Studies on utilization of rhizospheric pseudomonads in preventing seed biodeterioration of *Arachis hypogaea* L, enhancement in seed germination and crop production

SUMMARY OF

Thesis

SUBMITTED TO

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

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BHIMRAO
AMBEDKAR
UNIVERSITY

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प्रज्ञा शील करुणा

ESTABLISHED 1996

FOR THE DEGREE OF

Doctor of Philosophy

IN

ENVIRONMENTAL MICROBIOLOGY

Submitted By

Rishabh Chitranshi

(Enrolment no. 906/13)

Under the Supervision of

Prof. Naveen Kumar Arora

Head

DEPARTMENT OF ENVIRONMENTAL SCIENCE

SCHOOL FOR ENVIRONMENTAL SCIENCES

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

(A Central University, NAAC Accreditation 'A' Grade)

VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226 025

UTTAR PRADESH, INDIA

2019

SUMMARY

Groundnut (*Arachis hypogaea L.*) is an economical crop worldwide, having a rich source of all the nutrients. Being an oilseed, groundnut seeds are very much affected by predominant storage deteriorating fungi *A. niger* and *A. flavus*, which causing aflatoxin contaminations in storage seeds. These fungal agents cause various physiological and biochemical changes in the nutritive composition of seeds. Moreover, it is very difficult to pause or stop deteriorative alterations permanently. The after effects are clearly seen immediately or after sowing such as weak seedling, unhealthy plant, immature seeds and various plant diseases at the time of maturation.

Aflatoxin is basically a group of toxin such as G1, G2, B1, B2, M1, and M2 that are produced by the plant pathogen. These toxins occur naturally and have been found in a wide range of commodities, including groundnuts used for animal and human consumption. Aflatoxins are toxic, mutagenic, and carcinogenic compounds. Depending on their levels, toxins can severely affect human beings.

A group of bacteria found in the vicinity of plant roots network called rhizobacteria which helps in enhancing plant growth. So this group is also known as PGPR (Plant Growth Promoting Rhizobacteria). PGPRs include a large number of bacterial populations, in which *Pseudomonas* is one of a very efficient member of this group. They produce antibiotics and other metabolites like production of siderophore, hydrolytic enzymes such as phenazine (PHE), 2,4-diacetylphloroglucinol (PHL), pyoverdin (PYO) (pseudobactin),

indoleacetate and pyoluteorin (Plt) plays an important role in generating immune system of plants against various pathogens. *P. fluorescent* shows antagonistic property against plant pathogens. It is also reported that application of these beneficial microbes in plants under stress conditions may resist them against various pathogenic fungi. The bio-priming of seeds with microbial metabolites are very useful to protect seeds against pathogens during storage; they are more effective target based environmental friendly and cost effective alternatives of harmful chemicals based pesticides. *P. fluorescence* shows biocontrol activity against several phytopathogens such as *Alternaria, Aspergillus, Fusarium, Rhizoctonia, Phytophthora*.

In the present work thus, the utilization of *P. fluorescens* in prevention and control of seed deteriorating fungi (*A. niger*) during storage of groundnut seeds were analyzed. The preferred area for this study is natural environmental and agriculture conditions and the targeted sampling sites for collection of rhizospheric soil were located in three different districts (Mainpuri, Farrukhabad and Unnao) of Uttar Pradesh, India. Further, for the isolation of fungal pathogens, contaminated groundnut seeds were singled out form the warehouse of a local farmer situated near by the sampling site in district Farrukhabad.

For the isolation of fungal pathogen, surface sterilized groundnut seeds were placed on PDA plats and incubated at 29ºC for one week. After incubation different fungal colonies were observed on groundnut seeds which were further purified on PDA plates. Among all, three dominant fungal isolates

were identified as *A. flavus* (RF-02, RF-03) and *A. niger* (RF-07) after 18S rRNA sequencing. These identified fungal strains were separated for further study. Secondly, total 7 bacteria were isolated from rhizospheric soil of groundnut on fluorescent pseudomonas specific agar medium (Hi-media). These bacterial isolates were further analyzed for morphological, biochemical and PGP activity tests.

All the bacterial isolates have mucilaginous colonies with smooth margins. Moreover, the isolates were identified as Gram negative and rod shaped bacteria. All the isolates showing yellowish fluorescent and were fast growers. These isolates were further analyzed for various bio-chemical activities, for most of the analyses KB-002 Hi media biochemical test kit specific for gram negative rods were used. All seven isolates were positive for catalase activity, positive oxidase, nitrate reductase, urease production and citrate utilization while none of the isolates showed positive H_2S Production activity. Along with this enzymatic activity of lipase showed negative by all the isolates and TDA shows more likely to be positive. Presence of glucose was positive for all the isolates but in case of lactose it was about 89% positive and in arabinose it was not actually determined by the biochemical test kit. On the basis of these biochemical tests all the isolates were observed to be very close with *P. fluorescens*.

All the bacterial isolates were further go through with PGP activity analyses and observed that all bacterial isolates show positive IAA production ability checked by UV visible spectrophotometer (Evolution 201 UV visible)

analysis and colour change. Among all, RC-07 was the best IAA producer at optimum cultural conditions. Only one isolate RC-07 shows positive siderophore production on CAS agar plates during qualitative analysis with yellowish zone. Strain RC-01, RC-04 and RC-07 were showing positive HCN production by colour change of filter paper soaked in picric acid from yellow to orange.

On the bases of biochemical and PGP activity RC-07 with best results was selected for further studies. The antagonistic potential of RC-07 was checked on fungal deteriorating agent RF-07 (*Aspergillus niger*) through dual culture and spray dilution antifungal assays. In dual culture assay inhibition was clearly observed when compared with growth of deteriorating pathogen in control plate. Further, in present study RC-07 shows positive and maximum inhibition of fungal deteriorating agent *A. niger*. It was observed that *Pseudomonas* sp. secreted various enzymes and metabolites such as phenazine and 2,4-diacetylphloroglucinol, which not only inhibit the pathogens but also promote plant growth. The presence of phenazine and 2,4-diacetylphloroglucinol (2,4 DAPG) in the supernatant of RC-07 ware clearly observed in gas chromatography and mass spectrophotometry (GCMS).

Antagonistically active bacterial strain RC-07 was screened by 16s rRNA sequencing. The strain was showing 99% similarities with genus *Pseudomonas*. Moreover, a comparative study with available database on NCBI and The phylogenetic analysis of that strain RC-07 shows the direct

relation with *Pseudomonas fluorescens*. Sequence was submitted in DDBJ data base with the accession number LC375795.

During the pot study it was observed that groundnut seed treated with supernatant of *P. fluorescens* RC-07 shows best results against *A.niger* RF-07 pathogen in comparison to direct application of broth on seeds. After maturation, the difference between control and supernatant treated seed are clearly shown the enhancement of crop. While seeds infected with pathogen was not even grow properly. Direct bacterial contact with seed was not as effective as needed. The control grows in normal speed during pot study. So that this study found that the supernatant spray method is more effective for prevention of seed to be sown in pot from *A.niger*.

Similar results were obtained from field study of groundnut seeds. The supernatant spray of *P. fluorescens* RC-07 shows best results against *A. niger* RF-07 pathogen in comparison to application of broth spray on seeds. After maturation, the difference between control and supernatant treated seed are clearly visible.

High performance liquid chromatography profile of ethyl acetate extract of groundnut seeds oil clearly showed the high level presence of vitamins E, D2 & K which was confirmed through the compression with standard references of fat soluble vitamins. Fatty acid methyl Easter analysis (FAME) by GC of groundnut oil clearly showed the presence of oleic, linoleic and some other oils which was confirmed through the slandered library with an oil

content of about 43%. Different characteristic peaks were obtained in chromatogram between retention times (RT) as depicted in the chromatogram.

In concluding remark, it was observed during this study that *Pseudomonas fluorescens* was potentially able to suppress the growth of fungal pathogens on storage groundnut seeds. However, supernatant of *P. fluorescens* seems to be more effective in comparison to bacterial suspension. As bio-control agent, *P. fluorescens* have been shown beneficial prospects on seed health during storage. Various scientific reports are published worldwide and described the ability of different *pseudomonas* strains to significantly control a number of fungal, bacterial and nematode diseases in cereals, horticultural crops, oil seeds and others. However, the bacterial antagonisms in storage area for oil seed crops need more attention and in that case this work seems to be very beneficial in controlling deterioration and their after effects on crop production. Besides preventing seed deterioration, it may also improve seed health which helps in healthy seedling and healthy crop production to fulfill the yield gap for sustainable agro-economic development worldwide.