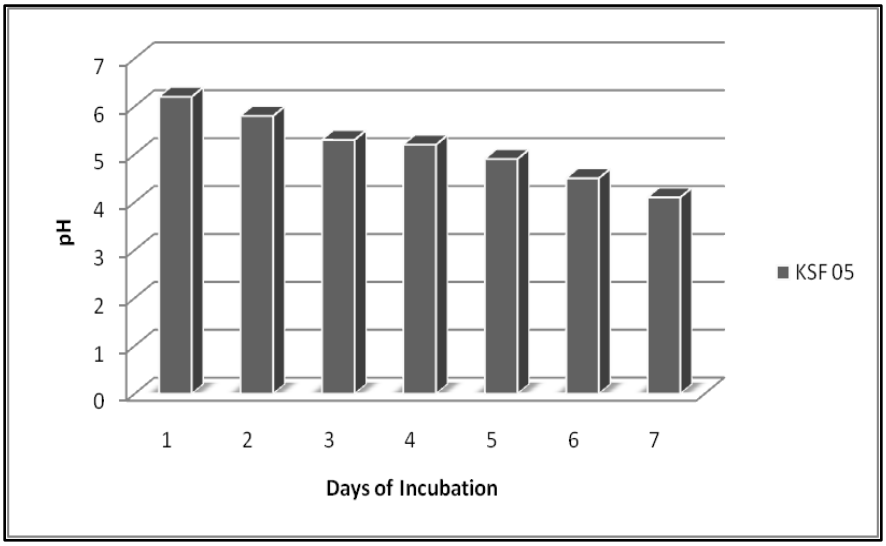

Fig 2:- pH of KSF 05 in Aleksandrov Broth during the 7 days of incubation.

Morphology & Molecular Characterization of KSF 05:-

Fungal strain KSF 05 showed black – brown colonies on Potato Dextrose agar. Conidiophores were short smooth walled .Conidia were globose, brown, dark with rough walls. Further genomic DNA was extracted from the pure culture which was 621bp long. Based on 18s rRNA sequence analysis, fungus was found 99% similar to *Aspergillus*

niger. The phylogenetic tree of KSF 05 was constructed with the help of MEGA software *(Fig.3). These* sequences were submitted in GenBank of National Centre for Biotechnology Information (NCBI) entitled as **"*Aspergillus niger strain SAM6*" and** the accession number obtained was KR007968. Similar results were obtained by Prajapati, *et al (* 2013).

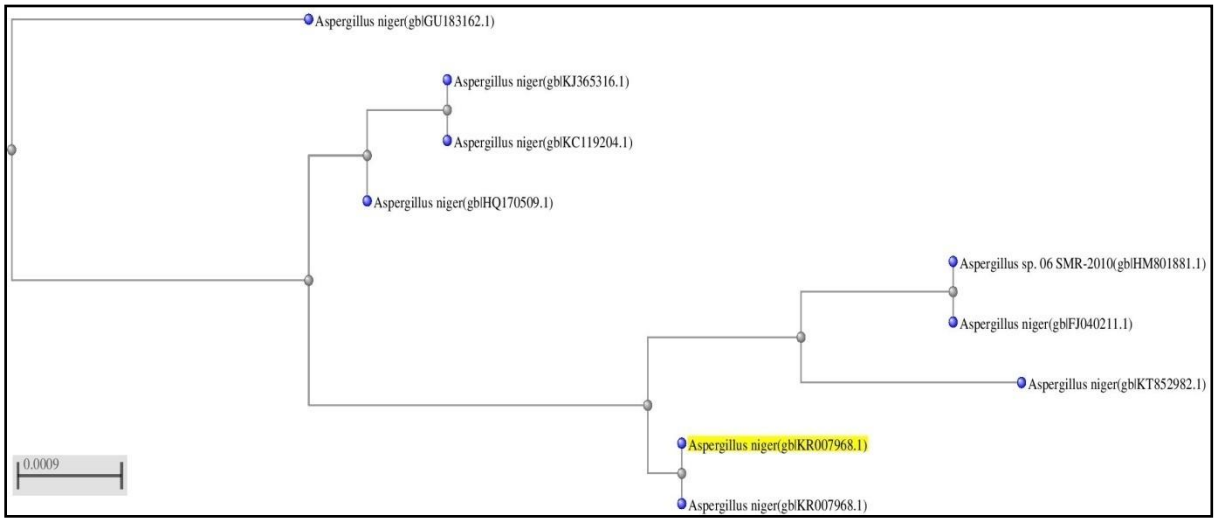

Fig 3:- The phylogenetic tree of KSF 05 (*A.niger SAM 6)* isolated from Sugarcane rhizosphere

Liquid bioinoculant of A.niger:-

Liquid formulations of *A.niger* was prepared by growing the fungus in Potato dextrose broth amended with different concentrations of glycerol. The survival of the isolate was seen uptil 180 days after incubation. The cells amended with additive survived in better way compared to the control. The best formulation found was Potato dextrose broth amended with 5% glycerol till complete 180 days (Table 2) .The reason for this survival is glycerol being an osmoprotectant retains the moisture content of the cell which is essential for the biochemical activities of cell. Similar study on liquid formulation was conducted by Kalavati & Modi, (2014) but this study is of its own kind as it includes addition of additives along with the liquid carrier.

Table 2:- Survival of KSF 05 *(A.niger)* in Potato Dextrose broth amended with additive Glycerol.

Different concentrations of Glycerol	Glycerol (1%)	Glycerol (2%)	Glycerol (5%)	Control (Without additive)
Fungal Count (PFU/mL) on 60^{th} day of incubation	$4.5 X10^{8}$	$7.6 X10^{9}$	$5.6 X10^{9}$	$5.6 X10^{7}$
Fungal Count (PFU/mL) on 120^{th} day of incubation	$3.6 X10^{8}$	$4.3 X10^{9}$	$5.7 X10^{9}$	$4.5 X10^{7}$
Fungal Count (PFU/mL) on 180^{th} day of incubation	$5.86 X10^{7}$	$12.34 X10^{8}$	$15.78X10^{8}$	$4.56X10^{6}$

Further, the efficacy of this fungal isolate can be checked on plants for its growth promoting effects. This can be a competent strain for production of fungal liquid bioinoculants which are very rare as compared to the bacterial liquid bioinoculants.

Conclusion:-

In the present study nine isolates showed zone of Potassium solubilization that used mica as source. KSF 09 was found to be most efficient strain which solubilized insoluble Potassium. The morphological & molecular identification indicated it to be *Aspergillus niger* .It was deposited in the NCBI and an accession number was obtained. An attempt was made to formulate liquid bioinoculant of this potential fungus. Such type of study is necessary as it promotes the use of Potassium solubilizing fungi as potash biofertilizer and is an efficient approach to substitute chemical fertilizers. Further there is necessity for field demonstration studies of these isolates along with standardization of the dose so as to achieve optimum growth and also that the technology can be easily

implemented by farmers for multiplication at their own level. Hence these liquid bioinoculants can be the means towards sustainable agriculture development.

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