

**EFFICACY OF RHIZOSPHERE PHOSPHATE
SOLUBILIZERS IN SOME WEEDS OCCURING IN
SORGHUM AND COTTON CROPS**

THESIS

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Submitted to

**Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
in partial fulfilment of the requirements
for the Degree of**

**MASTER OF SCIENCE
IN
AGRICULTURE
(PLANT PATHOLOGY)**

By

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DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the Thesis entitled **"EFFICACY OF RHIZOSPHERE PHOSPHATE SOLUBILIZERS IN SOME WEEDS OCCURING IN SORGHUM AND COTTON CROPS"** or part thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

Place: Akola

Date 27th 2013



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Enrolment No. JJ/2195

CERTIFICATE

This is to certify that thesis entitled "**EFFICACY OF RHIZOSPHERE PHOSPHATE SOLUBILIZERS IN SOME WEEDS OCCURING IN SORGHUM AND COTTON CROP**" submitted in partial fulfilment of the requirements for the degree of "**Master of Science in Agricultural (Plant Pathology)**" of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by **Sadgir Madan Dada** under my guidance and supervision.

The subject of the thesis has been approved by the Student's Advisory Committee.

Place: Akola
Date: 29/05/2013

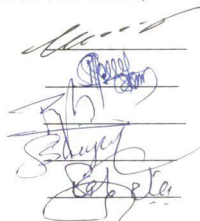

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List of Abbreviations

%	–	Per cent
/	–	Per
⁰ C	–	Degree celcius
C.D	–	Critical difference
cm	–	Centimetre
AICRP Project	–	All India Coordinated Research Project
e.g.	–	Exempli gratia (For example)
<i>et al.</i>	–	Et alia (and others)
etc.	–	Etcetra
Fig.	–	Figure
g	–	Gram
h	–	Hours
HCl	–	Hydrochloric acid
i.e.	–	That is
J.	–	Journal
ml	–	milliliter
mm	–	millimeter
μ	–	Micron
μg	–	Microgram
mg	–	milligram
l	–	Liter
-1	–	per
μl	–	micro liter
PDA	–	Potato dextrose agar
ppm	–	Part per million
PSB	–	Phosphate Solubilizing Bacteria
PSF	–	Phosphate Solubilizing Fungi
PSM	–	Phosphate Solubilizing Microorganism
SE (m) ±	–	Standard error of mean
Sig.	–	Significant
spp.	–	Species
viz.,	–	Videlicet (Namely)
–ve	–	Negative

(F)

THESIS ABSTRACT

- a) Title of thesis : "EFFICACY OF RHIZOSPHERE PHOSPHATE SOLUBILIZERS IN SOME WEEDS OCCURING IN SORGHUM AND COTTON CROPS"
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ABSTRACT

In vitro studies were conducted to find out most efficient Phosphate solubilizers. The 23 fungal and 9 bacterial Phosphate solubilizing microorganisms were isolated from rhizospheric soil of different weeds occurring in sorghum and cotton crops by serial dilution method. Most efficient 'P' solubilizers were identified on the basis of halo zone formation on Pikovskaya's agar medium, reduction in pH, organic acid production, and P_2O_5 solubilized in broth culture. The

result indicated that among fungi *Aspergillus niger*-20 and *Aspergillus niger*-5 and among all bacterial isolates Dr. PDKV strain of PSB and PSB-3 were produce maximum halo zone (5.33 to 4.66 mm) and they solubilized more tricalcium phosphate i.e. 26.24 to 18 P_2O_5 μ g/ml with reduction in pH (3.1 to 3.30) with increasing in titrable acidity i.e. 3.60 to 3.0. PSB-3, PSB- 4, PSB-6 and PSB-8 were produced Indole Acetic Acid (IAA).

Compatibility of Phosphate solubilizing fungi with agrochemicals were tested *in vitro*. Both the isolates of *Aspergillus niger* and *Aspergillus flavus* were not compatible at recommended dose of different agrochemicals viz., thiram (0.3%), mancozeb (0.25%) and propiconazole (0.05%) and quizalfop-ethyl (0.2%). However only *Aspergillus flavus* was compatible with recommended dose of carbendazim. Also *Aspergillus niger* not compatible with carbendazim (0.1%) and fenoxaprop-ethyl (0.2%). *Aspergillus niger* isolates were compatible with COC (0.25%) + streptocycline (100 ppm), streptocycline (100 ppm), glyphosate (0.6%) and insecticides viz., cypermethrin (0.03%), emamectin benzoate (0.04%) and imidacloprid (0.3%). *Aspergillus flavus* was compatible with COC (0.25%) + streptocycline (100 ppm), streptocycline (100 ppm), glyphosate (0.6%) and insecticides viz., cypermethrin (0.03%), emamectin benzoate (0.04%) and imidacloprid (0.3%).

Compatibility of PSB with agrochemicals, PSB-1 and PSB-8 isolates were tolerant to thiram (0.15, 0.3 and 0.45%) doses, PSB-1, PSB-5 and PSB-9 tolerant to mancozeb (0.12, 0.25 and 0.38%) doses remaining PSB isolates were sensitive to all doses whereas all the PSB isolates were compatible with carbendazim (0.05, 0.1, and 0.15%) and propiconazole (0.025, 0.05 and 0.075%) doses. All the PSB isolates were sensitive to COC + streptocycline and streptocycline (50,100 and 150 ppm). The PSB-3 and PSB-5 tolerant to glyphosate at (0.3, 0.6 and 0.9%) doses, remaining isolates were sensitive to glyphosate (0.3, 0.6 and 0.9%), however PSB-4 and PSB-6 were

tolerant to quizalofop-ethyl at different doses (0.1, 0.2 and 0.3%), while remaining isolates showed sensitive to all doses of quizalofop-ethyl, whereas all the PSB isolates were compatible with fenoxaprop-ethyl (0.1, 0.2 and 0.3%). All the isolates of PSB were tolerant to tested insecticides at different concentrations, they did not show negative effect on growth of PSB isolates i.e. all PSB isolates were compatible with cypermethrin (0.010, 0.03 and 0.045%) emamectin benzoate (0.02, 0.04 and 0.06%) and imidacloprid (0.1, 0.3 and 0.45%).

CHAPTER I

INTRODUCTION

1.1 Background Information

Phosphorus is an important plant nutrient along with major nitrogen and potassium plant nutrient elements. It is associated with several vital functions and is responsible for several characteristics in plant growth such as utilization of sugar and starch, photosynthesis, nucleus formation and cell division, fat and albumin formation, cell organization and the transfer of heredity. It is also essential in seed formation and early maturity of crop particularly in cereals. It plays important role as higher energy phosphate bonds (ATP, ADP) in respiratory and photosynthetic process. Phosphorus is constituent of nucleic acid, phytin, phospholipids. Phosphorus stimulates root growth also reported by some workers. Phosphorus is essential in seed formation and present in large quantities of plant, seed and fruit (Gaur 1990).

Phosphorus uptake by plants $1/3$ to $1/4^{\text{th}}$ that of potash. About 50 % of cultivable land in India is deficit in available form of soil phosphorus and only 20-25% of applied phosphatic fertilizers are utilized by crop plants, remaining being fixed or rendered unavailable to crop by forming insoluble complexes with metal cations and clay mineral complexes (Yadav and Dadarwal, 1997). Phosphorus is a major growth limiting nutrient, and unlike case for nitrogen, there is no large atmospheric source that can be made biologically available (Ezawa *et al.*, 2002). Root development, stalk development, flower and seed formation, crop maturity, crop production, crop quality, stress tolerance and resistance to plant diseases are the attributes associated with phosphorus nutrition. (Gyaneshwar *et al.*, 2002).

Mostly the phosphatic fertilizers are applied through the inorganic fertilizers like single super phosphate, diammonium phosphate, rock phosphate etc. However, a large proportion of the available phosphorus present in these chemical fertilizers is converted in to insoluble form soon after its application. In acidic soils the phosphate ions are fixed with Fe and Al hydroxides whereas, under alkaline conditions its reacts with calcium and sodium forming insoluble tricalcium phosphate $\text{Ca}_3(\text{PO}_4)_2$ 'P'

availability mainly depends on soil pH, soluble Fe, Al, Mn and Ca ions, organic matter content and activities of soil microorganisms. Most available ionic form of phosphorus is PO_4 , moderately available as HPO_4^{-2} and least as HPO_4^{-3} . Generally plant take up their phosphorus as the primary orthophosphate $\text{H}_2\text{PO}_4^{-2}$. The secondary, orthophosphate $\text{H}_2\text{PO}_4^{-2}$ is also believed to be absorbed by plant roots in small quantities (Gaur, 1990 and Subbarao, 1982).

1.2 Importance of study

The present agricultural scenario signifies the importance of integrated nutrient management. Biofertilizers may be an effective alternative for nutrient management instead of conventionally used chemicals fertilizers.

Seed inoculation with 'P' solubilizing bacteria may help in establishment of bacterial microorganisms in the rhizosphere in the immediate vicinity of growing roots (Rokade and Patil, 1992).

In wheat, increase in yield, root, shoot weight, plant height, spike length, grain yield, seed P content, leaf protein and leaf sugar content was observed with application of Phosphate solubilizing bacteria alone and in combination with Rhizobium. The Rhizobium and PSB gave 29% higher yield (Afzal and Bano, 2008). In case of sorghum, application of PSB increased in earhead weight, grain yield, shoot length, root length, P uptake 72.7 g, 69.19 g, 212.5 cm, 32.33 cm and 379.32 mg plant⁻¹ respectively (Appanna, 2007). In case of rice, application of PSB alone and in combination with rock phosphate showed increase in grain yield and straw yield 22.3% and 29.7% q ha⁻¹ respectively (Mohod *et al.*, 1991).

In soybean, application of PSB alone and in combination with FYM + crop residues resulted increase in yield (11.24 q ha⁻¹) and protein (451.98 kg ha⁻¹) (Deshmukh *et al.*, 2005). Significantly increase in yield of paddy was observed due to *Bacillus firmus* which is responsible for solubilization of rock phosphate and production of phytohormones (Datta *et al.*, 1982). Hence, the PSM has been proved as potential source for phosphorus required by plant and need to be exploited for sustainable production.

1.3 Objectives of the study

Now a days, regular use of PSM in nutrient management of organic crop production has become more attractive due to added benefits of enhanced plant growth. Such enhancement and phosphate solubilization has been observed with PSM. Thus in view of phosphate solubilizing ability of PSM.

The present investigation was carried out with the following objective

1. To assess the phosphate solubilizing activity of different fungal and bacterial isolates.

1.4 Hypothesis

Increase in cost of fertilizers and worldwide energy crises, low purchasing power of farmers, increase in cost of production restricted the use of chemical fertilizers alone as a source of plant nutrient. Under such condition it has become alternative to use all available resources of plant nutrients including microorganisms like PSM for sustainable soil fertility and productivity. These PSM have ability to increase stress tolerant capacity in plant and induce disease resistance against soil borne pathogens. 'P' solubilizing capacity of PSM varies with soil and soil condition. Therefore, use of efficient isolates is necessary. Hence, here the attempts were made to isolates efficient 'P' solubilizing microorganisms from rhizosphere of various weeds occur in sorghum and cotton and studies were undertaken to test the efficiency of different PSM isolates in laboratory.

1.5 Scope and Limitations

Many soil microorganisms can solubilize unavailable forms of organic or inorganic bond. (Curl, 1986). The phosphate solubilizing microorganisms include Bacteria (*Bacillus megaterium*, *B. cereulans*, *B. subtilis*, *Pseudomonas fluorescens*, *P. striata*), and fungi (*Aspergillus niger*, *A. awamori*, *A. flavus*, *Penicillium digitatum* and *Fusarium* sp.) are more effective for solubilization of inorganic form of phosphorus (Gaur, 1990).

These microorganisms are involved in many activities in soil as solubilization, mineralization and immobilization of phosphate. The phosphate solubilizing microorganism have the ability to bring insoluble form of phosphates in to soluble form by secreting organic acids such as formic, acetic, propionic, fumaric and succinic acid. (Subbarao, 1993).

Phosphate solubilizing microorganism having potential to increase the plant growth by increasing availability of trace elements such as iron, zinc, etc and by production of growth promoting regulators (Ponmurugan and Gopi, 2006).

Production of food and forage in semiarid and arid region of the world can be increased by application of phosphate solubilizing microorganisms. Moreover phosphate solubilizing microorganisms may also show plant growth promoting activities such as indole acetic acid (IAA), gibberellic acid, cytokinins, ethylene production, hydrogen cyanide (HCN) production and resistance to soil borne pathogens (Cattelan *et al.*, 1999).

The rhizospheric beneficial microorganism greatly affected by various environmental (pH and salt and temperature) stresses. (Banerjee *et al.*, 2010).

CHAPTER II

REVIEW OF LITERATURE

Studies regarding the "Efficacy of rhizosphere phosphate solubilizers in some weeds occurring in sorghum and cotton crops. Emphasis given to isolation of PSM isolated from rhizosphere region of some weeds occurring in sorghum and cotton crops of different places. All the isolates of PSM tested for efficiency of phosphate solubilization in Pikovskaya's agar and broth medium and compatibility of these PSM with different agrochemical were subsequently studied *in vitro*.

Attempt were made to put forth recently published work reviewed critically under the following major heads

2.1 Isolation of phosphate solubilizing microorganism

2.2 'P' solubilization by different 'P' solubilizing microorganism

2.3 Indole Acetic acid production by P' solubilizing bacterial isolates

2.4 Compatibility of PSM with fungicides, herbicides, antibiotics and insecticides.

2.1 Isolation of phosphate solubilizing microorganism

Pikovskaya's (1948) suggested a medium containing tricalcium phosphate with glucose, yeast extract and other salts for growth and Isolation of phosphorus solubilizing microorganism. Microorganisms gave clear zones around their colonies due to dissolution of phosphate.

Sattar and Gaur (1987) isolated 24 bacteria and 2 fungi from the rhizosphere soil of Bangladesh and tested for the solubilization of rock phosphate in liquid medium. Fungi were more effective than bacteria and almost all cultures produced acidity in the medium.

Shingte *et al.* (1987) screened 21 fungal and 13 bacterial isolates from green gram, soybean and Lucerne rhizosphere for their phosphate solubilizing activity and observed that fungi were as better phosphate solubilizers than bacterial isolates.

Darmwal *et al.* (1989) isolated ten fungal and three bacterial cultures from different samples of rhizosphere of wheat, gram, garden soils and compost material.

Varsha *et al.* (1994) isolated twenty seven *Aspergillus* spp., seven *Penicillium* spp., and one *Rhizopus* spp. from the rhizosphere of twenty four crop plants, from compost and from garden soil and reported that these isolates solubilized tricalcium phosphate in Pikovskaya's broth medium.

Singh and Kapoor (1994) reported twenty seven isolates of bacteria capable of producing clear halo zone of solubilization on Pikovskaya's medium which were obtained from rhizosphere and non rhizospheric soils of various crop in Hissar.

Nahas *et al.* (1996) reported that the fungal isolates were more active than bacteria in solubilizing insoluble phosphate. Further, the fungi presented more acid phosphatase activity than the bacteria and conversely the bacteria produced more alkaline phosphatase activity than the fungi.

Bhattacharya *et al.* (1997) evaluated native phosphate solubilizing microorganism from vidarbha soils and revealed that bacteria were predominant phosphate solubilizing microorganism in all vidarbha soils followed by fungi.

Chen *et al.* (2002) isolated the PSM from rhizosphere soil of nineteen weeds in citrus orchard at China and suggested that weed conservation could be beneficial to soil microbe's development in agro-ecosystem, especially in the initial stage of agro-ecosystem development because there is less organic carbon in bare soil. He also suggested that weed conservation could increase phosphate solubilizing activity of phosphate solubilizing microorganism.

Bhamare (2006) isolated the PSB from pulse, cereals, cotton, oilseed crops and reported that PSB solubilized tricalcium phosphate in Pikovskaya's agar medium by forming halo zone around their colonies also state that these isolates solubilized tricalcium phosphate 9 to 59 % in Pikovskaya's broth medium.

Ponmurugan and Gopi (2006) isolated the PSB from groundnut and showed that these isolates solubilized tricalcium phosphate with reducing pH (5.9 to 5.0) and increased in titrable acidity (2.4 to 3.9) due to organic acid production.

Balamurugan *et al.* (2010) isolated the PSB from rhizosphere soil of tea and reported that these isolates reduced pH from 3.8 to 4.62 of medium with increase in titrable acidity ranged from 3.9 to 7.9 ml.

Padmavathi and Seshachala (2010) Isolated PSM from rhizospheric soil of betel vine plants, and found that *Aspergillus* spp. shown 26.1 mm clear zone, *Bacillus subtilis* produced 46.6 mm clear zone on Pikovskaya's medium.

Humaira and Bano (2011) isolated and characterized phosphate solubilizing bacteria from rhizosphere soil of weeds of Khewra salt range of Attock on the basis of clear zone formation around their colony.

Sanjotha *et al.* (2011) they isolated PSM from saline soil samples and reported that the fungi viz., *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. have the more solubilizing ability of inorganic insoluble phosphate than bacteria.

Sonam *et al.* (2011) isolated the PSM and reported that a clear halo zones was evident surrounding the colony on the Pikovskaya's agar medium.

2.2 Phosphate solubilization by different Phosphate solubilizing microorganism

Arora and Gaur (1979) reported that all the microorganisms tested on Pikovaskaya's solid and liquid medium, including species of *Pseudomonas*, *Bacillus*, *Aspergillus* and *Penicillium* effectively solubilized tricalcium phosphate and hydrooxyaptite. Rock phosphate was more resistant to solubilization.

Wani *et al.* (1979) tested phosphate solubilizing microorganisms for their ability to solubilized tricalcium phosphate and rock phosphate as source of insoluble 'P' carrier amounting to 50 mg of phosphorus. They found that tricalcium phosphate was easily and efficiently solubilized by all organisms as compared to rock phosphate and there was no correlation between the degree of phosphate solubilization and final acidity produced by microorganisms.

Illmer and Schinner (1995) showed that *Penicillium aurantiogriseum* and *Pseudomonas* spp. (P18/89) have high abilities in

solubilizing inorganic phosphate also showed that the 'P' concentration in solution did not increase according to a sigmoid curve type.

Bilollikar *et al.* (1996) isolated phosphate solubilizing bacteria from rhizospheric soil of sorghum and reported that efficiency of phosphate solubilizing bacteria (*Bacillus* sp. and *Pseudomonas* sp.) ranged from 10 to 12 mg phosphate solubilized per ml in Pikovskaya's broth.

Alam *et al.* (2002) isolated ten most efficient phosphate solubilizing bacteria and three fungi (PSM) from maize and analyzed solubilization index, pH change, phosphate solubilized, 'P' immobilized and organic acids produced under *in vitro* conditions and found that 'P' solubilization index of these isolates ranged from 1.63 to 3.29 with decreasing in pH of the medium ranged from 7 to 3.2 with continuous growth of isolates for seven days.

Lal (2002) reported that reduction in pH increase titrable acidity this might be due to secretion of organic acids by phosphate solubilizing microorganism.

Nautiyal *et al.* (2002) isolated the four PSB strain from alkaline soil and reported that NBRI2601 strain was most efficient for phosphate solubilization in the presence of 10 % salt and pH 12.

Patil (2002) isolated the PSM from different cereal, pulses and oil seed, fruit crops and concluded that *Aspergillus flavus* among fungi produce maximum clear zone (7.8 mm), *Aspergillus niger* showed 4.6 mm clear zone and *Penicillium* spp. form 2 mm halo zone on Pikovskaya's agar medium also reported that these isolates solubilized tricalcium phosphate (17.4 to 144.2 mg/ 100ml) in Pikovskaya's broth medium.

Rashid *et al.* (2004) showed among fungal culture *Aspergillus niger* solubilized maximum (0.45%) 'P' in broth culture also showed that solubilizing index of 13 isolates of PSM ranged from 2.13 to 6.23 and reported that PSM produce organic acid.

Chen *et al.* (2006) characterized thirty six strains of phosphate solubilizing bacteria (PSB) from Central Taiwan and reported that these bacteria solubilized tricalcium phosphate up to 31.5 to 519.7 mg/l with lowering the pH.

Gupta *et al.* (2007) reported that the 12 fungi and 19 bacteria PSM and found that these isolates solubilized tricalcium phosphate (TCP) on solid and liquid Pikovskaya's medium. *Penicillium* sp.-21 solubilized and released 81.48 mg ml⁻¹ whereas *Penicillium* sp.-2 showed better efficiency of rock phosphate solubilization and produced 4.87 mg ml⁻¹ into the liquid medium, bacterial isolates exhibited 16.0 to 18.5 mm halo zones.

Appanna (2007) reported that the beneficial effect of 16 isolates of phosphate solubilizing bacteria able to promote growth and yield parameter of sorghum under greenhouse condition.

Khan *et al.* (2009) observed that soil phosphorus precipitated as orthophosphate and absorbed by Fe and Al oxides is likely to become bio available by bacteria through their organic acid production and acid phosphatase secretion. Phosphorus solubilizing bacteria mainly *Bacillus*, *Pseudomonas* and *Enterobacter* are effective for solubilizing in organic phosphorus.

Oliveira *et al.* (2009) reported several phosphate solubilizing microorganism from maize rhizosphere soil. The strains of bacteria, fungi and most of the actinobacteria were confirmed as PSM with high 'P' solubilization activity. Among the bacteria isolates, B17 and B5, identified as *Bacillus* sp. (B17) and *Burkholderia* sp. (B5) respectively, were the most efficient 'P' solubilizing strains from P-Ca source culture solution solubilizing 67% and 58.5% of the total 'P' respectively. The B5 isolate also had the highest reduction in pH in the growth solution to 4.46.

Banerjee *et al.* (2010) observed that activity of phosphate solubilizing microorganism is greatly affected by environmental stresses such as salt stress, pH, water, temperature stress.

Chakraborty *et al.* (2010) isolated 90 fungal isolates predominantly spp. of *Aspergillus* spp. and *Penicillium* spp. and reported that these isolates solubilized tricalcium phosphate ranged 799 to 856 mg l⁻¹ in Pikovskaya's broth culture.

Kukreja *et al.* (2010) isolated nine *Pseudomonas* spp. from rhizosphere and seven from graveyard soil, out of which eleven showed phosphate solubilization activity. Isolates PSP IV, V, VI, VIII, IX, X and XI

showed maximum phosphate solubilization while isolates PSP V, VI, VIII, IX and X were also found to produce IAA ranging from 1.06 to 4.12 µg/ml.

Manivannan *et al.* (2011) reported six different Phosphate solubilizing microorganisms (PSM) (*Pseudomonas* spp. *Bacillus* spp. *Penicillium* sp. and 3 strains of *Aspergillus* spp.) from the rhizosphere soils of chilli and tomato plants. He found that *Penicillium* sp. solubilized tricalcium phosphate 1045 µg/ml and *Pseudomonas* sp. 985 µg/ml after 14th days of incubation in Pikovskaya's broth medium also reported the pH of all cultures was decreased due to organic acid production.

Sharma *et al.* (2011) concluded that phosphate solubilizing fungi *Aspergillus awamori* gave highest dry matter and ear emergence of the wheat crop, also concluded that inoculation of *Aspergillus awamori* and *A. niger* significantly increased the uptake of Nitrogen, Phosphorus, and Potash in wheat in all stage of crop growth.

Shruti and Pathak (2011) conducted an experiment assess the effect of inoculation of *Pseudomonas fluorescens* on wheat (*Triticum aestivum*) in Doon valley by conventional method. Treatments involved *P. fluorescens* inoculation with phosphorus fertilizer and result that application of phosphate solubilizing bacteria in combination with half dose (50%) of superphosphate significantly increased the yield (36%) as well as phosphorus status in soil and plant.

Humaira and Bano (2011) studied on rhizospheric soil of four halophytic weeds for isolating phosphate solubilizing bacteria and concluded that, these isolates were able to solubilized tricalcium phosphate in broth culture.

Qureshi *et al.* (2012) isolated the PSB and conducted field trial of PSB with chemical fertilizer and revealed that bacterial inoculums produced significantly higher seed cotton yield 1630 as compared to 1511 kg/ ha. also reported that solubilizing efficiency and solubilizing index of bacterial isolates ranged 233 to 275 and 3.3 to 3.8 respectively.

Sharma *et al.* (2012) reported that several naturally occurring PSB isolates from tea rhizosphere of Darjeeling hills are capable of producing plant growth promoting substance IAA ranged from 10 to 30 mg/l

organic acids and capable of solubilizing inorganic phosphate ranged from 40.62 ± 1.1 to 136.73 ± 1.7 mg/l thereby decreasing the pH of the medium.

2.3 Indole Acetic Acid production by different phosphate solubilizing bacterial isolates

Sattar and Gaur (1987) tested eight phosphate dissolving bacterial and fungal isolates such as *Bacillus polymaxa*, *Bacillus pulvifaciens*, *Pseudomonas striata*, *Aspergillus awamori*, *A. niger*, *Penicillium digitatum* for synthesis of auxin and gibberellins and reported that PSB produce auxin and gibberellins.

Afzal *et al.* (2008) reported that *Rhizobium leguminosarum* and *Pseudomonas* spp. (54RB) synthesized IAA and GA phytohormones.

Hayat *et al.* (2010) studied soil beneficial microorganism and reported that PSB produce plant hormones such as auxin, cytokinins, gibberellins ethylene and abscisic acid.

Manivannan (2011) isolated the PSB and showed that bacteria produce IAA and organic acid.

Sanjani Samuel *et al.* (2011) reported that PSB (*Bacillus* spp., *Pseudomonas* spp.) were positive reaction to IAA production.

Sagervanshi *et al.* (2012) studied five strain of PSB and reported that two isolates were gram negative, round shape also reported that two isolates positive reaction to IAA production.

2.4 Compatibility of PSM with agrochemicals Fungicides, herbicides, antibiotics and insecticides.

Dey and Dutta (1981) concluded that application of four phenoxy herbicides-two pre-emergence tok E-40 (nitrofen-2,4-dichlorophenoxy-1, 2-nitrophenyl ether) and modown (5-methyl (2,4-dichlorophenoxy)-2-nitrobenzoate) and two post-emergence, fernoxone-sodium salt of 2,4-dichlorophenoxy acetic acid (2,4-D) and weeder-96-amine derivative of 2,4-D as spray solutions at their recommended doses exerted a significantly detrimental effect on phosphate solubilizing bacteria.

Moubasher *et al.* (1981) concluded that 2,4-D (2,4-dichlorophenoxyacetic acid) had a stimulating effect on the total count of *Aspergillus* sp., *A. niger*, *A. fumigatus* and *Fusarium* spp. at the low dose (6.3 ppm), but it was toxic to high doses (25.2 and 50.4 ppm). VCS-438[2-

(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] reduced the growth of *Aspergillus* spp. (*A. niger* and *A. fumigatus*) by the three doses (6.8, 27.2 and 54.8 ppm).

Helmecci *et al.* (1988) studied the effect of 14 herbicides on *Fusarium* sp., *Rhizopus* sp., *Aspergillus* sp. and concluded that higher doses of the herbicides prevented the growth of these fungus. Tiocarbamate derivatives Alirox B 80% EC, Anelda Plus 80% EC and Anelda III found most inhibitory effect on these fungus.

Rache and Coats (1988) reported that numbers of soil microorganisms *Aspergillus* sp. *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp., *Rhizopus* sp., *Helminthosporium* sp. *Pseudomonas* sp. were stimulated the growth through the utilization of chlorinated hydrocarbon, organophosphate and synthetic pyrethroid insecticides.

Jayaswal *et al.* (1990) isolated both antagonistic and nonantagonistic strains of *Pseudomonas* spp. and reported that RJ3 strain sensitive to tetracycline, neomycin, chloramphenicol, ampicillin, gentamicin, kanamycin, erythromycin, novobiocin, streptomycin and penicillin.

Oliveira *et al.* (1995) reported that the effects of Kanamycin and streptomycin added to soil on the survival of transposon Tn5 modified *Pseudomonas fluorescens* strain R2f was investigated.

Heydari *et al.* (1997) reported that pre and post emergence herbicide i.e. pendamethilin, promatryn and trifluralin were significantly reduced the growth of *Pseudomonas fluorescens* and *Burkholderia cepacia*.

Sands and Rovira (1997) studied 186 *Pseudomonas fluorescens* from 15 different soil and rhizosphere of wheat growing area and tested against 8 antibiotics and found that all isolates were resistant to chloromphenicol, erythromycin, cloxacillin, penicillin and ampicillin and sensitive to streptomycin and tetracycline.

Das and Mukharjee (1998) studied the tolerance of insecticides and reported that hexachlorocyclohexane (phorate) stimulated the population of bacteria, actinomycetes, fungi, N₂ fixing bacteria while fenvalerate stimulated the population of PSB. phorate, carbofuran, fenvalerate stimulated the proportion of *Penicillium* sp. in soil.

Omar (1998) reported that *Aspergillus niger*, *A. tamari* and *A. terreus* were not affected by organophosphosphate insecticide i.e. melathion, dursban and cyolan.

Das and Mukharjee (2000) reported that the most predominant genera of microorganisms, such as *Bacillus*, *Micrococcus* and *Aspergillus* were not affected by most of the insecticides viz., HCH, phorate, carbofuran and fenvalerate at their field application rates (7.5, 1.5, 1.0 and 0.35 kg a.i. ha⁻¹ respectively).

Debnath *et al.* (2002) reported that butachlor and basalin were shows stimulating effect on phosphate solubilizing microorganism.

Das *et al.* (2003) conducted that two herbicides viz., oxadiazon [5-terbutyl-3-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol 2-one] and oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenyl)-4 (trifluoromethyl) benzene] at rates of 0.4 and 0.12 kg a.i. ha⁻¹ respectively, stimulated the population and activities of phosphate solubilizing microorganisms and also the availability of phosphorus in the rhizosphere soil.

Mathew (2003) showed that *Pseudomonas fluorescens* was compatible with all the nine pesticides used. Amongst them imidacloprid gave best compatibility.

Shahin and Malik (2003) studied the tolerance of 64 bacterial isolates (40 *P. fluorescens*, 12 *Azotobacter*, 12 *Rhizobium* spp.) to pesticides and antibiotic. 12.5 % of the *Pseudomonas* isolates from soil tolerated concentration of 1600 µg/ ml. All the isolate further tested for their antibiotic susceptibility against seven different antibiotics viz., nalidixic acid, cloxacillin, chloromphenicol, tetracycline, amoxycillin, methicillin, doxycyline, 100% of the *Pseudomonas fluorescens* isolates were resistance to cloxacillin and 57.5% were resistant to methicillin. 7.5% of the isolates exhibited multiple resistances to five different antibiotics in three different combinations whereas 25% of the isolate showed multiple resistant to four different antibiotics in seven different combinations.

Sayed (2004) reported that fluconazole inhibit the complete growth of *Aspergillus niger* and *Penicillium* spp. at 0.01%, 0.02% and 0.03% concentration.

Fathia (2004) reported that phosphate solubilizing bacteria i.e. *Agrobacterium* strain Ca-18 were sensitive to fungicide viz, mancozeb, darosal and tolerant to alert plus, benlate at 0.1%, 0.2%, 0.4% and 0.6% concentration.

Kishore *et al.* (2005) reported that the *Pseudomonas aeruginosa* GSE-18 and GSE-19 were tolerant to the recommended field application rate for chlorothalonil. Fungicides tolerance is not uncommon in *P. fluorescens*.

Fathia *et al.* (2006) reported that fungicides viz., Alert Plus, Darosal and Mancozeb have the inhibitory effect on Plant Growth Promoting Rhizobacteria (PGPR).

Sarnaik *et al.* (2006) reported that application of insecticide i.e. carbosulfan 25 DS had no adverse effect on the count of PSB.

Nongthombam *et al.* (2008) reported that application of systemic pre emergence herbicides, viz., antfos and pendimethalin alone and in combination at their recommended rates increased efficiency of phosphate solubilizing microorganism resulting in increased availability phosphorus in soil.

Ahemad and Khan (2010) reported that phosphate solubilizing activity of *Enterobacter asburiae* strain PS2 decreased with increase in fungicide concentration.

Ahemad and Khan (2010a) concluded that herbicides, viz., quizalafop-ethyl and clodinafop were inhibitory effect on phosphate solubilizing activity of *Enterobacter asburiae* strain PS2 at dose higher than recommended, while recommended dose had less inhibitory effect. He also stated that glyphosate found less toxic than quizalafop-ethyl and clodinafop.

Ahemad and Khan (2010b) isolated quizalafop ethyl and clodinafop tolerant PSB and PGPR *Pseudomonas aeruginosa* PS1 from the rhizospheric soil of mustard and reported that quizalafop-ethyl 120 ppb, clodinafop 400 ppb increased total chlorophyll content, leg hemoglobin, root N and P seed protein of green gram.

Balamurugan *et al.* (2010) tested compatibility of PSB with different agrochemical and reported that PSB had tolerated hexaconazole fungicide.

Johnson and Subramanyam (2010) evaluated that seed treating Fungicides i.e. tebuconazole and hexaconazole + captan were control the seed born and soil born diseases which cause by *Aspergillus niger* and *verticillium* sp.

Rathod *et al.* (2010) observed the effect of six fungicides on *Aspergillus flavus* by poison food technique and concluded that thiram, mancozeb, carbendazim were found to be more inhibiting as compare to copper oxychloride, captan and captafol.

Sen and Charaya (2010) reported that *Penicillium* sp. BG, *Penicillium* sp. DG, and *Aspergillus sulphureus*, *Aspergillus niger* and *Fusarium moniliforme* were tolerant to copper (copper sulphate) at high concentration i.e. 400 ppm.

Tanney and Hutchison (2010) studied the impact of glyphosate on fungal growth and reported that soil borne fungi significantly decreased the number of CFUs and exhibited colony diameters 50 % less than that of controls at concentration 50 µg ml⁻¹.

Ahemad and Khan (2011) monitored the growth of *Pseudomonas putida* strain PS9 recovered from the mustard rhizosphere under insecticidal stress by exposing to the graded concentration 0 to 3200 µg ml⁻¹ of each selected insecticides on C and N source deficient.

Ahemad and Khan (2011a) studied that rhizobacteria *pseudomonas putida* PS9 grow with the varying concentration of insecticides and showed varying degree of tolerance levels against the tested insecticides. The *Pseudomonas putida* strain PS9 might have utilized these insecticides as the energy source. The rhizobacterial strains used were tested for their tolerance against 12 pesticides (herbicides, insecticides, and fungicides). Generally the rhizobacterial strain tolerated all the pesticides.

Koche (2011) reported that the Incorporation of metalaxyl, Fosetyl-Al, COC and Bourdeux mixture in growth medium of *P. fluorescens* affects its growth. COC and B.M. was less compatible with all

Pseudomonas isolates may be due to the presence of antibacterial properties in both fungicides as compared to metalaxyl and Fosetyl-Al. Maximum growth of Pf₁₅ was observed in metalaxyl (0.2%) (35.33 cfu/ml). Whereas Pf₉ (37.33 cfu/ml) was most compatible to Fosetyl-Al followed by Pf₁₀ (35.00 cfu/ml).

Kumar et al. (2011) reported that *Bacillus subtilis* was good tolerance to hexaconazole, propiconazole, and validamycin, moderate tolerance to tricyclazole and poor tolerance to benomyl and mancozeb at 1000 ppm. Integral showed compatibility to carbendazim and azoxystrobin up to 400 ppm.

Vimal and Patel (2011) reported that pesticides viz., carbendazim, monocrotophos and cloripyriphos were increase the phosphate solubilizing activity of *Bacillus sphaericus* and *Burkholderia cepacia* at their recommended dose.

Wani and Taskeen (2011) studied the effect of systemic and non-systemic fungicides on *Aspergillus niger* and reported that amongst systemic fungicides, carbendazim brought about highest reduction followed by hexaconazole, bitertanol and myclobutanil, respectively. Among non-systemic fungicides, the mancozeb was found the most effective followed by captan and zineb, respectively. The higher concentrations (1000 ppm and 2000 ppm) of all the fungicides proved more effective than lower concentrations (125 ppm and 500 ppm).

Hefnawy et al. (2012) concluded that *Aspergillus niger* and *Aspergillus fumigatus* were not affected at lower concentrations of tested herbicides i.e. glyphosate and putraline up to 200 mg l⁻¹ and at 800 mg l⁻¹ phosphate solubilization by *Aspergillus niger* and *A. fumigates* decreased approximately 30% and 85% respectively.

CHAPTER III

MATERIAL AND METHODS

In present study attempts were made to isolate various phosphate solubilizing microorganisms from rhizosphere of some weeds occurring in sorghum and cotton crops. The soil samples were collected from Yeola taluka of Nashik district and Dr. PDKV. Research Farm Akola, for studying their 'P' solubilizing efficiency so as to get efficient phosphate solubilizing microorganisms.

3.1 Materials

Following materials were used for experimentation.

3.1.1 Glassware

The glassware used during the course of research work were of Borosil make. The glasswares viz., Petriplates, test tubes, conical flasks of 250 ml, 500 ml and 1000 ml, funnel, beakers, pipette, measuring cylinders, slides, cover slips and glass rods were used.

3.1.2 Equipments used

Standard laboratory equipments viz., autoclave, BOD incubator, laminar airflow, research microscope, stereoscopic binocular microscope, refrigerator, hot air oven, digital weighing balance, Bunsen burner, water distillation unit etc. were used.

3.1.3 Chemicals used

Chemicals used during the course of research work are thiram, mancozeb, carbendazim, propiconazole, copper oxychloride, streptocycline, glyphosate, quizalofop-ethyl, fenoxaprop-ethyle, cypermethrin, emamectin benzoate and imidacloprid etc.

3.1.4 Other materials used

Blotter paper, non absorbent cotton, muslin cloth, polythene bags, cork borer, inoculating needle, forceps, paper bags, potato, dextrose, Agar-Agar, pipettes etc. were used during research programme.

3.1.5 Collection of soil samples

Rhizosphere soil from different commonly occurring weeds of sorghum and cotton were collected from two different locations of

Maharashtra viz., Yeola Dist. Nashik and Central Research Station, Dr. PDKV. Akola. Soil samples were drawn from 5-25 cm depth of rhizosphere region, air drying in laboratory at room temperature and then used for isolation. The soil samples were collected in June- July 2012. Details of soil samples collected are given in Table 1.

3.2 Methods

3.2.1 Sterilization of glass wares, media and water

The glass materials viz., Petriplates, test tubes and conical flask "Borosil" make were used during study. All glasswares were sterilized in hot air oven at 180°C for one hour. The culture media and distilled water were sterilized in autoclave at 1.05 kg/cm² for 15 minutes.

3.2.1.2 Precaution to eliminate contamination

The isolation and inoculation work of microbial culture were carried out in laminar air flow. The laminar air flow was sterilized by glowing ultraviolet lamp ½ hrs. prior to commencement of work.

3.2.2 Isolation of 'P' solubilizing microorganism screening and maintenance

Preparation of media

Addition of 2% agar for solid medium, all the ingredients in g l⁻¹ for distilled water.

The standard Pikovskaya's medium (1948) as

Glucose	-	10.0
Ca ₃ (PO ₄) ₂	-	5.0
(NH ₄) ₂ SO ₄	-	0.5
NaCl	-	0.2
MgSO ₄ .7H ₂ O	-	0.1
KCl	-	0.2
Yeast extract	-	0.5
MnSO ₄	-	Trace
FeSO ₄ . 7H ₂ O	-	Trace
Distilled water	-	1000 ml

Table 1. Location from where of soil samples were collected from which PSM could be isolated

Sr no.	Common name / local Name	Botanical name	Family	Location Of Collection
A Sorghum				
1	Harali	<i>Cynadon dactylon</i>	<i>Grammineae</i>	Yeola
	Harali	<i>Cynadon dactylon</i>	<i>Grammineae</i>	Dr. PDKV. Sorghum Research Unit
2	Mothi Dudhani	<i>Euphorbia geniculata</i>	<i>Euphorbiaceae</i>	Yeola
	Mothi Dudhi	<i>Euphorbia geniculata</i>	<i>Euphorbiaceae</i>	Dr. PDKV. S. R. Unit
3	Tandulja	<i>Amaranthus polygamus</i>	<i>Amaranthaceae</i>	Yeola
	Tandulja	<i>Amaranthus polygamus</i>	<i>Amaranthaceae</i>	Dr. PDKV S. R. Unit
4	Hajardani	<i>Phyllanthus niruri</i>	<i>Euphorbiaceae</i>	Yeola
	Hajardani	<i>Phyllanthus niruri</i>	<i>Euphorbiaceae</i>	Dr. PDKV. S. R. Unit
5	Chandvel	<i>Convolvulus arvensis</i>	<i>Convolvulaceae</i>	Yeola
	Chandvel	<i>Convolvulus arvensis</i>	<i>Convolvulaceae</i>	Dr. PDKV. S. R. Unit
6	Piwala dhotra	<i>Argemonia mexicana</i>	<i>Papavaraceae</i>	Yeola
	Piwala dhotra	<i>Argemonia mexicana</i>	<i>Papavaraceae</i>	Dr. PDKV. S. R. Unit
7	Gajar gawat	<i>Parthenium hysterophorus</i>	<i>Asteraceae</i>	Yeola
	Gajar gawat	<i>Parthenium hysterophorus</i>	<i>Asteraceae</i>	Dr. PDKV. S. R. Unit
8	Aghada	<i>Achyranthus aspera</i>	<i>Amaranthaceae</i>	Yeola
	Aghada	<i>Achyranthus aspera</i>	<i>Amaranthaceae</i>	Dr. PDKV. S. R. Unit
B Cotton				
1	Harali	<i>Cynadon dactylon</i>	<i>Gramineae</i>	Yeola
	Harali	<i>Cynadon dactylon</i>	<i>Gramineae</i>	Dr. PDKV. Cotton Research Unit
2	Choti Dudhi	<i>Euphorbia thymifolia</i>	<i>Euphorbiaceae</i>	Yeola
	Choti Dudhi	<i>Euphorbia thymifolia</i>	<i>Euphorbiaceae</i>	Dr. PDKV. C. R. Unit
3	Gajar gawat	<i>Parthenium hysterophorus</i>	<i>Asteraceae</i>	Yeola
	Gajar gawat	<i>Parthenium hysterophorus</i>	<i>Asteraceae</i>	Dr. PDKV. C. R. Unit
4	Cock comb	<i>Celosia argentea</i>	<i>Amaranthaceae</i>	Yeola
	Cock comb	<i>Celosia argentea</i>	<i>Amaranthaceae</i>	Dr. PDKV. C. R. Unit
5	Hajardani	<i>Phyllanthus niruri</i>	<i>Euphorbiaceae</i>	Yeola
	Hajardani	<i>Phyllanthus niruri</i>	<i>Euphorbiaceae</i>	Dr. PDKV. C. R. Unit
6	Reshimkata	<i>Alternanthera triandra</i>	<i>Amaranthaceae</i>	Yeola
	Reshimkata	<i>Alternanthera triandra</i>	<i>Amaranthaceae</i>	Dr. PDKV. C. R. Unit
7	Deepmal	<i>Acalypha indica</i>	<i>Euphorbiaceae</i>	Yeola
	Deepmal	<i>Acalypha indica</i>	<i>Euphorbiaceae</i>	Dr. PDKV. C. R. Unit
8	Kunjaru	<i>Digera arvensis</i>	<i>Amaranthaceae</i>	Yeola
	Kunjaru	<i>Digera arvensis</i>	<i>Amaranthaceae</i>	Dr. PDKV C. R. Unit
9	Math	<i>Amaranthus viridis</i>	<i>Amaranthaceae</i>	Yeola
	Math	<i>Amaranthus viridis</i>	<i>Amaranthaceae</i>	Dr. PDKV. C. R. Unit

3.2.2.1 Isolation of Phosphate solubilizing microorganism (Sanjotha *et al.*, 2011)

Efforts have been made to isolate PSM from rhizosphere region of different weeds of sorghum and cotton following serial dilution method using 10^{-3} and 10^{-7} dilution on Pikovskaya's medium (Gaur, 1990). The soil samples collected were stored on air drying at room temperature and one gram soil sample used for isolation. The proper dilution was obtained by following procedure.

1. Distilled sterilized water was used for preparing nine ml capacity water blanks.
2. One gram soil sample was taken from composite sample in to first number water blank to make 1:10 (10^{-1}) dilution.
3. Placed the sterile soil carefully in the test tube to replaced the cotton plug and vigorously shaken on vortex mixture.
4. Made soil 1:100 (10^{-2}) dilution of the sample by adding 1 ml of the water specimen diluted 1:10 to 9 ml of sterilize water.
5. Replaced the cotton plug with a sterile cork and mixed in step (3).
6. Made a 1:100 (10^{-3}) dilution of the sample by adding 1 ml of the water specimen diluted 1:100 to 9 ml sterile water.
7. Replaced the cotton plug with a sterile cork and mixed in step (3).
8. The original sample had been diluted 10^{-7} using every time a fresh sterilized pipette.
9. 0.2 ml from each 10^{-3} for fungi and 10^{-6} for bacteria was taken by sterilized fresh pipette and transferred in sterilized Petri dishes aseptically in laminar air flow work station.
10. The sterilized Pikovskaya's agar media were melted in hot water bath and allowed to cool at 45°C and poured approximately 15 ml in Petri dishes which had been inoculated with 0.2 ml water specimen of 10^{-3} and 10^{-6} dilution separately. Plates were rotated with hands for even distribution for each dilution and allowed to set.
11. The plate were incubated at $28 \pm 2^{\circ}\text{C}$ for 48 to 72 hours in BOD incubator triplicate were made for each sample. The identical colonies of fungi and bacteria which shown clear halo zone were

isolated and transferred in Petri dishes aseptically containing Pikovskaya's medium.

These fungal and bacterial cultures were maintained on Pikovskaya's agar medium and further used for present study.

3.2.3 Solubilization of tricalcium phosphate on Pikovskaya's agar medium

Sterilized Pikovskaya's agar medium was poured in to sterilized Petri dishes after solidification of media a pin point inoculation of test fungal/ bacterial isolates was inoculated in centre of Petri dishes under aseptic condition. The plates were incubated at $28 \pm 2^\circ\text{C}$ for 7 days and observed for colony diameter, diameter of solubilization (clear zone) regularly during incubation period. Solubilization index was evaluated accordingly (Clear halo zone = Total zone – colony diameter) in mm. The incubation period for fungal isolates was 3, 5 and 7 days and 2, 3 and 4 days for bacterial isolates at $28 \pm 2^\circ\text{C}$.

3.2.4 Microbial solubilization of insoluble phosphate in Pikovskaya's broth (Gaur, 1990).

Phosphate solubilization potential of PSM was studied *in vitro* by estimating available phosphorus in Pikovskaya's broth medium with known amount of tricalcium phosphate ($0.5 \text{ g } 100 \text{ ml}^{-1}$) as a substrate before sterilization. Each of 5 mm mycelial bit of test fungal culture and 0.5 ml suspension of each bacterial culture was inoculated in 250 ml conical flask containing 100 ml sterilized Pikovskaya's broth medium and make triplicate. A control without any PSM was also maintained. The fungal and bacterial isolates were allowed to grow for seven and fourteen days at $28 \pm 2^\circ\text{C}$ in BOD incubator. For PSF broth was filtered through whatman filter paper no. 42 and clear solution was collected in 100 ml volumetric flasks and volume was made up to 100 ml with sterilized distilled water. For estimation of PSB broth culture centrifuged at 15,000 rpm for 30 min. in centrifuge. The supernatant was collected in 100 ml conical flask and volume made up to 100 ml with glass distilled water. Thus extract of each test fungal and bacterial solution was prepared then the available phosphorus in broth culture were determined (Gaur, 1990).

Preparation of reagent

A. Chloromolybdic acid

15.0 g of ammonium molybdate was dissolved in 400 ml of warm distilled water, 400 ml of 10 N HCl was added to it.

B. Chlorostannous acid

10.0 g of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ crystals was dissolved in 25 ml of concentrated HCl and stock solution was prepared.

C. Dilute solution

1 ml of stock solution was taken to which 132 ml of distilled water was added. Prepared fresh dilution for each set of experiment

D. Standard phosphorus solution 100 ppm 'P'

0.4390 g of dried KH_2PO_4 was dissolved in 400 ml distilled water to which 25 ml of 7 N H_2SO_4 was added and made up to 1 lit.

Procedure –

One ml of filtrate/ suspension added in a 50 ml volumetric flask to which 10 ml of chloromolybdic acid was added and with the help of distilled water volume was made up to 45 ml. 2-3 drops of chlorostannous acid was immediately added and the volume was made to 50 ml with distilled water. A blue colour was developed and the absorbance at 600 nm was recorded using systronic make Spectrophotometer.

Construction of standard graph:

The standard graph was constructed by using KH_2PO_4 as substrate. Standard solution of KH_2PO_4 (3, 6, 9, 12 and 15 ppm). The graph was plotted on graph paper with standard concentration of KH_2PO_4 on the X- axis and optical density on the Y- axis.

Sr. no.	PPM (X-axis)	Optical Density (Y-axis)
1	3	0.3
2	6	0.6
3	9	0.8
4	12	1.1
5	15	1.5

3.2.4.1 Measurement of pH

The filtrate taken for chemical analysis was used for measurement of pH, inorganic phosphate solubilization is due to production of organic acids by 'P' solubilizing fungi and bacteria which results in a change in pH of the medium. The pH of the filtrate after separating the fungal mycelium and bacterial cells was measured by pH meter.

3.2.4.2 Estimation of acid production by different phosphorus solubilizing fungal and bacteria isolates by titration method (Sharma *et al.*, 2012)

Pikovskaya's broth was prepared and distributed in 250 ml conical flask (100 ml each) and sterilized in autoclave. Flasks were inoculated with test organism separately and incubated for 7 days at room temperature.

Seven days after inoculation the broth culture of fungal isolates and centrifuged suspension of bacterial culture were used for titration against NaOH (0.01 N).

Indicator: Phenolphthalein indicator

Procedure

1. Five ml of filtrate /suspension of respective organism was taken in a conical flask.
2. Added 2 to 3 drops of phenolphthalein indicator.
3. Then titrated against 0.01 N NaOH. Total titrable acidity was expressed in ml of 0.01N NaOH consumed per 5.0 ml of culture filtrate.

3.2.5 Indole Acetic Acid (IAA) production by different phosphate solubilizing bacterial isolates

1. Indole Acetic Acid (IAA) production

Auxin production was confirmed by conducting Indole production test. Indole is nitrogen containing compound formed from degradation of the amino acid tryptophan by various bacteria. Pure tryptophan is not used in the test medium Tryptone-a digestion product of certain proteins was used as substrate since it contains considerable amount of tryptophan.

Medium

Trypton broth = 1% aqueous solution of tryptone

Trypton broth (as a control) = Trypton broth + 1% glucose

Test reagent:

Kovac's reagent = P-dimethylaminobenzaldehyde 50 g, amyl alcohol 750 ml, HCl (conc.) 250 ml.

The media was distributed in test tubes and autoclaved. The bacteria was inoculated and incubated for 24 hrs. 3 ml of Kovac's reagent was then added to each test tube. The alcohol layer was separated from aqueous layer upon standing and a rendering of alcohol layer from tryptone broth within a few minutes was taken as positive test for indole.

In culture which contains both, protein and carbohydrate (1% tryptone + 1% glucose broth) rapid acid production from carbohydrate in habits the formation or activity of an enzyme.

2. Gram's reaction

Identification was made by gram staining and by studying the morphological characters of the isolates.

Procedure :

- i) A loopful of bacterial suspension was transferred in the center of slide, with the help of wire loop
- ii) First a smear was prepared of bacterial cells by holding a clean slide by grapping at edges
- iii) Then dried smear was fixed by passing the slide 3-4 times rapidly over the flame.
- iv) The smear was flooded with crystal violet for 30 seconds, washed in the tap water.
- v) Then the smear was immersed in potassium iodide/ Lugol's iodine solution for 30 seconds washed in tap water then decolorized with 95% alohol and rinsed with water.
- vi) Counterstained with saffranin for 10 second, again washed with tap water and air dried.
- vii) Drop of cedar wood oil was placed on the slide and examined the smear under oil immersion lense.

3.2.6 Compatibility of PSM with fungicides, herbicide, antibiotics and insecticide (Fathia *et al.*, 2006)

3.2.6.1 Compatibility of Bacteria by Disc diffusion method

PSB isolates were checked for their resistance towards different agrochemicals (fungicides, herbicides, antibiotics, insecticides) through disc diffusion method. Stock solutions of test fungicide, herbicides, antibiotics, insecticides were prepared by adding various doses of commercial formulation of fungicides, herbicides, antibiotics and insecticides in distilled water and filter sterilized using 0.45 µm filter papers. Filter paper discs (5 mm diameter) were prepared by adding three different concentrations of these stock solutions to evaluate the concentrations equivalent to, above and below the recommended dose of test fungicides, herbicides, antibiotics and insecticides. 1 ml of individual bacterial culture (10^7 cells/ml harvested at early logarithmic growth) was spread on Pikovskaya's agar plates with sterile glass spreader aseptically. Autoclave sterilized 5mm disc of Whatman's filter paper were soaked in each concentration of 5 minutes. These discs were then kept on sterilized blotter paper to drain out the excess water from the disc. These discs were placed at equidistance on the agar surface in triplicate. The plates were incubated for 2-3 days at $28 \pm 2^\circ \text{C}$, and the diameter of the inhibition zones was measured in mm.

3.2.6.2 Compatibility of phosphate solubilizing fungi by poisoned food technique (Rathod *et al.*, 2010).

Potato dextrose agar medium was prepared, equally distributed measuring 100ml in 250 ml conical flask and sterilized in autoclave. Requisite quantity of each of the fungicide was added in sterilized melted (45°C) PDA separately so as to obtain desired concentration. Flask containing poisoned medium was shake well to have even and uniform distribution of fungicide. About 20ml of melted poisoned PDA was poured in each sterilized Petriplate and allowed to solidify. These Petriplates were inoculated by test fungus separately. Six mm disc of one week old fungus culture was cut with a sterilized cork borer, lifted and transferred aseptically in the centre of a Petriplate containing the medium poisoned with test fungicide. The control plates were kept where the culture

disc were grown in same condition on PDA without fungicide. Treated plates were incubated at room temperature ($27 \pm 2^{\circ}\text{C}$) for a period of seven days. Colony diameter was recorded in mm and per cent of mycelial inhibition was calculated as per formula given below based on the average of colony diameter. The data of mycelial growth was also subjected to statistical analysis and conclusions were drawn (Gomez and Gomez, 1984).

$$\text{PI}(\%) = \frac{C - T}{C} \times 100$$

Where,

PI = Per cent inhibition

C = Growth in control plate

T = Growth in treatment plates

CHAPTER IV

RESULTS AND DISCUSSION

In the present study emphasis has been given on isolation of phosphate solubilizing microorganism and 'P' solubilizing efficiency of these isolates, isolated from rhizosphere region of some weeds occurring in sorghum and cotton crops of different places i.e. from Yeola taluka of Nashik district and Sorghum Research Unit Dr. PDKV., Akola and Cotton Research Unit Dr. PDKV., Akola. Fungi were tentatively identified based on colony and morphological characters as per CMI description plates. The fungi commonly noticed are *Aspergillus niger* and *Aspergillus flavus*. The efficiency of isolated PSM to solubilized tricalcium phosphate in Pikovskaya's agar medium, broth medium and compatibility of these PSM with different agrochemicals were subsequently studied *in vitro*.

4.1 Isolation of phosphate solubilizing microorganism from weeds occurring in sorghum and cotton crops.

Twenty three *Aspergillus* spp. and nine bacterial isolates were isolated from different rhizosphere region of weeds of sorghum and cotton crops on the basis of halo zone formation around their colony on Pikovskaya's agar medium.

The data presented in Table 2 showed isolates from weeds rhizosphere of sorghum and cotton crop. *Aspergillus* isolates i.e. *Aspergillus niger*-1 isolated from *Cynodon dactylon*, *A. niger*-2 from *Euphorbia geniculata*, *A. niger*-3 from *Amaranthus polygamus*, *A. niger*-5 from *Phyllanthus niruri*, *A. niger*-8 from *Argemone maxicana*, *A. niger*-10 from *Parthenium hysterophorus* and *A. flavus*-1 from *Convolvulus arvensis* from sorghum field of Yeola taluka of Nashik District, while *A. niger*-4, *A. niger*-6, *A. niger*-7 and *A. niger*-9 isolates from weeds of *Amaranthus polygamus*, *Phyllanthus niruri*, *Convolvulus arvensis* and *Argemone maxicana* respectively from sorghum Research Unit of Dr. PDKV., Akola. Isolates i.e. *A. niger*-11 isolated from *Cynodon dactylon*,

Table 2. Isolation of phosphate solubilizing fungi from rhizosphere of weeds

Sr. no.	Name Of Crop	Common name / local Name of weed	Scientific Name	location Of Collection	Name of isolates
A Sorghum					
1		Harali	<i>Cynadon dactylon</i>	Yeola	<i>Aspergillus niger</i> -1
2		Mothi Dudhani	<i>Euphorbia geniculata</i>	Yeola	<i>A. niger</i> -2
3		Tandulja	<i>Amaranthus polygamus</i>	Yeola	<i>A. niger</i> -3
		Tandulja	<i>Amaranthus polygamus</i>	Dr. PDKV. Sorghum Research Unit	<i>A. niger</i> -4
4		Hajardani	<i>Phyllanthus niruri</i>	Yeola	<i>A. niger</i> -5
		Hajardani	<i>Phyllanthus niruri</i>	Dr. PDKV. S. R. Unit	<i>A. niger</i> -6
5		Chandvel	<i>Convolvulus arvensis</i>	Yeola	<i>A. flavus</i> -1
		Chandvel	<i>Convolvulus arvensis</i>	Dr. PDKV. S.R. Unit	<i>A. niger</i> -7
6		Piwala dhotra	<i>Argemonia mexicana</i>	Yeola	<i>A. niger</i> -8
		Piwala dhotra	<i>Argemonia mexicana</i>	Dr. PDKV. S. R. Unit	<i>A. niger</i> -9
7		Gajar gawat	<i>Parthenium hysterophorus</i>	Yeola	<i>A. niger</i> -10
B Cotton					
1		Harali	<i>Cynadon dactylon</i>	Yeola	<i>A. niger</i> -11
		Harali	<i>Cynadon dactylon</i>	Dr. PDKV. cotton Research Unit	<i>A. niger</i> -12
2		Gajar gawat	<i>Parthenium hysterophorus</i>	Yeola	<i>A. niger</i> -13
3		Cocks comb	<i>Celosia argentea</i>	Yeola	<i>A. niger</i> -14
		Cocks comb	<i>Celosia argentea</i>	Dr. PDKV. C.R. Unit	<i>A. flavus</i> - 2
4		Hajardani	<i>Phyllanthus niruri</i>	Dr. PDKV. C. R. Unit	<i>A. niger</i> -15
5		Reshimkata	<i>Alternanthera triandra</i>	Yeola	<i>A. niger</i> -16
		Reshimkata	<i>Alternanthera triandra</i>	Dr. PDKV. C. R. Unit	<i>A. niger</i> -17
6		Deepmal	<i>Acalypha indica</i>	Yeola	<i>A. niger</i> -18
7		Math	<i>Amaranthus viridis</i>	Yeola	<i>A. niger</i> -19
8		Piwala dhotra	<i>Argemonia mexicana</i>	Yeola	<i>A. niger</i> -20
9		Piwala dhotra	<i>Argemonia mexicana</i>	Dr. PDKV. C.R. Unit	<i>A. niger</i> -21

A. niger-13 from *Parthenium hysterophorus*, *A. niger*-14 from *Celosia argentea*, *A. niger*-16 from *Alternanthera triandra*, *A. niger*-18 from *Acalypha indica*, *A. niger*-19 from *Amaranthus viridis* and *A. niger*-20 from *Argemonia mexicana*, were isolated from weeds of cotton field of Yeola taluka of Nashik District. *A. niger*-12 isolated from *Cynadon dactylon*, *A.*

niger-15 from *Phyllanthus niruri*, *A. niger*-17 from *Alternanthera triandra*, *A. niger*-21 from *Argemone mexicana* and *A. flavus*-2 from *Celosia argentea* were isolated from weeds of cotton field from Cotton Research Unit of Dr. PDKV., Akola. All these 23 fungal isolates were selected by observing the clear zone formation around their colonies on Pikovskaya's agar medium containing insoluble tricalcium phosphate (Plate 1 a and b).

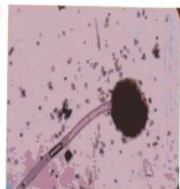
Present finding corroborates the studies reported by Pikovskaya's (1948) he reported microorganism gave clear zone due to dissolution of phosphate. This result is similar to the result of Chen *et al.* (2002) who isolated PSM (*Aspergillus niger*, *A. flavus*, *Penicillium* spp. and PSB) from nineteen weed species from citrus orchard in China. *Aspergillus* spp. and *Penicillium* spp. isolated from rhizosphere soil of pulses, cereals cotton and vegetables which solubilized tricalcium phosphate reported by Patil (2002). Similar results were also found by Alam *et al.* (2002) and Manivannan *et al.* (2011) who reported that *Aspergillus* spp. and *Penicillium* spp. isolated from rhizosphere of chilli and tomato plant.

The data presented in Table 3 revealed that bacterial isolates i.e. PSB-1 isolated from *Euphorbia geniculata*, PSB-3 from *Amaranthus polygamus*, PSB-4 from *Phyllanthus niruri* of sorghum field of Yeola taluka of Nashik district, whereas isolates i.e. PSB-2 isolated from *Euphorbia geniculata*, PSB-5 from *Achyranthus aspere* weeds of sorghum Research Unit of Dr. PDKV., Akola. Isolates i.e. PSB-6 isolated from *Celosia argentea* and PSB-7 from *Acalypha indica* of cotton field from Yeola taluka of Nashik district and isolates i.e. PSB-8 and PSB-9 were isolated from weeds i.e. *Acalypha indica* and *Digera arvensis* of cotton field from Cotton Research Unit Dr. PDKV., Akola (Plate 1c).

This result were similar to Humaira and Bano (2011) they reported that phosphate solubilizing bacteria isolated from halophytic weeds on the basis of halo zone formation around the colonies. Phosphate solubilizing bacteria isolated from rhizosphere of pulses, cereals, cotton and oilseed crops on the basis of clear zone formation around the colonies reported by Bhamare (2006). This result is on the line with the findings of



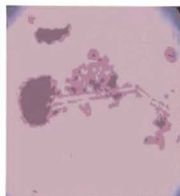
a) *Aspergillus niger*



b) Conidiophore of
A. niger



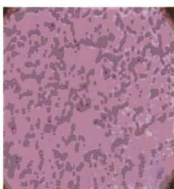
c) *Aspergillus flavus*



d) Conidiophore of
A. flavus



e) Phosphate solubilizing
bacteria



f) Bacterial cells

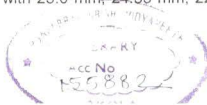
Plate 1: Isolates of phosphate solubilizing microorganisms

Table 4. Measurement of clear zone formation by fungal isolates around their colony on Pikovskaya's agar medium

Sr. no.	isolates	3 rd Day of Inoculation			5 th Day of Inoculation			7 th Day of Inoculation		
		TZ (mm)	CD (mm)	CZ (mm)	TZ (mm)	CD (mm)	CZ (mm)	TZ (mm)	CD (mm)	CZ (mm)
1	<i>A. niger</i> - 1	23.00	20.00	3.00	48.00	44.00	4.00	70.33	65.33	5.00
2	<i>A. niger</i> - 2	25.66	23.33	2.33	48.33	45.33	3.00	71.66	67.66	4.00
3	<i>A. niger</i> - 3	21.00	19.00	2.00	45.66	42.33	3.33	68.00	64.00	4.00
4	<i>A. niger</i> -4	23.66	21.33	2.33	47.33	44.33	3.00	70.00	66.33	3.67
5	<i>A. niger</i> -5	24.33	21.33	3.00	47.00	43.00	4.00	69.66	64.33	5.34
6	<i>A. niger</i> -6	26.33	24.00	2.33	49.33	46.00	3.33	72.66	68.00	4.66
7	<i>A. niger</i> -7	27.00	24.33	2.67	49.33	45.33	4.00	74.00	69.00	5.00
8	<i>A. niger</i> -8	24.66	22.00	2.33	47.00	43.66	3.34	70.66	66.33	4.33
9	<i>A. niger</i> - 9	25.33	23.00	2.33	48.00	45.00	3.00	71.00	67.00	4.00
10	<i>A. niger</i> -10	22.00	19.00	3.00	44.00	40.33	3.66	67.00	62.00	5.00
11	<i>A. niger</i> - 11	21.33	19.00	2.33	45.66	42.33	3.33	68.66	64.33	4.33
12	<i>A. niger</i> -12	23.00	21.00	2.00	46.00	42.33	2.67	69.33	66.00	3.33
13	<i>A. niger</i> -13	24.00	22.00	2.00	46.66	43.33	2.33	71.33	68.33	3.00
14	<i>A. niger</i> - 14	22.66	20.00	2.66	44.66	41.33	3.33	67.33	62.66	4.34
15	<i>A. niger</i> - 15	26.66	24.00	2.66	49.00	45.33	3.67	73.00	68.00	5.00
16	<i>A. niger</i> - 16	24.00	21.33	2.67	46.66	43.00	3.66	69.00	64.33	4.67
17	<i>A. niger</i> -17	22.33	20.00	2.33	46.33	42.33	4.00	70.00	65.00	5.00
18	<i>A. niger</i> -18	23.66	21.33	2.33	47.66	45.00	2.66	72.00	69.00	3.00
19	<i>A. niger</i> -19	25.00	23.00	2.00	49.00	46.33	2.67	74.66	71.33	3.33
20	<i>A. niger</i> - 20	25.66	22.66	3.00	47.66	44.33	3.33	72.66	67.33	5.34
21	<i>A. niger</i> -21	26.00	23.67	2.33	49.00	45.66	3.34	73.00	68.33	4.67
22	<i>A. flavus</i> - 1	9.66	8.33	1.33	12.66	11.00	1.66	19.66	17.33	2.33
23	<i>A. flavus</i> - 2	8.66	7.66	1.00	12.33	10.33	2.00	18.66	16.00	2.66
	'F'- Test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
	SE(M)±	0.38	0.37	0.33	0.43	0.45	0.31	0.45	0.46	0.29
	CD.(p=0.01)	1.47	1.41	1.26	1.65	1.72	1.20	1.74	1.79	1.13

TZ = Total Zone, CD = Colony Diameter, CZ = Clear Zone

The data presented in Table 4 showed that on 3rd day of incubation fungal isolates formed clear halo zone around their colony. Isolates i.e. *A. niger*-1, *A. niger*-5 *A. niger*-10 and *A. niger*-20 showed maximum clear halo zone i.e. 3.0 mm with 23.0 mm-24.33 mm, 22.0 mm



and 25.66 mm total zone and 20.0 mm, 21.33 mm, 19.00 mm and 22.66 mm colony diameter respectively, followed by *A. niger*-7 and *A. niger*-16 which formed 2.67 mm clear zone with 27.0 mm and 24.00 mm total zone and 24.33 mm and 21.33 mm colony diameter respectively while minimum clear zone i.e. 1.00 mm with 8.66 mm total zone and 7.66 mm colony diameter recorded by *A. flavus*- 2 (Plate 2a).

On 5th day of incubation *A. niger*-1, *A. niger*-5, *A. niger*-7 and *A. niger*-17 showed maximum halo zone of solubilization i.e. 4.00 mm with 48.00 mm, 47.00 mm, 49.33 mm, and 46.33 mm of total zone and 44.00 mm, 43.00 mm, 45.33 mm and 42.33 mm colony diameter respectively, while *A. flavus*-2 formed minimum halo zone i.e.1.66 mm with 12.66 mm total zone and 11.00 mm colony diameter respectively.

On 7th day of incubation *A. niger*-5 and *A. niger*-20 showed maximum solubilization clear halo zone i.e. 5.33 mm with 69.66 mm and 72.66 mm total zone and 64.33 mm and 67.33 mm colony diameter respectively, whereas minimum halo zone i.e.2.33 mm with 19.66 mm total zone and 17.33 mm colony diameter was recorded in *A. flavus*-1 and remaining fungal isolates showed halo zone ranged from 2.33 to 5.0 mm on Pikovskaya's agar medium.

This result indicated that those fungal isolates formed maximum halo zone on Pikovskaya's agar medium these isolates were effective phosphate solubilizers which confirmed the finding of Wani and Patil (1979). Padmawathi Tallapragada and Seshachala (2010) who reported that *Aspergillus* spp. formed 26.00 mm halo zone and *Bacillus subtilis* produce 46.10 mm clear zone on Pikovskaya's agar medium. In a similar study Patil (2002) reported that *Aspergillus niger* and *A. flavus* formed clear zone ranged from 2.00 to 7.80 mm. PSM (*Aspergillus* spp. and bacteria) isolated from rhizosphere of maize having solubilizing index ranged from 1.63 to 3.29 with decrease in pH is reported by Alam *et al.* (2002) and Rashid *et al.* (2004).

The data from Table 5 revealed that on 2nd day of incubation maximum halo zone was observed in Dr. PDKV strain of PSB i.e. 2.66 mm with 7.66 mm total zone and 5.00 mm colony diameter. Among all bacterial isolates PSB-3 showed maximum halo zone (2.33 mm) with 5.66 mm total

zone and 3.33 mm colony diameter while minimum halo zone was recorded in PSB-2 i.e. 1.00 mm with 4.66 mm total zone and 3.66 mm colony diameter. Other PSB isolates (PSB-1, PSB-4, PSB-5, PSB-6, PSB-7, PSB-8 and PSB-9) showed halo zone ranged from 1.00 mm to 1.66 mm with 4.66 to 5.66 mm total zone and 3.66 mm to 4.33 mm colony diameter respectively (Plate 2a).

Table 5. Measurement of clear zone formation by bacterial isolates around their colony on Pikovskaya's agar medium

Sr. no.	Isolates	2 nd day of inoculation			3 rd day of inoculation			4 th day of inoculation		
		TZ (mm)	CD (mm)	CZ (mm)	TZ (mm)	CD (mm)	CZ (mm)	TZ (mm)	CD (mm)	CZ (mm)
1	PSB-1	5.33	3.33	2.00	9.33	7.00	2.33	12.33	9.00	3.33
2	PSB-2	4.66	3.66	1.00	7.66	6.00	1.66	10.66	8.33	2.33
3	PSB-3	5.66	3.33	2.33	9.33	6.33	3.00	11.66	8.00	3.66
4	PSB-4	4.66	3.66	1.00	8.33	6.33	2.00	11.00	8.33	2.67
5	PSB-5	5.33	3.66	1.66	9.33	6.66	2.67	11.00	8.00	3.00
6	PSB-6	5.66	4.33	1.33	8.00	6.00	2.00	10.33	8.00	2.33
7	PSB-7	6.00	4.66	1.33	8.33	6.00	2.33	10.00	7.33	2.67
8	PSB-8	5.00	3.33	1.67	9.33	6.66	2.67	11.00	8.00	3.00
9	PSB-9	4.66	3.33	1.33	8.33	5.66	2.66	11.33	8.66	2.66
10	Dr. PDKV strain	7.66	5.00	2.66	11.00	7.33	3.67	13.66	9.33	4.33
	'F'- Test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
	SE(M)±	0.34	0.30	0.25	0.34	0.26	0.27	0.37	0.28	0.29
	CD,(p=0.01)	1.36	1.22	1.01	1.38	1.03	1.09	1.49	1.13	1.17

TZ = Total Zone, CD = Colony Diameter CZ = Clear Zone

On 3rd day of incubation maximum clear zone was recorded by Dr. PDKV strain of PSB i.e. 3.67 mm with 11 mm total zone and 7.33 mm colony diameter. Among all isolated bacterial isolates PSB-3 showed maximum halo zone i.e. 3 mm with 9.33 mm total zone and 6.33 mm colony diameter followed by PSB-8 and PSB-5 which produced 2.67 mm clear zone, while minimum halo zone was recorded in PSB-2 i.e. 1.66 mm

with 7.66 mm total zone and 6.00 mm colony diameter. Other PSB isolates (PSB-1, PSB-4, PSB-6, PSB-7 and PSB-9) formed 2.0 mm clear zone on Pikovskaya's agar medium.

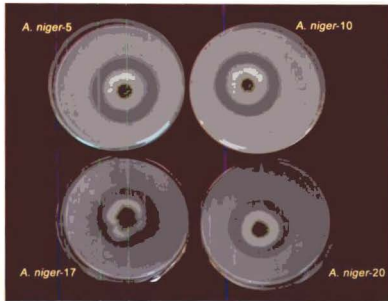
On 4th day of inoculation maximum halo zone was recorded in Dr. PDKV strain of PSB i.e. 4.33 mm with 13.66 mm total zone and 9.33 mm colony diameter. Among all bacterial isolates PSB-3 showed maximum halo zone i.e. 3.66 mm with 11.66 mm total zone and 8.0 mm colony diameter while PSB-2 formed minimum clear zone i.e. 1.66 mm with 7.66 mm total zone and 6.00 mm colony diameter (Plate 2b).

PSB-3 and PSB-1 were efficient phosphate solubilizers (Table 5). The result were similar to the result of Rashid *et al.* (2004) and Alam *et al.* (2002) that described the solubilizing index of some isolated bacterial and fungal isolates ranged from 1.69 to 3.29. Similar result were also found by Balamurugan *et al.* (2010) who reported that bacterial isolates i.e. PSB-37 and PSB-25 were formed 5.2 mm and 4.50 halo zone respectively. Humaira and Bano (2011) who reported that five different bacterial isolates isolated from different weed species they showed halo zone ranged from 0.64 to 2.23 cm on Pikovskaya's agar medium.

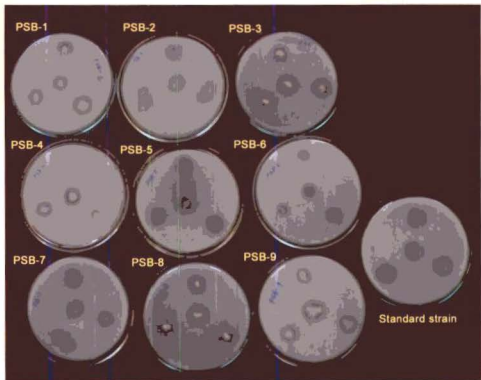
4.3 Assessment of phosphate solubilizing activity of phosphate solubilizing microorganism in broth medium

The fungal and bacterial isolates were tested for phosphate solubilizing activity *in vitro*. Test organisms were inoculated in Pikovskaya's broth medium containing tricalcium phosphate (0.5 g/100 ml) and incubated for 7th and 14th days at $28 \pm 2^{\circ}$ C and observations were recorded for reduction in pH and amount of solubilized phosphate (P_2O_5 µg/ml) in broth medium. Initial pH of the broth was 7.00 before inoculation.

The data presented in Table 6 revealed that after 7th day of inoculation with different fungal isolates the change reduction in pH of medium ranged from 7.00 to 3.10 and amount of P_2O_5 solubilized measured between 12.00 to 23.33 P_2O_5 µg/ml was observed, the pH of the medium was decreased due to production of organic acids in the medium



A) Clear halo zone formed by phosphate solubilizing fungi



B) Clear halo zone formed by phosphate solubilizing bacteria

Plate 2: Phosphate solubilizing activity (clear halo zone) of different phosphate solubilizing microorganisms

by the isolates. Among all *Aspergillus* spp. maximum reduction in pH recorded in *Aspergillus niger*-20 i.e. 3.50 with maximum P_2O_5 solubilization i.e. 19.33 P_2O_5 $\mu\text{g/ml}$ followed by *Aspergillus niger*-5 (3.53 pH) with 19.00 P_2O_5 $\mu\text{g/ml}$, *Aspergillus niger*-17 (3.56 pH) 18.66 P_2O_5 $\mu\text{g/ml}$ and *Aspergillus niger*-15 (3.56 pH) with 19.00 P_2O_5 $\mu\text{g/ml}$, whereas minimum reduction in pH recorded in *Aspergillus flavus*-1 (4.2 pH) with 12.00 P_2O_5 $\mu\text{g/ml}$ as compared to control (6.9 pH) with 00.0 P_2O_5 $\mu\text{g/ml}$. Other isolates of *Aspergillus* spp. recorded reduction in pH ranged from 4.13 to 3.50 and 18.33 to 13.00 P_2O_5 $\mu\text{g/ml}$.

After 14th day of incubation *Aspergillus niger*-20 exhibited maximum reduction in pH i.e. 3.1 with maximum P_2O_5 solubilization i.e. 23.33 P_2O_5 $\mu\text{g/ml}$ followed by *Aspergillus niger*-5 (3.13 pH) with 23.00 P_2O_5 $\mu\text{g/ml}$, while *Aspergillus flavus*-2 showed minimum reduction in pH (3.90) with minimum P_2O_5 solubilization i.e. 17.66 P_2O_5 $\mu\text{g/ml}$ as compare to control (6.76 pH) with 2.00 P_2O_5 $\mu\text{g/ml}$. *Aspergillus niger*-20 and *Aspergillus niger*-5 recorded high organic acid production, maximum reduction in pH and maximum P_2O_5 solubilization in Pikovskaya's broth medium on 7th and 14th day of incubation.

Arora and Gaur (1979) noticed that as pH decreases solubilization activity increases and also reported that *Aspergillus* spp. and *Penicillium* spp. effectively solubilized tricalcium phosphate in liquid medium. *Aspergillus awamori* and *Pseudomonas striata* were found to solubilize maximum amount of tricalcium phosphate. In a similar study it was reported that isolates of *Aspergillus* and *Penicillium* isolated from agricultural soil showed maximum level of phosphate solubilization activity *in vitro* when liquid medium was supplemented with both tricalcium phosphate and rock phosphate separately. Chakraborty *et al.*, (2010), Alam *et al.* (2002) and Rashid *et al.* (2004) reported maximum solubilization of tricalcium phosphate by PSM the ranged from 0.04 to 0.147 %. Among fungal isolates *Aspergillus niger* showed maximum solubilization i.e. (0.45%) P_2O_5 in broth.

Table 6. Estimation pH and phosphate solubilization by different fungal isolates in Pikovskaya's broth medium

Sr. no.	'P' solubilizing fungal isolates	Initial pH 7			
		7 th day of incubation		14 th day of incubation	
		pH	P ₂ O ₅ µg/ ml	pH	P ₂ O ₅ µg/ ml
1	<i>Aspergillus niger</i> - 1	3.63	18.33	3.20	22.33
2	<i>Aspergillus niger</i> - 2	3.83	16.00	3.56	20.00
3	<i>Aspergillus niger</i> - 3	3.80	16.33	3.50	20.33
4	<i>Aspergillus niger</i> - 4	3.90	15.66	3.63	19.33
5	<i>Aspergillus niger</i> - 5	3.53	19.00	3.13	23.00
6	<i>Aspergillus niger</i> - 6	3.73	17.33	3.33	21.33
7	<i>Aspergillus niger</i> - 7	3.66	18.33	3.23	22.33
8	<i>Aspergillus niger</i> - 8	3.73	17.00	3.43	20.66
9	<i>Aspergillus niger</i> - 9	3.83	16.00	3.60	19.66
10	<i>Aspergillus niger</i> -10	3.66	18.00	3.26	22.00
11	<i>Aspergillus niger</i> - 11	3.76	16.66	3.40	20.66
12	<i>Aspergillus niger</i> - 12	3.93	15.33	3.70	18.66
13	<i>Aspergillus niger</i> - 13	4.00	14.66	3.76	18.33
14	<i>Aspergillus niger</i> - 14	3.73	17.00	3.36	21.00
15	<i>Aspergillus niger</i> - 15	3.56	18.66	3.16	22.66
16	<i>Aspergillus niger</i> - 16	3.63	17.66	3.30	21.66
17	<i>Aspergillus niger</i> - 17	3.56	19.00	3.26	22.00
18	<i>Aspergillus niger</i> - 18	4.03	14.33	3.80	18.00
19	<i>Aspergillus niger</i> - 19	3.96	15.00	3.76	18.33
20	<i>Aspergillus niger</i> - 20	3.50	19.33	3.10	23.33
21	<i>Aspergillus niger</i> - 21	3.70	17.66	3.40	21.00
22	<i>Aspergillus flavus</i> -1	4.20	12.00	3.83	17.00
23	<i>Aspergillus flavus</i> -2	4.13	13.00	3.90	17.66
24	Control	6.90	00	6.73	2.0
	'F'- Test	Sig	Sig	Sig	Sig
	SE(M)±	0.04	0.38	0.03	0.29
	CD,(p=0.01)	0.13	1.44	0.12	1.09

Manivannan *et al.* (2011), Patil (2002) and Chakraborty *et al.* (2010) reported that *Aspergillus* spp. and *Penicillium* spp. solubilized maximum tricalcium phosphate ranged from 799 to 856 mg/ l in Pikovskaya's broth medium due to organic acid production.

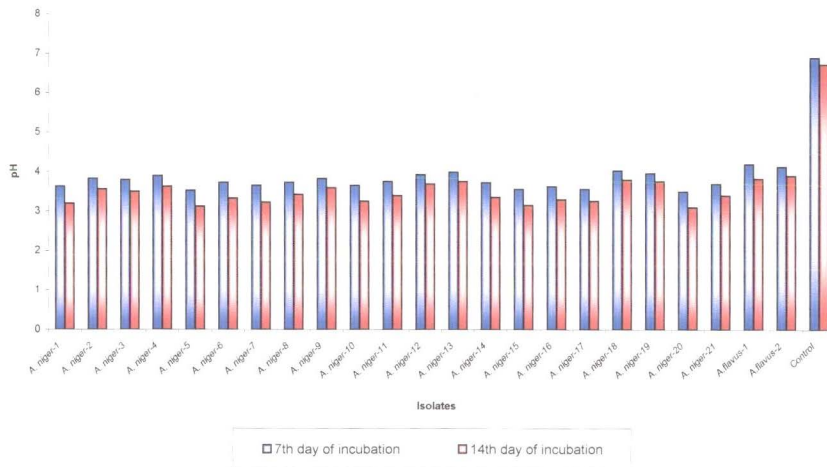


Fig.1.Effect of fungal isolates on pH in Pikovskaya's broth medium

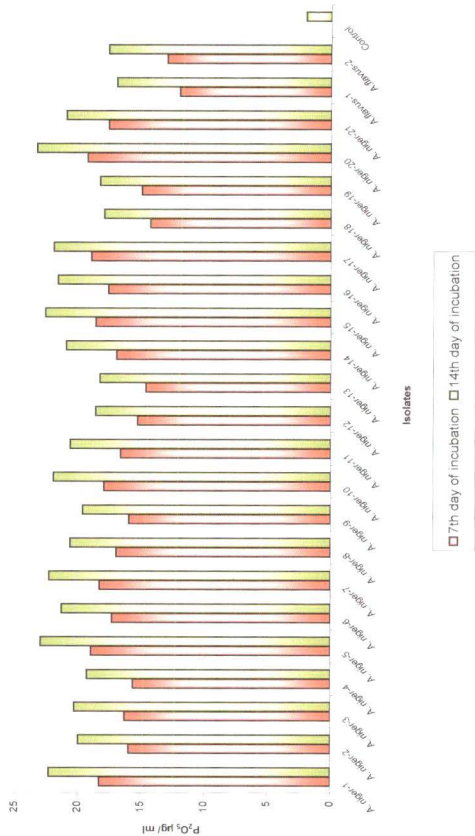


Fig.2. Effect of fungal isolates on solubilization of P_2O_5 in Pikovskaya's broth medium

The data presented in Table 7 revealed that on 7th day of incubation PSB isolates produced organic acid which decreased the pH of the medium and solubilized tricalcium phosphate in broth medium. Maximum reduction in pH recorded in Dr. PDKV strain of PSB i.e. 3.53 with 18.66 P₂O₅ µg/ml solubilization. Among all PSB isolates, PSB-3 showed maximum reduction in pH i.e. 3.7 with 17.33 µg/ml P₂O₅ solubilization followed by PSB-1 (3.8 pH) with 16.00 P₂O₅ µg/ml and minimum reduction in pH was recorded in PSB-2 i.e. 4.6 pH with 11.00 P₂O₅ µg/ml as compare to control (6.9 pH) and 0.00 P₂O₅ µg/ml.

Table 7. Estimation of pH and phosphate solubilization by different bacterial isolates in Pikovskaya's broth medium

Initial pH 7

Sr. no.	'P' solubilizing bacterial isolates	7 th day of incubation		14 th day of incubation	
		pH	P ₂ O ₅ µg/ml	pH	P ₂ O ₅ µg/ml
1	PSB- 1	3.80	16.00	3.40	21.00
2	PSB- 2	4.60	11.00	3.96	16.00
3	PSB-3	3.70	17.33	3.30	22.00
4	PSB-4	4.30	14.00	3.70	18.00
5	PSB-5	4.00	15.00	3.50	20.00
6	PSB-6	4.40	12.00	3.90	16.33
7	PSB-7	4.20	14.33	3.66	18.33
8	PSB-8	3.90	15.33	3.46	20.33
9	PSB-9	4.30	14.00	3.80	17.66
10	Dr. PDKV strain	3.53	18.66	3.20	22.66
	control	6.90	0.00	6.80	1.66
	'F'- Test	Sig	Sig	Sig	Sig
	SE(M)±	0.04	0.40	0.03	0.37
	CD,(p=0.01)	0.17	1.59	0.12	1.49

On 14th day of incubation, Dr. PDKV. strain of PSB recorded maximum reduction in pH (3.2) with 22.66 µg/ml P₂O₅ solubilization. Among PSB isolates, PSB-3 showed maximum decreased in pH (3.30) with 22.00 µg/ml P₂O₅ solubilization, whereas minimum reduction in pH recorded in PSB- 2 i.e. 3.96 pH with 16.00 P₂O₅ µg/ml solubilization. Other isolates having reduction in pH ranged from 3.9 to 3.4 with 11.0 to 20.00 µg/ml P₂O₅

solubilization as compared to control. Among all PSB isolates PSB- 3 and PSB-1 were efficient phosphate solubilizers.

Similar result were reported by Chen *et al.* (2006) who observed that isolate 36 strain of phosphate solubilizing bacteria solubilized tricalcium phosphate 1.5 to 519.7 mg/l with decreasing the pH of the medium. Phosphate solubilizing bacteria isolated from rhizosphere of tea, bacteria produced organic acid (3.6 to 7.9 ml) which decreased the pH (4.65 to 3.88) and solubilized tricalcium phosphate (33.9 to 46.6 ppm) reported by Balamurugan *et al.* (2010). Manivannan *et al.* (2011) reported PSB (*Pseudomonas* spp.) solubilized tricalcium 98.5 mg/ 100ml in broth culture. Five PSB solubilized tricalcium phosphate ranged from 6.00 to 16.7 µg/ml in broth medium with lowering the pH (6.9 to 5.26) reported by Humaira and Bano (2011). It may be due to the production of organic acids by these strains that lowers the pH of medium. The inverse relationship between pH and soluble phosphate was reported by Rashid *et al.* (2004). Sharma *et al.* (2012) they reported that PSB isolates, isolated from tea rhizosphere of Darjeeling hills are capable of producing plant growth promoting substance IAA ranged from 10-30 mg/l organic acids and capable of solubilizing inorganic phosphate ranged from 40.62 ± 1.1 to 136.73 ± 1.7 mg/l thereby decreasing the pH of the medium.

4.4 Estimation of change in titrable acidity and pH by different phosphate solubilizing fungal and bacterial isolates by titration method

Isolated phosphate solubilizing microorganisms were tested to measuring their change in pH and titrable acidity by titration method *in vitro*. The Pikovskaya's broth medium was inoculated with test organism and incubated for 7 day at $28 \pm 2^{\circ}$ C and observations were recorded for change in titrable acidity and reduction in pH in broth medium. Initial pH of the broth medium was recorded at 7.00 before inoculation.

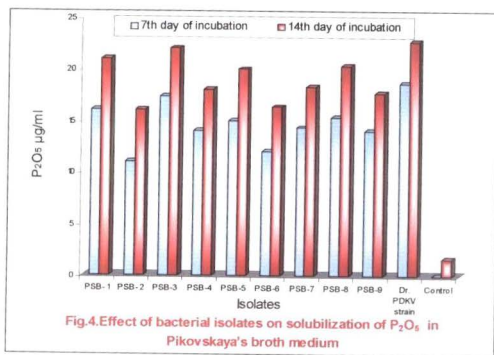
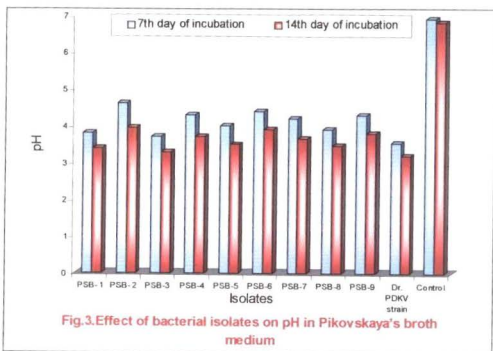


Table 8. Estimation of change in titrable acidity and pH by 'P' solubilizing fungal isolates

Sr. no.	'P' solubilizing fungal Isolates	7 th day of incubation	
		Titration acidity (ml)	pH
1	<i>Aspergillus niger</i> - 1	3.43	3.63
2	<i>Aspergillus niger</i> - 2	3.00	3.83
3	<i>Aspergillus niger</i> - 3	3.06	3.80
4	<i>Aspergillus niger</i> - 4	2.90	3.90
5	<i>Aspergillus niger</i> - 5	3.53	3.53
6	<i>Aspergillus niger</i> - 6	3.33	3.73
7	<i>Aspergillus niger</i> - 7	3.36	3.66
8	<i>Aspergillus niger</i> - 8	3.20	3.73
9	<i>Aspergillus niger</i> - 9	2.96	3.83
10	<i>Aspergillus niger</i> -10	3.36	3.66
11	<i>Aspergillus niger</i> - 11	3.16	3.76
12	<i>Aspergillus niger</i> - 12	2.86	3.93
13	<i>Aspergillus niger</i> - 13	2.70	4.00
14	<i>Aspergillus niger</i> - 14	3.26	3.73
15	<i>Aspergillus niger</i> - 15	3.46	3.56
16	<i>Aspergillus niger</i> - 16	3.40	3.63
17	<i>Aspergillus niger</i> - 17	3.56	3.56
18	<i>Aspergillus niger</i> - 18	2.63	4.03
19	<i>Aspergillus niger</i> - 19	2.83	3.96
20	<i>Aspergillus niger</i> - 20	3.60	3.50
21	<i>Aspergillus niger</i> - 21	3.30	3.70
22	<i>Aspergillus flavus</i> -1	2.43	4.20
23	<i>Aspergillus flavus</i> -2	2.50	4.13
24	Control	0.56	6.90
	'F'- Test	Sig	Sig
	SE(M)±	0.04	0.03
	CD,(p=0.01)	0.18	0.13

Data presented in Table 8 revealed that on 7th day of incubation all the fungal isolates produce various amount of organic acid. Among fungi *Aspergillus niger*-20 recorded maximum titrable acidity i.e. 3.6 ml with reduction in pH i.e. 3.5 as compared to control, followed by *A. niger*-17 (3.56 ml) with reduction in pH (3.56) and *A. niger*-5 (3.53 ml) with pH (3.53), whereas *A. flavus*-1 showed minimum titrable acidity i.e. 2.43 ml with pH 4.2 and other isolates of *Aspergillus niger* having titrable acidity ranged between 2.50 to 3.43 ml with pH ranged from 3.63 to 4.13. The result showed that all the fungal isolates produce organic acid with decrease in pH.

The similar results were reported by Patil (2002) reported that *Aspergillus flavus*, *Aspergillus niger* and *Penicillium* spp. produced organic acid and reduced the pH of the medium with P₂O₅ solubilization which ranged from 65.40 to 137.4 P₂O₅ mg/100 ml. Alam *et al.* (2002) observed that *Aspergillus* and *Penicillium* produce organic acid which reduced the pH of medium and solubilized tricalcium phosphate (0.22%) in liquid medium on 7th day of incubation.

The data presented in Table 9 revealed that on 7th day of incubation PSB isolates produce organic acid which decreased the pH of the medium. Maximum reduction in pH (3.53) with maximum titrable acidity (3.13 ml) showed by Dr. PDKV strain of PSB and among all PSB isolates, PSB-3 showed reduction in pH up to 3.7 with 3 ml titrable acidity followed by PSB- 8 pH (3.9) with 2.83 ml titrable acidity, while minimum reduction in pH (4.6) recorded in PSB-2 with 2.30 ml titrable acidity as compared to control and other PSB isolates (PSB-1, PSB-4, PSB-5, PSB-6, PSB-7 and PSB-9) having titrable acidity, ranged from 2.30 to 2.80 ml with pH reduction ranged from 4.8 to 3.8.

Table 9. Estimation of change in titrable acidity and pH by PSB isolates

Sr. no.	'P' solubilizing bacterial isolates	7 th day of incubation	
		Titrable acidity (ml)	pH
1	PSB- 1	2.90	3.80
2	PSB- 2	2.30	4.60
3	PSB-3	3.00	3.70
4	PSB-4	2.60	4.30
5	PSB-5	2.80	4.00
6	PSB-6	2.50	4.40
7	PSB-7	2.66	4.20
8	PSB-8	2.83	3.90
9	PSB-9	2.60	4.30
10	Dr. PDKV strain	3.13	3.53
	Control	0.53	6.90
	'F'- Test	Sig	Sig
	SE(M)±	0.04	0.05
	CD,(p=0.01)	0.16	0.18

This result similar is to the results reported by Lal (2002) and Humaira and Bano (2011) they reported that reduction in pH and increase titrable acidity this might be due to secretion of organic acids by PSB. Balamurugan *et al.* (2002) isolated the PSB from rhizosphere soil of tea and reported that these isolates reduced pH 3.88 to 4.62 of medium with increase in titrable acidity ranged from 3.9 to 7.9 ml. The result also similar to Ponmurugan and Gopi (2006) who isolated the PSB from groundnut and reported that these isolates solubilized tricalcium phosphate which reduce pH (5.9 to 5.0) and increases in titrable acidity (2.4 to 3.9) due to organic acid production.

4.5 Indole Acetic Acid (IAA) production by phosphate solubilizing bacteria

All nine isolates were tested for IAA production *in vitro*. All isolates were Gram -ve, rod shaped, irregular colonies. The result obtained

that among all PSB isolates three isolates i.e. PSB-3, PSB-4, PSB-6 and PSB-8 were able to produce Indole Acetic Acid (Plate 3).

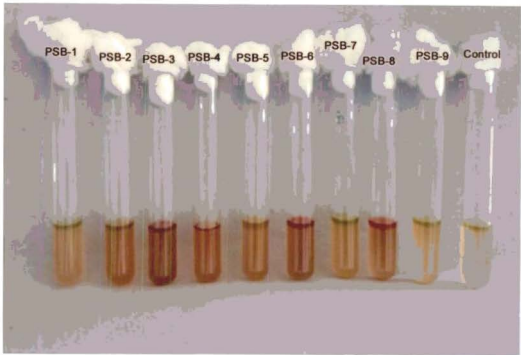
Mahantesh and Patil (2011) and Sagervanshi *et al.* (2012) isolated the PSB and reported that PSB isolates produce IAA and organic acid. Manivannan *et al.* (2011) isolated the PSB and reported that bacteria produce IAA and organic acid. The findings also in the line of Sanjani Samuel *et al.* (2011) who reported that PSB (*Bacillus* spp. and *Pseudomonas* spp.) were able to produce Indole Acetic Acid (IAA).

4.6 Compatibility of phosphate solubilizing microorganism with agrochemicals (fungicide, antibiotics, herbicide and insecticide)

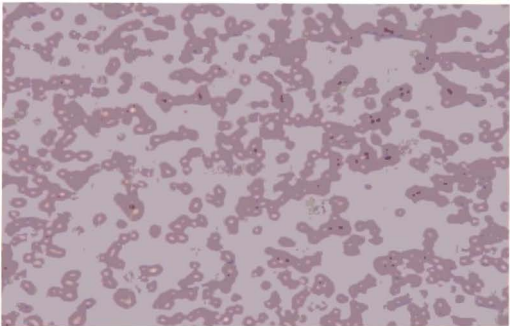
4.6.1 Compatibility of phosphate solubilizing fungi (*Aspergillus niger* and *A. flavus*) with fungicides and antibiotics

Compatibility of phosphate solubilizing fungi were tested *in vitro* by using commonly fungicides viz., Thiram, Carbendazim, Mancozeb and Propiconazole and antibiotic viz., Streptocycline and in combination with COC + Streptocycline by adopting "Poisoned Food Technique" given by Rathod *et al.* (2010).

The data presented in Table 10 revealed that 100 per cent growth inhibition of *Aspergillus niger*-20 and *A. niger*-5 was observed with thiram (0.3%), carbendazim (0.1%), mancozeb (0.25%) and propiconazole (0.05%). All tested fungicides with different concentration showed detrimental effect on growth of *A. niger*-20 and *A. niger*-5. In case of COC (0.25%) + streptocycline (100 ppm) inhibited growth of *A. niger*-20 and *A. niger*-5 i.e. 38.34 per cent and 53.6 per cent respectively, whereas streptocycline (100 ppm) alone did not show any detrimental effect on growth of both *A. niger* isolates. From the result it is concluded that all the fungicides were not compatible with tested organisms, however COC + streptocycline and streptocycline were compatible with *A. niger* isolates (Plate 4a).



A) Indole acetic acid production



B) Gram staining

Plate 3: Indole Acetic Acid (IAA) production and gram staining test of phosphate solubilizing bacteria

Table10. Compatibility of PSF *Aspergillus niger* with fungicides and antibiotics.

Treatment no.	Fungicides and antibiotics	Concentration	<i>A. niger</i> -20		<i>A. niger</i> - 5	
			Colony diameter in (mm)	Per cent growth inhibition %	colony diameter in (mm)	Per cent growth inhibition %
T ₁	Thiram 75 % WP	0.3%	0.00	100	0.00	100
T ₂	Carbendazim 50 % WP	0.1%	0.00	100	0.00	100
T ₃	Mancozeb 75% WP	0.25%	0.00	100	0.00	100
T ₄	Propiconazole 25% EC	0.05%	0.00	100	0.00	100
T ₅	COC 50 % WP + Streptocycline	0.25% + 100 PPM	55.33	38.64	41.76	53.6
T ₆	Streptocycline	100PPM	90	0	90	0
T ₇	Control		90		90	
	'F'- Test		Sig		Sig	
	SE(M)±		0.08		0.27	
	CD,(p=0.01)		0.34		1.16	

Wani and Taskeen (2011) studied the effect of systemic and non-systemic fungicides on *A. niger* and reported that among systemic fungicides, carbendazim brought about highest reduction followed by hexaconazole, bitertanol and myclobutanil respectively. Among non-systemic fungicides, the mancozeb was found the most effective followed by captan and zineb respectively. The higher concentrations (1000 ppm and 2000 ppm) of all the fungicides proved more effective than lower concentrations (125 ppm and 500 ppm). Similar result also found by Rathod *et al.* (2010) and Sen and Charaya (2010) reported that *Aspergillus sulphureus*, *Aspergillus niger* and *Fusarium moniliforme* were tolerant to copper (copper sulphate) at high concentration i.e. 400 ppm.

4.6. Compatibility of PSF *Aspergillus niger* with herbicides and insecticides

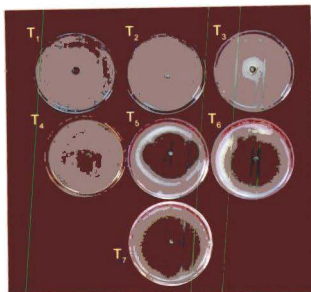
In vitro effect of herbicides and insecticides on growth of *Aspergillus niger* isolates was tested. The herbicides viz., Glyphosate

(0.6%), quizalfop-ethyl (0.2%) and fenoxaprop-ethyl (0.2%) and insecticides viz., cypermethrin (0.03%), emamectin benzoate (0.04%) and Imidacloprid 0.03% were used to test their compatibility against *A. niger*-20 and *A. niger*-5 stains.

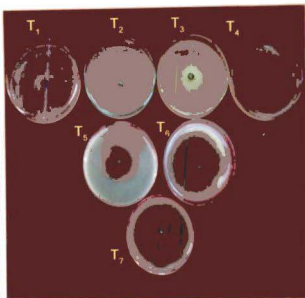
Table 11. Compatibility of PSF *Aspergillus niger* with herbicides and insecticides

Treatment no.	Herbicides and insecticides	Concentration	<i>A.niger</i> -20		<i>A. niger</i> -5	
			colony diameter in (mm)	Per cent growth inhibition %	colony diameter in (mm)	Per cent growth inhibition %
T ₁	Glyphosate 41% SL	0.6%	42.66	52.60	35.06	61.04
T ₂	Quizalfop-ethyl	0.2%	0.00	100	0	100
T ₃	Fenoxaprop-ethyl 9.3% EC	0.2%	0.00	100	0	100
T ₄	Cypermethrin 25 % EC	0.03%	90	0	90	0
T ₅	Emamectin benzoate 5 % SG	0.04%	90	0	90	0
T ₆	Imidacloprid 17.8 % SL	0.3%	0.00	100	0	100
T ₇	Control		90		90	
	'F'- Test		Sig		Sig	
	SE(M)±		0.12		0.48	
	CD,(p=0.01)		0.49		2.01	

The data presented in the Table 11 showed quizalfop-ethyl (0.2%), fenoxaprop-ethyl (0.2%) and imidacloprid (0.3%) showed 100 per cent growth inhibition of *A. niger*-20 and *A. niger*-5 followed by glyphosate i.e. 52.6 and 61.04 per cent growth of *A. niger*-20 and *A. niger*-5 isolates respectively, whereas in case of insecticides viz., cypermethrin (0.03%) and emamectin benzoate (0.04%) did not show no negative effect on growth of both isolates of *A. niger* strain. It concluded that quizalfop-ethyl, fenoxaprop-ethyl and imidacloprid with tested concentration were not compatible with both the strains of *A. niger*. However glyphosate and tested insecticides were compatible with both the isolates of *A. niger* (Plate 4b).



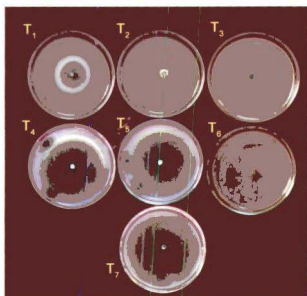
Aspergillus niger-20



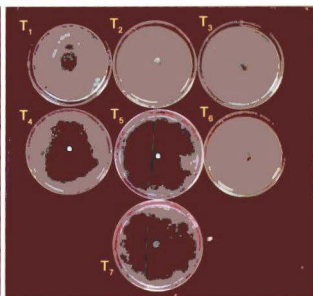
Aspergillus niger-5

T1=Thiram @ 0.3%, T2= Carbendazim @ 0.1%, T3=Mancozeb @ 0.25%,
 T4=Propiconazole @ 0.05%, T5= COC @ 0.25% + Streptocycline 100ppm,
 T6= Streptocycline @ 100 ppm, T7=Control

A) Compatibility of *Aspergillus niger* with different fungicides and antibiotics



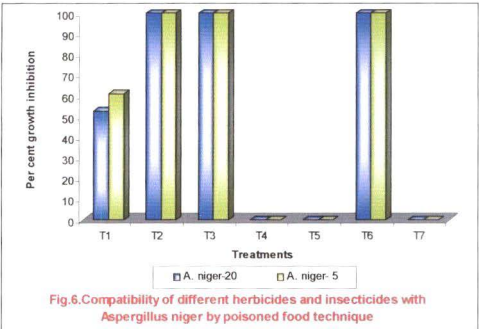
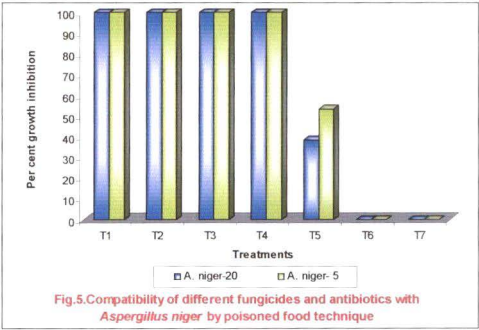
Aspergillus niger-20



Aspergillus niger-5

T1= Glyphosate @ 0.6%, T2= Quizalofop ethyl @ 0.2%, T3= Fenoxaprop p ethyl @ 0.2%,
 T4=Cypermethrin @ 0.03%, T5=Emamectin benzoate @ 0.04%,
 T6= Imidacloprid @ 0.3%, T7=Control

B) Compatibility of *Aspergillus niger* with different herbicides and insecticides



Similar results were observed by Hefnawy *et al.* (2012) who concluded that *Aspergillus niger* and *Aspergillus fumigatus* were not affected at lower concentrations of tested herbicides i.e. glyphosate and putraline up to 200 mg l⁻¹ and at 800 mg l⁻¹ concentration. Das and Mukharjee (1998) and Das and Mukharjee (2000) reported that the most predominant genera of microorganisms, such as *Bacillus*, *Micrococcus* and *Aspergillus* were not affected by most of the insecticides viz., HCH, phorate, carbofuran and fenvalerate at their field application rates (7.5, 1.5, 1.0 and 0.35 kg a.i. ha⁻¹), respectively this result also on the line of present findings.

The data presented in Table 12 showed that thiram (0.3%), mancozeb (0.25%) and propiconazole (0.05%) were showed 100 per cent inhibition growth of *A. flavus*-2, followed by carbendazim (0.1%) which inhibited 34.64 per cent growth. In case of antibiotics, COC (0.25%) + streptocycline (100 ppm) showed 33.04 per cent growth inhibition, whereas streptocycline (100 ppm) showed 14.06 per cent growth inhibition of *A. flavus*-2. It concluded that among all tested fungicides thiram (0.3%), mancozeb (0.25%) and propiconazole (0.05%) were not compatible with *A. flavus*-2 while carbendazim (0.1%) and antibiotics i.e. COC+ streptocycline (0.25+ 100 ppm) and streptocycline (100 ppm) were moderately compatible with *A. flavus*-2 (Plate 5a).

Table 12. Compatibility of *Aspergillus flavus*-2 with fungicides and antibiotics.

Treatment no.	Fungicides and antibiotics	Concentration (%)	Mean colony diameter (mm)	Per cent growth inhibition (%)
T ₁	Thiram 75 % WP	0.3	0.00	100
T ₂	Carbendazim 50 % WP	0.1	13.43	34.64
T ₃	Mancozeb 75% WP	0.2	0.00	100
T ₄	Propiconazole 25% EC	0.05	0.00	100
T ₅	COC 50% WP + Streptocycline	0.25 + 100 PPM	13.76	33.04
T ₆	Streptocycline	100 PPM	17.66	14.06
T ₇	Control		20.55	
	'F'- Test		Sig	
	SE(M)±		0.33	
	CD,(p=0.01)		1.43	

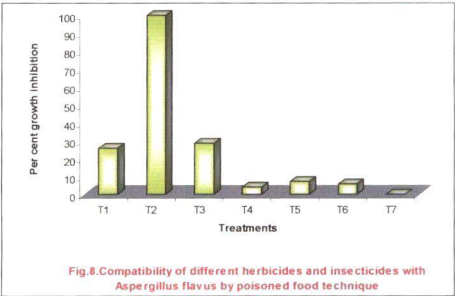
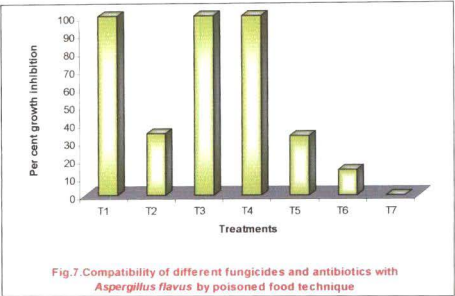
Sen and Charaya (2010) and Rathod *et al.* (2010) reported the effect of six fungicides on *Aspergillus flavus* by poisoned food technique and concluded that thiram, mancozeb, carbendazim were found to be more inhibite as compared to copper oxychloride, captan and captafol.

The data presented in Table 13 revealed that three herbicides and three insecticides were used to test compatibility against *A. flavus*-2. Among them quizalfop-ethyl (0.2%) showed 100 per cent growth inhibition followed by fenoxaprop-ethyl showed 28.88 per cent growth inhibition, whereas glyphosate (0.6%) inhibited 25.74 per cent growth of *A. flavus*-2. In case of insecticides i.e. cypermethrin (0.03%) showed 3.99 per cent, imidacloprid (0.3%) showed 5.93 per cent and emamectin benzoate (0.04%) showed 7.05 per cent growth inhibition respectively. It showed that quizalfop-ethyl (0.2%) was not compatible and other tested chemicals were compatible with *A. flavus*-2 (Plate 5b).

Table 13. Compatibility of *Aspergillus flavus*- 2 with herbicides and insecticides

Treatment no.	Herbicides and Insecticides	Concentration (%)	Mean colony Diameter (mm)	Per cent growth inhibition (%)
T ₁	Glyphosate 41% SL	0.6	15.26	25.74
T ₂	Quizalfop-ethyl 5% EC	0.2	0.0	100
T ₃	Fenoxaprop-ethyl 9.3 % EC	0.2	14.63	28.80
T ₄	Cypermethrin 10% EC	0.03	19.73	3.99
T ₅	Emamectin benzoate 5% SG	0.04	19.10	7.05
T ₆	Imidacloprid 17.8 % SL	0.3	19.33	5.93
T ₇	Control		20.55	
	'F'- Test		Sig	
	SE(M)±		0.57	
	CD _i (p=0.01)		2.41	

Present findings corroborate with the findings of Hefnawy *et al.* (2012) who reported that *Aspergillus niger* and *Aspergillus fumigatus* were not affected at lower concentrations of tested herbicides i.e.



glyphosate and putraline up to 200 mg l⁻¹ and at 800 mg l⁻¹. The result is on the line of Das and Mukharjee (2000) finding that *Aspergillus* spp. were not affected by most of the insecticides viz., HCH, phorate, carbofuran and fenvalerate at their field application rates (7.5, 1.5, 1.0 and 0.35 kg a.i. ha⁻¹).

4.6.3 Compatibility of phosphate solubilizing bacteria with agrochemicals (fungicides, antibiotics, herbicides and insecticides) by paper disc method (mm)

Different agrochemicals (fungicides, antibiotics, herbicides and insecticides) with different concentration and were assayed to ascertain the effect on growth of phosphate solubilizing bacteria by paper disc method. The filter paper discs containing different concentration were placed in Pikovskaya's agar plate already inoculated with the isolates. The zone of inhibition was recorded after 48 hrs. of incubation.

4.6.3.1 Compatibility of fungicides with phosphate solubilizing bacteria

All the isolates tolerant to 0.15% and 0.12% concentration of thiram and mancozeb respectively. At 0.3 % concentration, of thiram showed maximum growth by PSB-1, PSB-8 and PSB-9 (6 mm inhibition zone) followed by PSB-2 and PSB-5 (6.33 mm), whereas minimum growth was recorded in PSB-3, PSB-4, PSB-6 and PSB-7 (6.66 mm). At one and half than recommended dose (0.45%) maximum growth was observed in PSB-7 and PSB-8 (8.00 mm inhibition zone) followed by PSB-1 (8.33 mm), PSB-3 and PSB-5 showed least growth (8.66 mm), whereas minimum growth showed in PSB-6, and PSB-9 (9.33 mm).

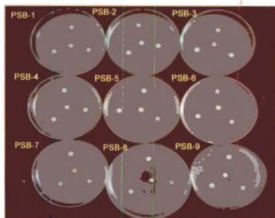
Regarding mancozeb, at 0.25% concentration, PSB-7 and PSB-8 showed maximum growth (6.33 mm), whereas PSB-2 and PSB-4 showed minimum growth (7.66 mm). At 0.38% maximum growth showed by PSB-8 (7.33 mm inhibition zone) followed by PSB-7 (7.66 mm), least growth recorded in PSB-1, PSB-5 and PSB-8 (8.0 mm), whereas minimum growth showed in PSB-2 (8.66 mm). Similar observation was recorded in carbendazim at 0.05, 0.1 and 0.15 % concentration and propiconazole at

0.025%, 0.050% and 0.075% concentration and result obtained that no significant inhibitions of PSB isolates were recorded with these concentrations. These results showed that all isolates were compatible with carbendazim and propiconazole at different concentrations (Plate 6).

Table 14. Compatibility of PSB isolates with different concentration of fungicides by paper disc method (mm)

Sr. no.	PSB isolates	Inhibition zone (mm)											
		Thiram			Mancozeb			Carbendazim			Propiconazole		
		0.15 Conc. → %	0.3 %	0.45 %	0.12 %	0.25 %	0.38 %	0.05 %	0.1 %	0.15 %	0.025 %	0.05 %	0.075 %
1	PSB-1	0.00	6.00	8.33	0.00	7.00	8.00	0.00	0.00	0.00	0.00	0.00	0.00
2	PSB-2	0.00	6.33	9.00	0.00	7.66	8.66	0.00	0.00	0.00	0.00	0.00	0.00
3	PSB-3	0.00	6.66	8.66	0.00	7.33	8.66	0.00	0.00	0.00	0.00	0.00	0.00
4	PSB-4	0.00	6.66	9.00	0.00	7.66	8.33	0.00	0.00	0.00	0.00	0.00	0.00
5	PSB-5	0.00	6.33	8.66	0.00	7.00	8.00	0.00	0.00	0.00	0.00	0.00	0.00
6	PSB-6	0.00	6.66	9.33	0.00	7.33	8.33	0.00	0.00	0.00	0.00	0.00	0.00
7	PSB-7	0.00	6.66	8.00	0.00	6.33	7.66	0.00	0.00	0.00	0.00	0.00	0.00
8	PSB-8	0.00	6.00	8.00	0.00	6.33	7.33	0.00	0.00	0.00	0.00	0.00	0.00
9	PSB-9	0.00	6.00	9.33	0.00	7.00	8.00	0.00	0.00	0.00	0.00	0.00	0.00
	Standard Deviation	-	Avg- 6.37 ± 29	Avg- 8.33 ± 0.46		Avg- 7.07 ± 0.41	Avg- 8.11 ± 0.41	-	-	-	-	-	-

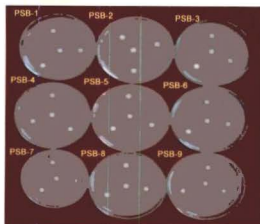
Fathia (2004) reported that phosphate solubilizing bacteria i.e. *Agrobacterium* strain Ca-18 was sensitive to fungicide viz., mancozeb, darosal and tolerant to alert plus, benlate at 0.1%, 0.2%, 0.4%, 0.6% concentration. Similar study carried by Kumar *et al.* (2011) and concluded that *Bacillus subtilis* was compatible to 1000 ppm of hexaconazole and propiconazole, also reported that strain was moderately compatible to tricyclazole and poorly compatible to benomyl 1000 ppm. The strain has good compatibility to carbendazim, at 200 and 400 ppm. Ahemad and Khan (2010) reported that phosphate solubilizing activity of *Enterobacter asburiae* strain PS2 decreased with increase in fungicide concentration. The findings reported by above scientists and Koche (2011) are on the similar line of present results.



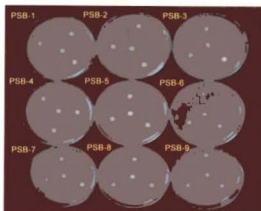
Thiram



Mancozeb



Carbendazim



Propiconazole

4.6.3.2. Compatibility of antibiotics with phosphate solubilizing bacteria

Antibiotics i.e. streptocycline and combination with COC+ streptocycline were tested at three different concentrations against phosphate solubilizing bacteria.

Table 15. Compatibility of PSB isolates with different concentration of antibiotics by paper disc method (mm)

Sr. no.	PSB Isolates	Inhibition zone (mm)					
		COC+ Streptocycline (% + ppm)			Streptocycline (ppm)		
		0.13+50	0.25+100	0.38+150	50	100	150
1	PSB-1	12.00	13.66	15.33	12.33	13.66	16.33
2	PSB-2	12.33	14.00	15.66	11.33	13.33	16.33
3	PSB-3	10.00	12.66	13.33	10.33	13.00	14.66
4	PSB-4	11.00	12.66	14.33	11.33	14.33	15.33
5	PSB-5	12.66	14.00	15.33	12.00	14.00	16.00
6	PSB-6	12.00	14.00	14.66	12.33	14.33	16.00
7	PSB-7	12.66	14.33	15.66	12.66	14.66	16.66
8	PSB-8	12.33	14.00	15.33	12.66	14.00	16.00
9	PSB-9	12.66	14.33	16.00	12.33	14.33	16.33
	Standard Deviation	Avg-	Avg-	Avg-	Avg-	Avg-	Avg-
		11.96	13.74	15.04	11.92	13.74	15.07
		± 0.85	± 0.54	± 0.78	± 0.73	± 0.60	± 0.78

Data presented in Table 15 revealed that at 0.13% + 50 ppm concentration of COC+ streptocycline, showed inhibitory effect on growth of isolates. Among all isolates PSB-3 and PSB-4 showed maximum growth (10.0 and 11.00 mm) inhibition zone respectively, whereas minimum growth recorded in PSB-5, PSB-7 and PSB-9 (12.66 mm). At 0.25% + 100 ppm concentration, PSB-4 showed maximum growth (12.66 mm inhibition zone), while PSB-7 and PSB-9 showed minimum growth (14.33 mm). At 0.38% + 150 ppm concentration, PSB-3 showed maximum growth (13.33 mm inhibition zone), than other isolates showed minimum growth.

At 50 ppm, 100 ppm and 150 ppm of concentrations streptocycline PSB-3 showed maximum growth (10, 13 and 14.6 mm inhibition zone) respectively, whereas PSB-7 showed minimum growth

(12.66, 14.66 and 16.66 mm) respectively. All the concentrations of antibiotic showed detrimental effect on growth of isolates, all isolates showed sensitivity to all tested concentrations of antibiotics (Plate 7).

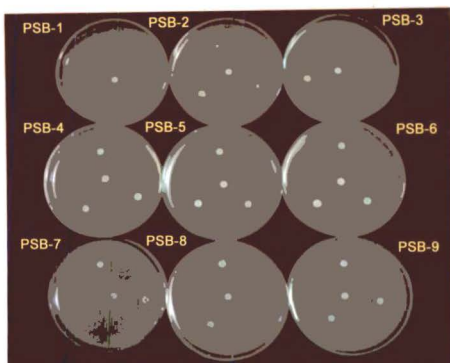
Present findings corroborates with the findings of Jayaswal *et al.* (1990) who reported that strain RJ3 found sensitive to tetracycline, chloromphenicol, ampicillin, gentamicin, kenamycin and streptomycin and RF2 sensitive to chloromphenicol, tetracycline and resistant to ampicillin, gentamicin, kanamycin and streptomycin. Sands and Rovira (1997) also reported that *Pseudomonas fluorescens* was resistant to chloromphenicol and ampicillin and sensitive to streptomycin and tetracycline.

4.6.3.3 Compatibility of herbicides with phosphate solubilizing bacteria

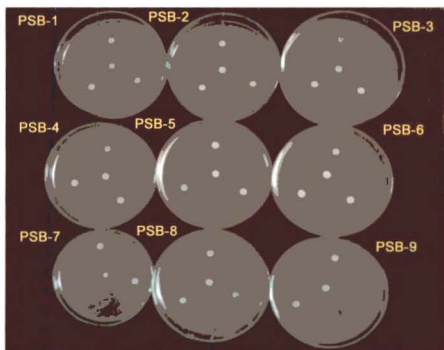
Application of selective and nonselective herbicides is general practice for management of weed. Hence to determine the effect of herbicides on growth and survival of phosphate solubilizing bacteria three herbicides at three different concentrations were tested.

Table 16 revealed that at 0.3% concentration of glyphosate maximum growth was recorded by PSB-3, PSB-5, PSB-9 (7.00, 7.33 and 7.33 mm inhibition zone respectively) followed by PSB-4 and PSB-6 (7.66 and 8.00 mm respectively), whereas minimum growth was recorded by PSB-2 (8.33 mm). At 0.6% concentration, PSB-3 and PSB-8 showed maximum growth (8.66 mm inhibition zone), whereas PSB-2, PSB-6 showed minimum growth (9.66 mm). At 0.9% concentration, maximum growth showed by PSB-3 and PSB-8 (11.0 mm inhibition zone), least growth was observed in PSB-5 and PSB-7 (11.33 and 11.66 mm) respectively, while minimum growth showed in PSB-2 (12.66 mm).

Regarding quizalofop-ethyl, at 0.1% concentration, maximum growth observed in PSB-6 and PSB-9 (8.66 mm inhibition zone) followed by PSB-2 (9.00 mm), PSB-3 and PSB-5 (9.33 mm), whereas minimum growth was recorded in PSB-7 (10.33 mm). At 0.2% concentration, PSB-6 showed maximum growth (9.66 mm inhibition zone), while least growth was recorded in PSB-4 (10.66 mm), whereas minimum growth showed in PSB-1 and PSB-7 (11.66 mm). At 0.3% concentration, PSB-4 and PSB-6 showed maximum growth (11.66 mm inhibition zone), least growth



COC + Streptocycline



Streptocycline

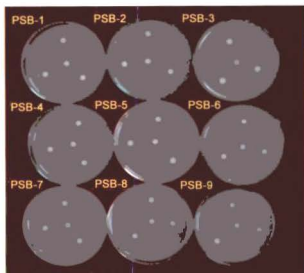
Plate 7: Compatibility of phosphate solubilizing bacteria with antibiotics

recorded in PSB-9 (12.33 mm), whereas PSB-1 and PSB-7 showed minimum growth (13.33 mm). Similar observation and growth pattern was observed when these isolates were exposed to 0.1, 0.2 and 0.3% concentration of fenoxaprop-ethyl, it showed that all isolates tolerated these concentrations. From the result of Table 16 indicated that PSB isolates were sensitive to tested concentration of glyphosate and quizalfop-ethyl i.e. isolates were not compatible with these herbicides and good compatible with fenoxaprop-ethyl herbicides (Plate 8).

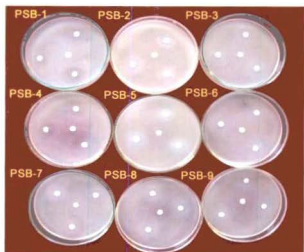
Table 16. Compatibility of PSB isolates with different concentration of herbicides by paper disc method (mm)

Sr. no.	PSB Isolates	Inhibition zone (mm)								
		Glyphosate			Quizalfop-ethyl			Fenoxaprop-ethyl		
	Conc. →	0.3 %	0.6 %	0.9 %	0.1 %	0.2 %	0.3 %	0.1 %	0.2 %	0.3 %
1	PSB-1	8.00	9.00	12.33	9.66	11.66	13.33	0.00	0.00	0.00
2	PSB-2	8.33	9.66	12.66	9.00	11.33	12.66	0.00	0.00	0.00
3	PSB-3	7.00	8.66	11.00	9.33	11.00	12.66	0.00	0.00	0.00
4	PSB-4	7.66	9.33	12.33	9.66	10.66	11.66	0.00	0.00	0.00
5	PSB-5	7.33	9.00	11.33	9.33	11.00	13.00	0.00	0.00	0.00
6	PSB-6	8.00	9.66	13.00	8.66	9.66	11.66	0.00	0.00	0.00
7	PSB-7	7.33	9.33	11.66	10.33	11.66	13.33	0.00	0.00	0.00
8	PSB-8	8.00	8.66	11.00	10.00	11.33	13.00	0.00	0.00	0.00
9	PSB-9	7.33	9.33	12.33	8.66	11.00	12.33	0.00	0.00	0.00
	Standard Deviation	Avg- 7.66 ± 0.42	Avg- 9.18 ± 0.35	Avg- 11.96 ± 0.69	Avg- 9.40 ± 0.54	Avg- 11.00 ± 0.67	Avg- 12.63 ± 0.60	-	-	-

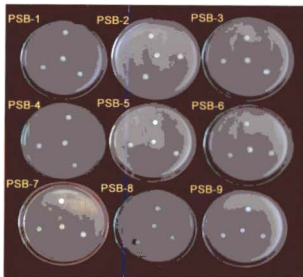
Debnath *et al.* (2002) reported that butachlor and basalin were showed stimulating effect on phosphate solubilizing microorganisms. Nongthombam *et al.* (2008) also reported that application of systemic pre emergence herbicides, viz., antfos and pendimethalin alone and in combination at their recommended doses increased efficiency of phosphate solubilizing microorganisms resulting in increased availability of phosphorus in soil. Heydari *et al.* (1997), Ahemad and Khan (2010a) concluded that herbicides, viz., quizalafop-ethyl and clodinafop had inhibitory effect on phosphate solubilizing activity of *Enterobacter asburiae*



Glyphosate



Quizalofop ethyl



Fenoxaprop p ethyl

strain PS2 at dose higher than recommended, while recommended dose had less inhibitory effect and also stated that glyphosate found less toxic than quizalofop-ethyl and clodinafop.

The quoted results of various workers are on the similar line of present investigation.

4.6.3.4. Compatibility of insecticides with phosphate solubilizing bacteria

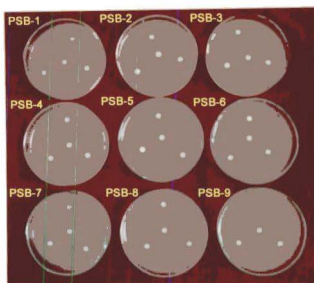
Under crop protection the use of insecticides is higher rate hence three level i.e. half than recommended, recommended dose and one half than recommended dose of insecticides were investigated against the growth of phosphate solubilizing bacteria. table 17 revealed that three concentrations of cypermethrin 10% EC (0.015, 0.03 and 0.045%), emamectin benzoate 5% SG (0.02, 0.04 and 0.06%) and imidacloprid 17.8% SL (0.15, 0.3 and 0.45%) were tested and result showed that no negative effect on growth of isolates, all isolates tolerant to tested concentration of insecticides, i.e. isolates showed compatibility with tested insecticides (Plate 9).

Table 17. Compatibility of PSB isolates with different concentration of insecticides by paper disc method (mm)

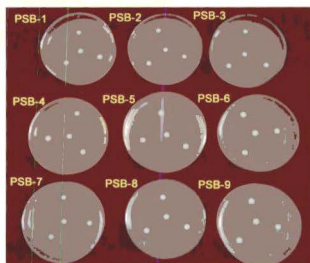
Sr. no.	Isolates	Inhibition zone (mm)								
		Cypermethrin			Emamectin benzoate			Imidacloprid		
		Conc.→	0.015%	0.03%	0.045%	0.02%	0.04%	0.06%	0.15%	0.30%
1	PSB-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	PSB-2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	PSB-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	PSB-4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	PSB-5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	PSB-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	PSB-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	PSB-8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	PSB-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Present findings corroborates with the findings of Das and Mukharjee (1998) who reported that hexachlorocyclohexane (phorate) stimulated the growth of bacteria, actinomycetes, fungi, N₂ fixing bacteria while fenvalerate stimulated the population of PSB. Similar result was recorded by Mathew (2003) who reported that *Pseudomonas fluorescens* was compatible with all the nine pesticides used. Amongst them

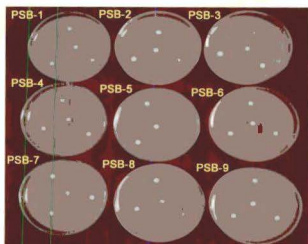
imidacloprid gave best compatibility also similar result were found by Ahemad and Khan (2011a) studied that rhizobacteria *pseudomonas putida* PS9 grew with the varying concentration of insecticides and showed varying degree of tolerance levels against the tested insecticides. The *Pseudomonas putida* strain PS9 might have utilized these insecticides as the energy source.



Cypermethrine



Emamectin benzoate



Imidacloprid

Plate 9: Compatibility of phosphate solubilizing bacteria with insecticides

CHAPTER V

SUMMARY AND CONCLUSIONS

Present investigations were undertaken to study the "Efficacy of rhizosphere phosphate solubilizers in some weeds occurring in sorghum and cotton crops" *in vitro* at Department of Plant Pathology Dr. PDKV., Akola.

The 'P' solubilizing microorganisms were isolated from rhizosphere of different weeds occurring in sorghum and cotton crops on Pikovskaya's agar medium by serial dilution method. Total 32 isolates showed 'P' solubilizing activities were of which 23 fungi (21 *Aspergillus niger* and 2 *Aspergillus flavus*) and 9 were bacterial isolates.

Most efficient phosphate solubilizers were identified on Pikovskaya's agar medium by measuring clear halo zones around the colony. Maximum halo zone i.e. 5.34 mm was recorded in *Aspergillus niger*-5 and *Aspergillus niger*-20 with 69.66 mm and 72.66 mm total zone and 64.33 mm and 67.33 mm colony diameter respectively, whereas minimum halo zone (1.00 mm) showed by *Aspergillus flavus*-1. Among bacterial isolates Dr. PDKV strain of PSB showed maximum halo zone (4.33 mm) followed by PSB-3 (3.66 mm), whereas minimum zone recorded in PSB-2 and PSB-6 (2.33 mm). The result indicated that *Aspergillus niger*-20 isolated from cotton weed of *Argemone mexicana* rhizosphere and Dr. PDKV strain of PSB isolates produce maximum clear zone around the colony i.e. 5 mm and 4.3 mm than rest of isolates. The isolated 'P' solubilizing fungi and bacteria were tested for 'P' solubilizing efficiency, reduction in pH and organic acid production *in vitro*. It was estimated as reduction in pH and amount of tricalcium phosphate solubilization (P_2O_5 $\mu\text{g/ml}$) in 7th and 14th day of incubation. After inoculation with different fungal isolates the reduction in pH measured between 4.20 to 3.10 (initial pH 7.00) and amount of tricalcium phosphate solubilization measured between 23.33 to 17.66 P_2O_5 $\mu\text{g/ml}$. All fungal and bacterial isolates showed 'P' solubilizing ability. The pH of the culture filtrate was measured by pH meter and observation recorded clearly indicated that among fungi

Aspergillus niger-20 isolated from weed i.e. *Argemone Mexicana* of cotton and *Aspergillus niger*-5 isolated from weed i.e. *Phyllanthus niruri* of sorghum solubilized maximum tricalcium phosphate i.e. 23.33 and 23.00 µg/ml with reduction in pH i.e. 3.1 and 3.4 respectively.

Bacterial isolates solubilized tricalcium phosphate measured between 22.66 to 16.00 P₂O₅ µg/ml with reduction in pH between 3.96 to 3.20 (initial pH 7.00). Among bacterial isolates, Dr. PDKV strain of PSB and PSB-3 recorded maximum tricalcium phosphate solubilization i.e. 22.66 and 22.00 P₂O₅ µg/ml with reduction in pH of the medium i.e. 3.2 and 3.3. Where incubation period increased the pH also differs.

Acid production by different PSM isolates during solubilization of phosphate in Pikovskaya's broth medium after 7th day of incubation by titration method. Among fungal isolates *Aspergillus niger*-20 was maximum titrable acidity i.e. 3.60 ml with reduction in pH (3.50) followed by *Aspergillus niger*-5 i.e. 3.56 ml and among PSB isolates maximum acidity recorded in Dr. PDKV strain of PSB and PSB-3 i.e. 3.13 ml and 3.00 ml.

Further PSB isolates were tested for Gram staining and Indole Acetic Acid (IAA) production. All isolates were Gram-ve and three isolates (PAB- 3, PSB-4, PSB-6 and PSB- 8) were produced Indole Acetic Acid (IAA).

Compatibility of PSM isolates (*Aspergillus* spp. and PSB) were tested with different agrochemicals (fungicides, antibiotics, herbicides and insecticides). Fungicides i.e. thiram (0.3%), carbendazim (0.1%), mancozeb 0.25% and propiconazole (0.05%) showed 100 per cent growth inhibition of *A. niger* strain i.e. (*Aspergillus niger*-20 and *Aspergillus niger*-5), whereas COC+ streptomycin inhibited 38.64 and 41.76 per cent growth inhibition of *A.niger*-20 and *Aspergillus niger*-5 strain, while streptomycin did not show inhibitory effect on both the *Aspergillus niger* strains.

Among herbicides, quizalofop-ethyl (0.2%) and fenoxaprop-ethyl (0.2%) showed 100 per cent growth inhibition, while glyphosate (0.6%) showed 52.60 and 61.04 per cent growth inhibition respectively. Insecticides did not show any detrimental effect on growth of *Aspergillus niger* i.e. *Aspergillus niger* tolerance to tested insecticides.

Regarding *Aspergillus flavus*-2, fungicides i.e. thiram (0.3%), mancozeb (0.2%) and propiconazole showed 100 per cent growth inhibition while carbendazim (0.1%) showed 34.64 per cent growth inhibition. Antibiotics i.e. COC (0.25%) + Streptocycline (100 ppm) showed 33.04 per cent growth inhibition. Among herbicides quizalofop-ethyl (0.2%) showed 100 per cent growth inhibition, glyphosate (0.6%) and fenoxaprop-ethyl (0.2%) showed 25.74 per cent 28.80 per cent growth inhibition respectively, whereas carbendazim (0.03%), emamectin benzoate (0.04%) and imidacloprid (0.3%) showed 3.99 per cent, 7.05 per cent and 5.93 percent growth inhibition.

Regarding compatibility of PSB isolates with different agrochemicals were used *in vitro*. PSB-1 and PSB-8 isolates were found tolerant and other PSB were sensitive to thiram (0.3 and 0.45%), PSB-1, PSB-5 and PSB-9 were tolerant to mancozeb (0.25 and 0.38%). All PSB isolates were sensitive to antibiotic i.e. COC (0.13, 0.25 and 0.38%) + Streptocycline (50, 100 and 150 ppm) and streptocycline (50,100 and 150 ppm). PSB-3, PSB-5 and PSB-9 were tolerant, while other isolates sensitive to glyphosate (0.3, 0.6 and 0.9%) and PSB-6 and PSB-6 were showed maximum growth, while other isolates sensitive to quizalofop-ethyl (0.3, 0.6 and 0.9 %). All PSB isolates were tolerant to carbendazim (0.05, 0.1 and 0.15%), propiconazole (0.025, 0.050 and 0.075%) and fenoxaprop p ethyl (0.1, 0.2 and 0.3 %). Insecticides were not shown negative effect on growth of PSB i.e. all PSB isolates were tolerant to cypermethrin (0.015, 0.03 and 0.045%), emamectin benzoate (0.02, 0.04, 0.06%) and imidacloprid (0.1, 0.3 and 0.45%).

On the basis of experiment conducted it can be concluded that-

1. Among fungal isolates, *Aspergillus niger*- 20 isolated from weed i.e. *Argemone Mexicana* of cotton and *Aspergillus niger*-5 isolated from weed i.e. *Phyllanthus niruri* of sorghum and *Aspergillus niger*-1 isolated from weed i.e. *Cynodon dactylon* of sorghum and bacterial isolates i.e. Dr. PDKV strain of PSB, PSB-3 isolated from weed i.e. *Amaranthus polygamus* of sorghum and PSB-1 isolated from weed i.e. *Cynodon dactylon* of sorghum showed maximum solubilization of

tricalcium phosphate on Pikovskaya's agar medium and broth medium with maximum reduction in pH and more titrable acidity.

2. Amount of 'P' solubilized by PSM increases with increases in incubation period and has inverse reaction in pH i.e. increases with decrease in pH.
3. Amount of 'P' solubilization increased with increase in acid production i.e. (maximum acidity).
4. All PSB isolates were Gram-ve and PSB-3, PSB-4, PSB-6 and PSB-8 produced Indole Acetic Acid (IAA).
5. *Aspergillus niger* and *Aspergillus flavus* were not compatible with fungicides i.e. thiram, carbendazim, mancozeb and propiconazole and herbicides i.e. glyphosate, quizalofop-ethyl and fenoaxaprop p ethyl, whereas COC + streptocycline, streptocycline and insecticides i.e. cypermethrin, emamectin benzoate and imidacloprid were found compatible.
6. PSB-1 and PSB-8 isolates were found tolerant to thiram, PSB-1, PSB-5 and PSB-9 tolerated to mancozeb.
7. PSB-3 and PSB-5 were compatible with glyphosate and PSB-4 and PSB-6 were compatible with quizalofop-ethyl.
8. All the PSB isolates were compatible with carbendazim, propiconazole, fenoaxaprop-ethyl and insecticides i.e. cypermethrin, emamectin benzoate and imidacloprid.

CHAPTER VI

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Signature of Student

APPENDIX

1) Media

i) Pikovaskayas media content

Glucose	-	10.0 g
$\text{Ca}_3(\text{PO}_4)_2$	-	5.0 g
$(\text{NH}_4)_2\text{SO}_4$	-	0.5 g
NaCl	-	0.2 g
$\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$	-	0.1 g
KCl	-	0.2 g
Yeast extract	-	0.5 g
MnSO_4	-	Trace
$\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$	-	Trace
Agar	-	15.0 g
Distilled water	-	1000 ml
pH adjusted to	-	7 to 0.2

iii) Tryptone broth medium

Tryptone	-	10 g
Sodium chloride	-	05 g
Calcium chloride	-	1.5 g
K_2HPO_4	-	1.5 g
Distilled water	-	1000 ml



iii) Agrochemicals

Sr. no	chemicals	Concentration (%)
A	Fungicides	
1	Thiram 75 % WP	0.3
2	Carbendazim 50 % WP	0.1
3	Mancozeb 75 % WP	0.25
4	Propiconazole 25% EC	0.05
B	Antibiotics	
1	COC+ Streptocycline 50 % WP + 100 ppm	0.25+ 100 ppm
2	Streptocycline	100 ppm
C	Herbicides	
1	Glyphosate 41% SL	0.6
2	Quizalofop ethyl 5% EC	0.2
3	Fenoxaprop p ethyl 9.3 % EC	0.2
D	Insecticides	
1	Cypermethrin 25 % EC	0.03
2	Emamectin benzoate 5 % SG	0.04
3	Imidacloprid 17.8 % SL	0.30