APPLICATION FOR FINANCIAL ASSISTANCE FOR "MINOR RESEARCH PROJECT"

: SUBMITTED TO:

The President,
Royal Education Society's
COLLEGE OF COMPUTER SCIENCE AND INFORMATION TECHNOLOGY (COCSIT)
LATUR

: Submitted Through:
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: Principal Investigator:
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Jan 2022

Proposal for Minor Research Project

Part-A

1. Broad Subject : "Rhizobium and Phosphate Solubilizing Bacteria effect on growth and productivity of soybean varieties"

2.	Area of Specialization		:	**Biotechnology**
3.	Duration		:	**1 Years**
4.	Principal Investigator		:	
	i)	Name	:	**Mr. K.P. Maknikar**
	i)	Sex	:	**Male**
	iii)	Date of Birth	:	**20-08-1980**
	iv)	Qualification	:	**M.Sc. (BT), NET Ph.D. (perusing)**
	v)	Designation	:	**Assistant Professor in Biotechnology**
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5) Name of the Institution Where the Project will be Undertaken:

a) Department: : **Department of Biotechnology, College of Computer Science and Information Technology, COCSIT Campus, Ambajogai Road,**

Latur – 413 531 (M.S.)

b) College/University : **Swami Ramanand Teerth Marathwada University, Nanded (M.S)**

6) Whether the College/University Is approved under section 2(f) And 12(B) of UGC Act? : **Yes**
please see 'Appendix- III'

7) Teaching and Research Experience of the Principal Investigator :

a) Teaching Experience : **U.G. Courses : 15 Years**
: **P.G. Courses : 10 Years**

b) Research Experience : 6.5 years

c) Publication :

Paper published

Accepted **: 03**

Communicated **: ----**

Books Published

Accepted **: ----**

Communicated **: ----**

PART – B

Proposed Research Work

8)

I) Project title:

1. Introduction :

Nitrogen and phosphorus play a crucial role in the growth and the development of soybean plants. Nitrogen is an indispensable part of amino acid, protein, chlorophyll, and many essential enzymes critical for photosynthesis and plant growth [1]. It is also necessary for partitioning photosynthate, stimulating root growth and enhancing uptake of other nutrients by plants [2]. On the contrary, another macronutrient, P has the utmost importance for pod formation and seed development in soybean [3]. It also plays an imperative role in photosynthesis, enzyme activation, ATP formation, energy transfer, metabolism of carbohydrates, and cell division [4]. Therefore, farmers use excessive N and P fertilizers to maintain high agricultural productivity. However, plants can use only 30–40% of these fertilizers and the rest is lost to soil and water, which is a big threat to the environment [5]. To check this environmental issue, eco-friendly and sustainable sources of nutrients should be used, reducing the necessity of synthetic fertilizers [6]. Hence, application of microbial inoculants as biofertilizers has emerged as a costeffective, environment-friendly, remunerative, and sustainable strategy to boost soil fecundity and crop growth [7,8]. The interaction between plants and microorganisms rhizobium and phosphate solubilizing bacteria (PSB) can reduce reliance on N and P [9]. Rhizobium and PSB have shown added benefit in legume cultivation by fixing N and solubilizing unavailable P [10]. Being a member of the Fabaceae family, soybean can build a symbiotic association with rhizobium, which converts atmospheric N2 into a useable form for plants [11]. With the help of symbiosis, the soybean can fix up to 200–250 kg N ha−1 [12], which is less prone to leaching loss and volatilization [5]. Biologically fixed N reduces production costs and protects the environment from the harmful effects of nitrogenous chemical fertilizer [13]. Previous studies documented that soybean requires up to 80 kg N per 1000 kg seeds depending on soil conditions to maintain optimum productivity [14] and approximately 80% of it is contributed by biologically fixed N [15]. However, in-built soil N-fixing bacteria is not sufficient to fix enough N [16]. That's why inoculating a rhizobial strain with seeds can be a cost-effective, environment-friendly, profitable, and sustainable strategy to supply N to plants [1]. A significant increase in plant growth and the yield of soybean because of seed inoculation with rhizobium was recorded by Zohaib et al. [16]. Additionally, the left-over nitrogen found in soil after the soybean crop harvest is equal to 30–80 kg N fertilizer per hectare, which is beneficial for the next crop [17]. Moreover, the biologically fixed N is sometimes inadequate for plants' growth and development [18]. To optimize yield, soybean needs biologically fixed N and N uptake by roots [19]. An exogenous supply of N as a starter dose during seedling development until the nodulation stage is critical for soybean to ensure optimal production [20]. Hardy et al. [21] documented the beneficent impact of applying N fertilizer before planting on the early vegetative growth of soybean until root nodules are formed. Furthermore, rhizobium requires P to drive energy for atmospheric N fixation and nodule formation [22]. Phosphorus also provides enough infection sites for rhizobium by promoting root growth [23]. Phosphorus assists rhizobium in building the mitochondrial and symbiosomal membranes of nodules and assimilation of ammonium as amino acids [24]. Mitran et al. [25] revealed a positive relationship between nodular P content and N fixation in legumes. Insufficient P impedes root growth and hinders photosynthesis and the accumulation and translocation of photosynthates and other functions directly related to biological N fixation [22]. Although soil contains a fair amount of P, it usually forms compounds with calcium, magnesium, and other minerals and becomes fixed in the soil [26]. Only 15–20% of soil applied P remains available to plants [27]. The mineralization and the solubilization of fixed organic P are performed by PSB [28]. PSB solubilizes the organic P by secreating various organic acids and enzymes [29], improves legume yield, and reduces dependency on organic and inorganic sources of P fertilizer [30]. Gaur et al. [31] documented the solubilization of approximately 30–40 kg P2O5 ha−1 due to inoculation of PSB. In addition, PSB produces plant growth hormone especially auxin and stimulates plant growth [32]. An increment of 23%, 15%, and 16% in nodules number per plant, grain yield and protein content were recorded in chickpea by Ditta et al. [33] due to PSB inoculation, respectively. Besides, dual inoculation of beneficial microbes was proven to be more effectual than single inoculation considering crop growth and yield [34]. The combined application of rhizobium and PSB has an augmented effect on nodulation, nitrogen fixation, root growth, and in turn the yield of soybean. In Bangladesh, soybean oil is the most popular edible oil but farmers produce only 5% of total demand [35]. The major reason behind this scenario may be the farmers' preference for other crops. But soybean has great potential in Bangladesh as it is the crop with the richest source of protein [36], and it requires less fertilizer, reducing the input cost. Previously neglected, soybean cultivation is now gaining popularity due to the availability of high-yielding, short-duration varieties and a suitable climatic condition [37]. Farmers will be more encouraged if they can reduce production costs by using biofertilizer such as rhizobium and PSB, which will also benefit the environment. In our experiment, we investigated the efficacy of nitrogen fixing bacteria (R. japonicum) and phosphate solubilizing bacteria (P. striata) to provide necessary N and P to soybean plants and to improve their growth, yield, Agriculture 2022, 12, 1136 3 of 18 and quality. We assumed that R. japonicum and P. striata with a reduced amount of N and P fertilizers as a starter dose would perform better than recommended N and P fertilizer doses. The present study aimed to explore the comparative effect of R. japonicum and P. striata inoculants with or without a reduced amount of synthetic N and P fertilizers on the growth, yield, and quality of soybean. The experiment also compared the effectiveness of microbial inoculants with chemical fertilizer on nodulation and the nutritional status of soil and plants

c. Review of Literature & Development in the Subject

Akka Samudin et. al. "Effect of Rhizobium inoculation to nodulation and growth of soybean [Glycine max (L.) Merrill] Germplasm" January 2018 Legume Research - An International Journal 41(of) DOI:10.18805/LR-385.

Nitrogen is an important macronutrient mineral that is needed in the largest amount by the plant and is the main limiting factor for plant development. Rhizobium is a group of bacteria capable of providing nutrients for soybean crops. When symbiotic with legume crops, this group of bacteria is able to infect plant roots and form root nodules. The study aimed to investigate the effect of Rhizobium on nodulation and growth of some soybean germplasm. The results showed no interaction between the inoculation treatment and the genotype. The significant effect of inoculation was shown by the number of nodules, nodule dry weight, root length, and root dry weight. Inoculation did not affect plant height and shoot dry weight. These traits were more influenced by genetic factors. In the condition without inoculation, the root length was higher but the root dry weight was lower. Plant height differed between the genotypes. KPT5 and KPT6 showed the highest plant height than any other genotypes. Both genotypes also had the highest shoot dry weight. The highest shoot dry weight was also achieved by KPT4

Fatima et.al. "Effect of Rhizobium strains and Phosphorus on growth of soybean (Glycine Max) and survival of Rhizobium and P solubilization bacteria". Pak. J. Bot., 38(2): 459-464, 2006.

Pot experiments were conducted to evaluate the effect of Rhizobium leguminosarum strains (TAL-377, TAL-379 and TAL-102) alone and in combination with Phosphorus on soybean. The
parameters studied were survival of Rhizobium at pod filling stage and after harvesting, and root/
shoot dry and fresh weight of soybean under natural condition. Surface stererilized soybean seeds
var. NARC-4 were sown in earthen pots filled with soil and sand 1:3. Phosphorus (P) was applied

as single super phosphate (SSP) at the time of sowing in the soil. Soybean seeds were inoculated
with Rhizobium strains as seed coating just before sowing. The effect of growth was highly significant (α0.05) with an increase in root/shoot dry and fresh weight in plants with mixture of
Rhizobium inoculums with phosphorus on soybean. Among three strains TAL-102 performed well
as compared to TAL-377 and 379 Rhizobium strains. The CFU count of Rhizobium and P solubilizing bacteria was found maximum both at pod filling and after harvesting stage when Rhizobium strains and P was applied in mixed culture. A mixture of effective strains with phosphorus is a promising way for enhancing the growth of legume crop.

d) International/ National status.

Various studies are being carried out on biofertilizer application. Organic Farming Mission in India led by Govt. of India is dedicated towards the awareness of farmers towards the organic farming. Global warming and disturbed soil health is the major concern throughout world. Extensive use of chemical fertilizers is causing serious health problems globally. Therefore global Universities and research organizations are funding the research in this field.

e) Significance of the study:

In Marathwada region the major crop cultivated by farmers is soyabean. Successful completion of this study will revolutionized the cultivation practice of sugarcane crop. Also it will help farmer to boost their economy with increased production.

III. Objectives :

1. Study/review various types of research activity in nitrogen fixing and phosphate solubilizing bacteria .
2. Isolation of nitrogen fixing and phosphate solubilizing bacteria .
3 Determing activity of nitrogen fixing bacteria .
4 Determing activity of phosphate solubilizing bacteria
5. Determing effect of nitrogen fixing bacteria on growth of soyabean plant.
6. Determing effect of phosphate solubilizing bacteria on growth of soyabean plant.
7. Determing effect of nitrogen fixing bacteria on yield of soyabean plant.
8. Determing effect of phosphate solubilizing bacteria on yield of soyabean plant.

9. To contribute the assumption for the future development and formulation of biofertilizer

IV. MATERIALS AND METHODS :

2.1. pot Experimental Setup

A pot experiment was conducted in the net house from January to April of 2021 to evaluate the impact of seed inoculation with *Rhizobium japonicum* and *Pseudomonas striata* on the growth, yield, and quality of soybean as well as soil nutrient availability.

The experiment have to design and replicate three times. Twelve treatment combinations will use comprising four rhizobium treatments namely, R0: Control (no nitrogen and R. japonicum), R1: 100% N (as recommended for soybean in FRG [38] without R. japonicum, R2: 50% N with R. japonicum, and R3: only R. japonicum inoculant and three treatments for Pseudomonas viz. P0: Control (no P and no P. striata), P1: 100% P (as recommended for soybean in FRG [38]) without P. striata and P2: 75% P with P. striata. The pot (88 cm × 33 cm × 23 cm) was filled with 35 kg of soil, and BARI Soybean-5 was grown as a test crop. Seeds were collected from Bangladesh Agricultural Research Institute, Gazipur. Seed will sown at a row to row distance of 30 cm. After 15 days, thinning have to do to make the plants in a row approximately 5 cm apart from each other. 2.2. Initial and Post-Harvest Soil Analysis For the pot experiment, soil from unexploited land in latur district.

The selected site belongs to the Harangul village farm series .The site has a general soil type of shallow red brown terrace soil with sandy clay loam texture under the taxonomical class of Ultic Ustochrepts [39]. Before the beginning of the experiment, soil samples have to collecte from 10 random spots of the selected site at a depth of 0–15 cm to analyze chemical properties. Then, a composite soil sample was prepared for analysis by air-drying, grinding, and sieving by a 2 mm sieve.

1. After crop harvest, a soil sample have to collecte from each pot, and the post-harvest soil nitrogen and phosphorushave to estimated from the average of three repetitions of each treatment. The soil pH have to detecte by the method proposed by Mehmood et al. [40] and soil organic carbon by Walkley and Black [41]. Soil organic matter was calculated by multiplying organic carbon with conversion factor 2 as mentioned by Douglas [42]. Available phosphorus, potassium, and sulphur were determined according to the method described by Olsen et al. [43], Brown and Lilleland [44], and Victor and Nearpass [45], respectively. .

Chemical properties of soil (initial) used for the study. Soil Properties Value pH 7.2 Soil organic carbon (%) 0.48 Soil organic matter (%) 0.96 Nitrogen (%) 0.057 Available phosphorous (mg kg^{-1}) 2.83 Available potassium (mg kg^{-1}) 140 Available sulphur (mg kg^{-1}) 11 Agriculture 2022, 12, 1136 4 of 18 2.3. Application of Bio-Fertilizer and Chemical Fertilizer Two peat soil based biofertilizers were used in this experiments, one for Rhizobium japonicum and another for Pseudomonas striata. Both biofertilizers contained approximately 1 × 108 cells per gram of soil. The R. japonicum strain was collected from the nodules of a soybean plant and cultured and stored in Yeast Manitol Agar media (1 liter media contains mannitol—10 g, K_2HPO_4—0.5 g, $MgSO_4 \cdot 7H_2O$—0.2 g, NaCl—0.1 g, yeast—0.5 g, and agar—15 g) at 4 ◦C for 2 months before being used in peat based biofertilizer. On the other hand, P. striata strains were isolated from the rhizospheric soil of mungbean and grown and stored in Pikovskaya's Agar media (10 g glucose, 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4.7H_2O$, 0.5 g yeast extract, 15 g agar powder and a trace amount of $MgSO_4$ and $FeSO_4$ for 1 liter) at 4 ◦C for 1 month before use. Seeds were treated with Rhizobium japonicum inoculant and Pseudomonas striata inoculant at 20 g kg^{-1} seed before sowing. Except for nitrogen and phosphorus, all the nutrients were supplied as per the recommended fertilizer dose [38]. Nitrogen and phosphorus have to apply according to the treatment requirement, where 2.45 g urea and 5.25 g triple superphosphate were equivalent to 100% N and 100% P, respectively. In addition, approximately 3.5 g of muriate of potash, 6.9 g gypsum, 0.16 g zinc sulphate, and 0.10 g boric acid were supplied during the final soil preparation for the recommended dose of 50-18-1-0.5 kg ha^{-1} KSZnB. All the fertilizers were incorporated with soil during final land preparation. 2.4. Data Collection Data on plant height, leaf area index, total chlorophyll content, number of branches $plant^{-1}$, number of pods $plant^{-1}$, number of seeds pod^{-1} , and 100-seed weight were recorded. At maturity, five plants were selected to measure plant height, number of branches $plant^{-1}$, and number of pods $plant^{-1}$. Leaf area was determined with the help of a leaf area meter, and the leaf area index was calculated using Watson's formula [46]. Leaf area index = Leaf area Ground area The methods described by Arnon [47] and Zohaib et al. [16] were followed to measure the total chlorophyll content of fresh leaves at 60 days after sowing (DAS). For measuring chlorophyll content, 0.5 g of fresh green leaves was chopped into small pieces and 5 mL of 80% acetone was used to extract chlorophyll. The extract was then centrifuged for 5 min at a speed corresponding to 14,000× g. Finally, the absorbance of a supernatant at 645 nm and 663 nm was recorded and total chlorophyll content was calculated using the formula provided by Zohaib et al. [16]. Two plants were uprooted from each pot to calculate the number of nodules and nodule dry weight (mg $plant^{-1}$) at 30, 50, and 70 DAS. For determining dry weight, nodules were dried in an oven for 72 h at 65 ◦C and the constant weight was measured. Seeds were harvested from 60 cm × 33 cm of each pot and the yield was converted into t ha^{-1} . Meanwhile, the number of seeds pod^{-1} and 100-seed weight were also determined. Shoot was fine-ground and digested in acid to measure the phosphorus content by Jones and Case [48]. The nitrogen percentage of shoot, nodules, and seeds, was determined by the Official Methods of Analysis, AOAC [49] recommended standard: the Micro-Kjeldahl method. In this method, approximately 1 g of ground sample was taken in a digestion tube and 15 mL of concentrated sulfuric acid and 4 g of catalyst mixer were added. Then, the sample was digested at 380 ◦C for 120 min and nitrogen present in the sample was converted to ammonium sulphate.

V. Year-wise Plan of work and targets to be achieved:

Tentative phase wise plan of proposed study we have designed as per the convenience of the study.

Phase 1:

10. Study/review various types of research activity in nitrogen fixing and phosphate solubilizing bacteria .
11. Isolation of nitrogen fixing and phosphate solubilizing bacteria .

Phase 2:

1. Determing activity of nitrogen fixing bacteria .
2. Determing activity of phosphate solubilizing bacteria

Phase 3:

12. Determing effect of nitrogen fixing bacteria on growth of soyabean plant.
13. Determing effect of phosphate solubilizing bacteria on growth of soyabean plant.
14. Determing effect of nitrogen fixing bacteria on yield of soyabean plant.

15. Determing effect of phosphate solubilizing bacteria on yield of soyabean plant.

Phase 4:

1. To contribute the assumption for the future development and formulation of biofertilizer
2. Report Writing.

VI. References:

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9. Financial Assistance Required

Sr No	Item	Estimated Expenditure
1	**Books**	**Rs. 5,000/-**
2	**Equipments**	**Rs. 25,000/-**
3	**Field Work and Travel**	**Rs. 5,000/-**
4	**Chemicals and glassware**	**10000**
5	**Contingency**	**Rs. 5,000/-**
6	**Hiring Services**	**--**
	Total	**Rs. 50,000/-**

10. Whether the teacher has received support for the research project from the UGC under Major, Minor, and scheme of support for research or from any agency? If so, please indicate:

------ Yes --

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i. Name of the agency from which the assistance was approved :

College of Computer Science and Information Technology, Latur

ii. Sanction letter No. and date under which the assistance was approved

Amount approved and utilized : **Rs.50,000/-**

iii. Title of the project for which assistance was approved : "Rhizobium and Phosphate Solubilizing Bacteria effect on growth and productivity of soybean varieties"

iv. In case the project was completed, whether the work on the project has been published

No

v. If the candidate was working for the doctoral degree, whether the thesis was submitted and accepted by the University for the award of degree.

No

(A summary of the report/thesis in about 1,000 words may please be attached with the application)

vi. If the project has not been completed, please state the reasons

Not Applicable

11. (a) Details of the project/scheme completed or ongoing with the P.I

(b) Institutional and Departmental facilities available for the proposed work:

------- Yes -----

Equipments:

We have two Research Center departments like biotechnology and Computer science for P.G. and U.G Courses. We also have collaboration with nearest research centers.

Other Infrastructural Facilities:

1. Library
2. Computer Systems(P4)
3. Broad band Internet Connection
4. Microbiology laboratory
5. Microbiology research journals
6. Magazines
7. Research Review Papers

Royal Education Society's
[Govt. of Maharashtra approved, affiliated to S.R.T. Marathwada University, Nanded and included under Section 2(f) and 12(B) of U.G.C. Act 1956]
COLLEGE OF COMPUTER SCIENCE AND INFORMATION TECHNOLOGY(COCSIT), LATUR
संगणकशास्त्र व माहितीतंत्रज्ञान महाविद्यालय(कॉक्सिट), लातूर.
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Ph.: 02382-229191, Fax : 02382-228646, Website : www.cocsit.org.in, E-mail : cocsit365@rediffmail.com

Ref. No.:
Date :

Dr. N. S. Zulpe
M.C.S., M.Phil., Ph.D.
Principal
Cell : 9970763030

Certificate

To certify that:

a) I shall abide by the rules governing the scheme in case assistance is provided to me from the College for the above project.
b) I shall complete the project within the stipulated period. If I fail to do so and if the College is not satisfied with the progress of the research project, the Commission may terminate the project immediately and ask for the refund of the amount received by me/us.

c) The above research Project is not funded by any other agency.

Place : Latur

Date : 05/01/2022

College of Computer Sci.& Information Technology, Latur ★ 365

I/C. PRINCIPAL
College of Computer Science
and Information Technology, Latur

Principal Investigator
(Mr. K.P.Maknikar)

Principal
(Dr. N. S Zulpe)