International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 12, Issue 4, 2020, pp.-1816-1820.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

EXPLORING NEW WATER-SOLUBLE POWDER FORMULATION OF *RHIZOBIUM* AND ARBUSCULAR MYCORRHIZAE MEDIATED NODULATION AND YIELD ENHANCEMENT IN REDGRAM

GNANACHITRA M.[1]*, KUMUTHA K.[1], MARIMUTHU P.[1], SENTHILKUMAR M.[2] AND MARY K.[1]
[1]Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, 641 003, Tamil Nadu, India
[2]Agriculture College & Research Institute, Eachangkottai, Thanjavur, 614 902, Tamil Nadu Agricultural University, Coimbatore, 641 003, Tamil Nadu, India
*Corresponding Author: Email - gnanachitradavid@gmail.com

Received: April 02, 2020; Revised: April 26, 2020; Accepted: April 27, 2020; Published: April 30, 2020

Abstract- Many legumes form tripartite symbiotic associations with rhizobia and arbuscular mycorrhizal fungi; but still *Rhizobium* and phosphobacteria are commonly applied bioinoculants in pulse production. In order to study the tripartite association effect of *Rhizobium* and AM fungi, both pot culture and field experiment was conducted with two different bioinoculant formulations, *viz*., the existing carrier based and new water-soluble powder formulations to enhance the nodulation, nitrogen fixation and yield in redgram. Both in pot culture and field experiment, *Rhizobium* alone inoculated treatments recorded highest number of nodules/ plant as 7.4 and 10.1 nodules /plant respectively followed by coinoculation with AM fungi. Combined application of *Rhizobium* with both the formulations of AM fungi recorded the highest grain yield (1362 kg/ha) and performed on par with each other followed by *Rhizobium* alone inoculated treatments. Similarly, irrespective of the bioinoculants and formulations, all the inoculated treatments recorded increased available N and P content than uninoculated control.

Keywords- *Red gram, Rhizobium, AM fungi, Nodulation, Yield enhancement*

Citation: Gnanachitra M., *et al*., (2020) Exploring New Water-Soluble Powder Formulation of *Rhizobium* and Arbuscular Mycorrhizae Mediated Nodulation and Yield Enhancement in Redgram. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 12, Issue 4, pp.-1816-1820.

Academic Editor / Reviewer: Kiranjot Kaur

Introduction

Red gram (*Cajanus cajan* L.), commonly known as 'Arhar' a common protein rich pulse crop which plays a vital component in Indian diet because of its high-quality protein. The crop has growing demand particularly in India, but the area under this crop is reduced. Hence, there is a need to enhance the productivity with the existing area in order to meet the demand of growing population. Normally, redgram grown as either rainfed crop or grown in less fertile soil condition leads to poor nodulation, nitrogen fixation and thereby the yield. Particularly, in acidic soil condition nutrient availability is still very less leads to poor nodulation, nitrogen fixation and causes nutrient imbalance [1]. Normally legumes form intimate associations with both rhizobia and arbuscular mycorrhizal fungi (AMF) which is referred as tripartite symbioses. *Rhizobium* is the common micro-symbiont associated with legumes and fixes nitrogen in the specialized structure called nodules. In addition, mycorrhizal fungi also associated with legume plants and forms a triple association capable of supplying both N and P to the plants [2]. Therefore, legumes form intimate associations with arbuscular mycorrhizal fungi and rhizobia. Both mycorrhizal fungi and *Rhizobium* act as biofertilizers and have the unique ability to convert nutritionally important elements from unavailable to available form through biological process [3]. Mycorrhiza benefits the host through mobilization of phosphorus from non-labile sources, whereas rhizobia fixes N2 [4]. In general, legumes have a higher P requirement for nodule formation, nitrogen fixation and optimum growth. Hence, plant benefits include plant growth and yield increases, improved N and P nutrition, drought resistance, disease control and P solubilisation due to combined application of both Rhizobia and AM fungi [5]. Though many research studies are conducted on dual inoculation of *Rhizobium* and AM fungi in variety of legume crops, application of *Rhizobium* and AM fungi is not currently followed. Because the commonly applied biofertilizers *viz*., *Rhizobium* and phosphobacteria are lignite based recommended for seed treatment; while AM fungal biofertilizer is vermiculite based normally recommended for soil-based application @ 50 kg /ha. Hence, it is not normally followed by the farmers except research persons. In general, the nodulation is poor in redgram compare to other pulses and it has to be improved for effective nitrogen fixation and thereby the yield. To consider both the point of view *i.e*., use of both the biofertilizers in the same formulation by one application method, the present study was conducted in redgram using two different formulations *viz*., the carrier based *Rhizobium* (lignite) and AM fungi (Vermiculite) and new powder based water soluble formulations of *Rhizobium* and AM fungi [6-9].

Materials and Methods

The present experimental studies *viz*., the pot culture and field experiments were conducted in National Pulses Research Centre, Vamban, Pudukottai using VBN(Rg)3 redgram variety during the year 2016-18. The two formulations of *Rhizobium* (CPR 9) and AM fungi were obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, TamilNadu and used for the experiments at the recommended doses.

Microbial Inoculation

For microbial inoculation *Rhizobium* and Arbuscular fungi were used with both carrier-based formulation and new water-soluble formulations. Normally seed treatment of *Rhizobium* and phosphobacteria is followed for redgram cultivation. In this study, the new water-soluble powder formulation of *Rhizobium* and AM spore were used by coating the redgram seeds using polymer to enhance the nodulation and yield in redgram. In conventional formulation the standard *Rhizobium* is used as lignite based at the rate of 600g/ha seed and AM fungi was used as vermiculite-based formulation [Fig-1] applied at the rate of 50 kg/ha. The water-soluble powder formulation contains high cell load of *Rhizobium* (1x 1011/ g) and spore load (10,000 spores/ g) of AM fungi applied as seed coating at the rate of 10g/ac of seed using polymer [Fig-2].

Hence, in both the formulations *Rhizobium* was given by seed treatment whereas, AM fungi was inoculated by seed treatment (new water-soluble powder formulation) and soil application (vermiculite formulation). The effect of both the formulations of *Rhizobium* and AM fungi on redgram were studied for effective nodulation by taking the biometric observations *viz*., number of nodules/ plants on 45th days after sowing.

Rhizobium (lignite based) AM fungi

Fig-1 Carrier based formulations

Rhizobium AM fungi

Fig-2 Water soluble Powder formulation

Pot culture Experiment

Pot culture experiment was conducted to study the synergistic interaction between AM fungi, *Rhizobium* and their effect on growth, nodulation with the above-mentioned treatments [Fig-3]. The experiment was conducted using sterilized soil and the redgram seeds were sown with the following treatment details. The biometric observations *viz*., root length, shoot length, number of nodules/ plant and AM fungal infection in the inoculated roots were recorded on 45th days after sowing. Soil analysis were analysed for available N, P, K and Fe.

Treatment details:

T1: Uninoculated control
T2: Seed treatment with lignite based formulation of *Rhizobium* (CPR 9)
T3: Soil application of vermiculite based formulation AM fungi
T4: Seed treatment of new water soluble powder formulation of AM fungi
T5: Seed treatment of water soluble powder formulation of *Rhizobium*+ AM fungi
T6: T2 +T3

Fig-3 Pot culture experiment on the effect of *Rhizobium* and AM fungi

AM colonization test

The Arbuscular mycorrhizal infection was tested in both vermiculite based formulation and in the new water soluble formulation. The inoculated redgram plant samples were collected and tested for the presence the Arbuscular fungal colonization interms of presence of fungal body structures *viz*.,vesicles and arbuscules. The AM colonization was estimated by adopting the procedure described by [10].

Fig-4 AM colonization test of red gram roots by root clearing and staining method

The roots were cut into 1 cm bits and immersed in FAA solution (Formaldehyde 5 ml: Glacial acetic acid 5 ml: Alcohol 90 ml) and were immersed in 10 percent potassium hydroxide. After autoclaving at 5 Lbs pressure for 10 minutes, potassium hydroxide was poured out and the root bits were washed with water for 3 - 4 times. Then immersed in 30 percent hydrogen per oxide solution for 10 - 15 minutes, then decanted and rinsed with water. These were then immersed in 2 percent hydrochloric acid for 5 minutes and the excess acid was decanted. The root bits were stained with 0.05 percent tryphan blue in lacto phenol (Lactic acid, Glycerol: water at 40 ml: 40ml: 20ml) and boiled for about 10 minutes [Fig-4]. One hundred root bits were examined for each sample under stereo zoom binocular microscope for studying AM colonization and colonization percent was calculated as below,

AM Colonization percent = Total number of root bits infected / Total number of root bits examined x 100

Field experiment

Based on the plant infection conducted at pot culture experiment, the two formulations of *Rhizobium* and Arbuscular fungi were taken for field study in order to confirm the combined effect of *Rhizobium* and Arbuscular Mycorrhiza and to compare the two different formulations of *Rhizobium* and AM fungi (carrier based & powder formulation) on growth, nodulation in redgram and on soil parameters another field experiment was conducted during kharif' 2017 on 21.09.2017 with the following 7 treatments and three replications using VBN(Rg) 3 variety. The biometric observations *viz*., root length, shoot length, number of nodules/ plant and nodule dry weight/plant were recorded on 45th days after sowing and the yield parameters taken at the time of harvest.

Treatment details:

T1- Uninoculated control
T2 -*Rhizobium* (CPR 9) -seed treatment
T3- AM fungi (Vermiculite based formulation- soil application @ 50 kg/ha)
T4- *Rhizobium* (Powder formulation @ 10g/ac seed)
T5 -AM spore (powder formulation @ 10g/ac seed)
T6 -T4+ T5
T7- Seed treatment of *Rhizobium* (lignite based)+Soil application of AM fungi (Vermiculite based)

Fig-5 Field experiment on the effect of bioinoculants (*Rhizobium* + AM spore) in redgram

Experimental Results & Discussion

Pot culture experiment

Effect on plant growth and nodulation

The result revealed that combined application of seed treatment of *Rhizobium* + soil application of AM fungi (T6) recorded significantly higher number of nodules (7.4/plant), root, shoot length and biomass dry weight as 20.3, 138.4 cm and 158.8 g/plant respectively followed by seed treatment of *Rhizobium* alone (T2) recorded 7.2 /plant. Un-inoculated control recorded the least no.of nodules as 5.4/plant [Table-1]. Similarly in plant dry weight also T6 recorded significantly higher plant dry weight as 158.8 g/plant followed by T2 (Seed treatment with *Rhizobium* alone) recorded 158.4 g/plant.

Effect of AM fungal colonization in redgram roots

Among the different treatments, irrespective of the formulations all AM fungi inoculated treatments (T3 to T6) showed better colonization interms of fungal hyphae and presence of vesicles both in individual and combined application with *Rhizobium*.

Table-1 *Effect of bioinoculants (Rhizobium + AM spore) on the growth and nodulation in redgram under pot culture condition*

SN	Treatments	Mean value of 3 replications			
		Nodule number/ plant	Root length (cm)	Shoot length (cm)	Plant biomass dry weight (g/plant)
1	T1- Uninoculated control	5.4	17.2	112.4	112.4
2	T2- Seed treatment with lignite-based formulation of *Rhizobium* (CPR 9)	7.2	16.8	129.6	158.4
3	T3- Soil application of vermiculite-based formulation AM fungi alone	5.8	18.0	125.4	124.2
4	T4- Seed trmt of water-soluble powder formulation of AM fungi	6.5	16.4	113.3	119.6
5	T5- Seed trmt. of water-soluble powder formulation of *Rhizobium*+ AM fungi	6.8	19.1	121.9	157.4
6	T6-T2 + T3	7.4	20.3	138.4	158.8
	SEd±	0.04	1.018	3.77	5.83
	CD @(0.05)	0.09	2.17	8.03	12.43

Table-2 *Effect of Rhizobium+AM fungi on soil available nutrient status in redgram under potculture condition*

SN	Treatments	Soil available major nutrients status (kg /ha)			Fe
		N	P	K	(ppm)
1	T1- Uninoculated control	190.0	65.0	89.0	2.528
2	T2- Seed treatment with lignite-based formulation of Rhizobium (CPR 9)	218.0	75.0	103.0	2.894
3	T3- Soil application of vermiculite-based formulation AM fungi	202.0	70.0	107.0	2.016
4	T4- Seed trmt of new water-soluble powder formulation of AM fungi	193.0	120.0	129.0	2.004
5	T5- Seed trmt. of water-soluble powder formulation of *Rhizobium*+ AM fungi	193.0	135.0	224.0	2.416
6	T6-T2 + T3	188.0	65.0	102.0	2.868

Table-3 *Effect of bioinoculants (Rhizobium + AM spore) on the growth in redgram under field condition*

SN	Treatments	Mean value of 3 replications					
		@ 45 DAS				@ harvest	
		Nodule number /plant	Nodule dry wt.(mg/pt)	Root length (cm)	Shoot length (cm)	Plant dry weight (g/pt)	Grain yield (kg/ha)
1	T1- Uninoculated control	7.2	5.1	12.6	83.1	79	683
2	T2- Seed trmt of *Rhizobium* (lignite based)	13.7	9.7	13.1	95.4	111	841
3	T3- Soil appln.of AM fungi (vermiculite based)	12.4	10.6	12.4	89.8	102	984
4	T4- Seed trmt.of *Rhizobium* (New powder formulation)	12.5	7.2	13.4	86.7	105	857
5	T5- Seed trmt. of AM spore (New powder formulation)	12.4	8.7	14.4	87.6	132	964
6	T6- Seed trmt. of new powder formulation of *Rhizobium* + AM fungi	13.7	8.6	14.0	97.7	132	923
7	T7- Seed trmt of *Rhizobium* + Soil appln. of AM fungi	11.7	13.5	13.2	84.4	120	1012
	SEd±	1.05	1.07	1.21	5.26	6.33	55.8
	CD @(0.05)	2.28	2.33	2.64	11.46	13.79	121.6

Table-4 *Effect of Rhizobium + AM fungi on soil available nutrient status under field condition*

SN	Treatments	Soil available major nutrients status (kg /ha)			Fe
		N	P	K	(ppm)
1	T_1- Uninoculated control	181	8.1	184	7.80
2	T_2- Seed trmt of *Rhizobium* (lignite based)	194	9.2	182	7.50
3	T_3- Soil appln. of AM fungi (Vermiculite based)	192	9.0	176	7.38
4	T_4- Seed trmt.of *Rhizobium* (New powder formulation)	184.0	8.9	190	7.39
5	T_5- Seed trmt. of AM spore (New powder formulation)	191.4	9.0	201	7.42
6	T_6- Seed trmt. of new powder formulation of *Rhizobium* + AM fungi	196	9.5	198	7.61
7	T_7- Seed trmt of *Rhizobium* + Soil appln. of AM fungi	190	9.4	204	7.64

Similarly, AM fungi inoculated plants recorded higher percentage of root infection in terms of vesicles and colonization compared to the *Rhizobium* alone (T2) inoculated plants [Table-2] & [Fig-1]. Among the two formulations, the new water soluble powder formulation of AM fungi recorded more percentage of AM fungi infection seen by the presence of more number of vesicles compared to conventional formulation of AM fungi [Fig-3a] & [Fig-3b].

Effect on soil available nutrient status

Soil analysis report clearly indicated that all the inoculated treatments recorded increased available N and P content than uninoculated control. Among the treatments, seed treatment of lignite-based *Rhizobium* alone inoculated (T2) recorded more available N (218 kg/ha) than other treatments. The increase in available P and K was observed more in AM fungi spore formulation treated (both alone and in combination with *Rhizobium*) plots (T4 & T5) as 120 & 135 kg/ha and 129 & 224 kg/ha respectively. Regarding the change in micronutrients, carrier-based formulation of AM fungi (T6) and *Rhizobium* (T2) recorded increase in available Zn and Fe content as 2.39 & 1.28 ppm and 2.87 & 2.89 ppm respectively. The available Fe content was reduced in all the inoculated treatments indirectly indicated the mobilization of soil Fe to the plants by the microbial inoculants [Table-3].

Hence, the results clearly indicated that in redgram, the available nitrogen was increased in the seed treatment of lignite based formulation of *Rhizobium* (T2) whereas, the available 'P' and 'K' content application increased in seed treatment of new powder formulation of AM spore both in individual (T4) and combined application with *Rhizobium* (T6). AM fungi increased the available P level and in turn available N than control indicated the synergistic effect between *Rhizobium* and AM fungi was observed as marginal.

Regarding the soil available Fe content, unlike the other parameters except uninoculated control, soil Fe content was reduced in all the inoculated treatments. The reduction in soil Fe may be due to the uptake of soil Fe in all inoculated plants and deposited in the plant biomass which reflected in increased plant biomass dry weight in all the inoculated plants than control.

Nodulation and plant growth effect under field condition

Under field condition also all the inoculated treatments recorded significantly higher number of nodules and on par with each other. Among the inoculated treatments, seed treatment of *Rhizobium* in both the formulations either alone (T2) or combined application with new powder formulation of AM fungi (T6) recorded the highest nodules as 13.7/plant and on par with each other.

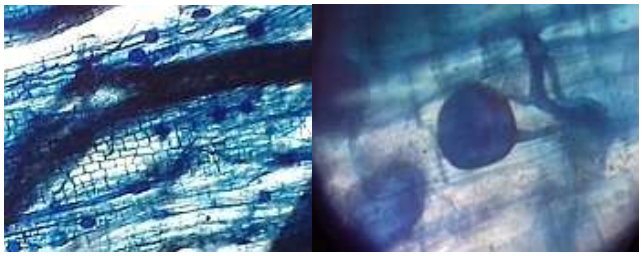

Fig-3a Fungal colonization in the infected redgram roots

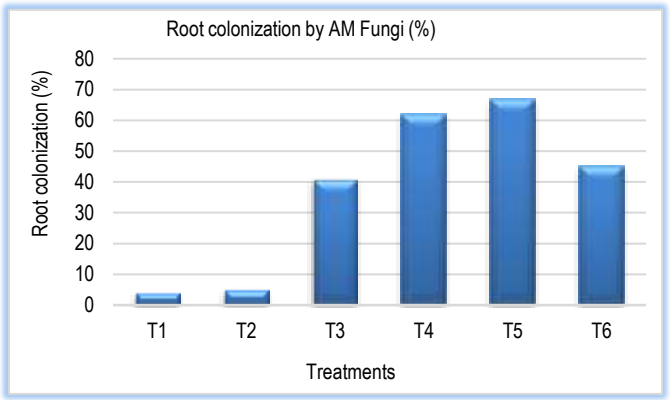

Fig-3b Effect of AM fungal formulations on root colonization in redgram

Regarding nodule dry weight, combined application of AM fungi (soil application)+ seed treatment of lignite based *Rhizobium* (T7) recorded the highest value of 13.5mg per plant followed by seed treatment of *Rhizobium* alone (T2) recorded 10.6 mg/plant [Table-4]. Among the two formulations of *Rhizobium*, lignite based recorded more nodulation in individual application; whereas, new formulation of *Rhizobium* recorded more nodulation in combined application with AM fungi.

Effect of *Rhizobium* and AM fungi normally increase root and shoot development; because they contribute for N and P supply respectively for the plant growth. In the present study also inoculated plants recorded more root and shoot length compared to control.

Effect on yield parameters

In Plant biomass dry weight except uninoculated control, all the other inoculated treatments recorded increased biomass dry weight. Among the treatments, the new water soluble powder formulation of AM fungi inoculated plants (T5) registered significantly highest dry weight (132 g/plant) followed by soil application of vermiculite based AM fungi (T7) recorded 120 g/plant. Since, application of AM fungi in general increases the root biomass than other bioinoculants that may be reason for the more plant biomass dry weight compared to other treatments. Regarding yield also, though numerically T7 recorded the highest grain yield (1012 kg/ha), it is on par with T3, (AM fungi - soil application of vermiculite based formulation- @ 20kg/ha), T5 (WSF @ 10g/ac seed) and T6 (*Rhizobium* (seed treatment) + AM (soil application) registered 984, 964 & 923 kg/ha respectively. Uninoculated control recorded 683 kg/ha which is significantly lower than all the other treatments [Table-4].

Overall, in terms of seed yield combined application of *Rhizobium* and AM fungi in both the formulations performed on par and recorded the highest. Irrespective of the formulations, the increase in yield in combined application is may be due the synergistic effect of both *Rhizobium* and AM fungi.

Effect on soil available nutrient status under field condition

In the field experiment also, all the inoculated treatments recorded increased available N and P content than uninoculated control. Among the inoculated treatments, combined application of AM fungi + *Rhizobium* (T5 & T6) recorded slightly more soil available P content (9.5 & 9.4 kg/ha) compared to other inoculated treatments. Regarding the soil available Fe content, unlike the other parameters except uninoculated control, soil Fe content was reduced in all the inoculated treatments. The reduction in soil Fe may be due to the uptake of soil Fe in all inoculated plants and deposited in the plant biomass which reflected in increased plant biomass dry weight in all the inoculated plants than control.

Discussion

Effect on nodulation

To increase redgram productivity in a sustainable way with the available area, bioinoculants play a vital role. Of which, *Rhizobium* and phosphobacteria are the common applied biofertilizers; application of AM fungi also could enhance the nodulation in tripartite symbiotic way. Based on this, the study was conducted with two different formulations of *Rhizobium* and AM fungi. Irrespective of the formulations, seed treatment of *Rhizobium* alone and co-inoculation with AM fungi performed better in terms of nodulation. In addition to *Rhizobium*, AM fungi also associated with many of the legume crops [2]. Associative action of mycorrhizal fungi in legumes has a great impact on root, shoot development and phosphorous uptake which results in the enhancement of nodulation and nitrogen fixation [11].

Similar result recorded that *Rhizobium* increased the nodulation number/plant both in individual application and combined application with Arbuscular mycorrhiza which supported the results of the present findings [12]. In general, the dual application with mycorrhiza and Brady *Rhizobium* significantly increased nodule number of soybean when compared to control [13]. Similar effect already recorded that, associative actions of mycorrhizal fungi in legumes improve phosphorus uptake which results in the enhancement of nodulation and nitrogen fixation [11, 14]. It has also been reported that dual inoculation of AM fungi and *Rhizobium* had a significant effect on the nodulation in green gram [15, 16]. There are many researches carried out in the past few decades on various aspects of root symbionts *viz*., *Rhizobium* and AM fungi and their dual interactions in many legume crops [17-21].

Effect on yield

Regarding the yield also, irrespective of the formulations, combined application of *Rhizobium* + AM fungi recorded significantly higher grain yield revealed that both *Rhizobium* + AM fungi are synergistically interacted. The result was in agreed with work, also revealed the synergistic effect in dual inoculation with micro-symbionts *viz*., *Glomus fasciculatum* and *Rhizobium* was remarkable in pigeon pea [20].

The results highlighted that mutualistic double symbiosis by *Rhizobium* and VAM fungi provided better growth than either of the single symbiotic microbial symbiosis with leguminous crop plants. Several workers have also examined the interactions between different AMF species and *Rhizobium* species strains, and concluded the growth and productivity of the legumes were dependent on the specific combination of AMF and rhizobia indicating that synergistic interactions between compatible micro-symbionts resulted in growth and yield increases [22, 23].

Effect on soil nutrient status

In general, all the inoculated treatments recorded increased available N P& than uninoculated control. Combined application of new powder formulations of AM fungi +*Rhizobium* inoculated treatments recorded increased available N and P content than application of existing carrier-based AM fungi + *Rhizobium* indicated that the synergistic effect between *Rhizobium* and AM fungi was observed as marginal. The available Fe content was reduced in all the inoculated treatments indirectly indicated the mobilization of soil Fe to the plants. Similar to the present result, the yield increase in pigeon pea observed in compatible AMF + rhizobia treatments were probably due to enhanced N and P uptake [5]. The growth and yield increase of legumes inoculated with AMF and rhizobia is generally due to enhanced N and /or P uptake [22-26]. An overall increase in nitrogen and phosphorus contents in the inoculated pigeon pea plants with both *G. fasciculatum* and *Rhizobium* as compared to control. The dual inoculation with microsymbionts revealed synergistic effect. They also suggested that dual inoculation have the capability to increase the nutrients content and chlorophyll content of pigeon pea [20]. There was overall increase in nitrogen and phosphorus contents in the treated plants as compared to control. However, maximum nitrogen and phosphorus contents were recorded in the plants dually inoculated with *G. fasciculatum* and *Rhizobium* [27]. Moreover, combined application of AM fungi and *Rhizobium* also increase rhizospheric microflora *viz*., acid producers and phosphate solubilizes causing more available phosphorus [28]. AM fungi supported nitrogen fixation by providing legumes with phosphorus and other immobile nutrients which are essential for nitrogen fixation [29].

An effective AM fungus can enhance the performance of rhizobial infection and vice versa which was also evident for the present investigation [30]. The results of the present study indicated that combined inoculation of both carrier based and powder formulations of AM fungi and *Rhizobium* had a synergistic effect resulting in the improvement of nitrogen and phosphorus.

Conclusion

With the less available area under cultivation and to meet the demand of the growing population, there is an immense need to enhance redgram productivity. To increase the productivity in a sustainable way, in addition to the regular biofertilizers *viz*., *Rhizobium* and phosphobacteria, another microsymbiont namely AM fungi also can be exploited. Though many studies are available on combined effect of *Rhizobium* with AM fungi, it is yet to be in application. Hence, to narrow down the disadvantages interms of dosage and usage in the existing carrier-based formulation of *Rhizobium* and AM fungi, in this study a new powder formulation of *Rhizobium* and AM fungi was introduced. This new powder formulation having more population and spore load and both can be coinoculated and applied through seed treatment. From the study it was concluded that either seed treatment of carrier based *Rhizobium* (@ 600g/ha) and soil application of AM fungi @ 50 kg/ha or seed treatment of new powder formulation of *Rhizobium* and AM fungi each @ 10 g/ac is better for enhancing nodulation, nitrogen fixation and yield in redgram under rainfed condition is an ecofriendly way.

Application of research: Seed treatment of powder formulation of *Rhizobium* and AM fungi for pulses for sustainable yield enhancement.

Research Category: Pulse productivity

Abbreviations: AM fungi-Arbuscular Mycorrhizal fungi

Acknowledgement / Funding: Authors are thankful to Dr K. Ramasamy, former Vice-Chancellor (TNAU), Coimbatore for advised to take up this study in redgram, The Professor and Head, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, 641 003, India for providing the new bioinoculant formulations and guidance; the Professor and Head, National Pulses Research Institute, Vamban for the successful conduct of the experiments and providing facilities.

****Principal Investigator or Chairperson of research: M. Gnanachitra**
University: Tamil Nadu Agricultural University, Coimbatore, 641 003, India
Research project name or number: Arbuscular Mycorrhizal mediated nodulation and nitrogen fixation in Redgram- NRM/VMB/AGM/RGR/2015/001

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: National Pulses Research Centre, Vamban, Pudukottai

Cultivar / Variety / Breed name: Redgram

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

References

[1] Foy C.D. (1984) *Soil acidity and liming. American Society of Agronomy, Madison*, 57-98.
[2] Silveira A. P. D. and Cardoso E. J. B. N. (2004) *Agricultural Science*, 61, 203-209.
[3] Hegde D.M., Dwivedi B.S. and Sudhakara S.N. (1999) *The Indian Journal of Agricultural Sciences*, 69,73-83.
[4] Scheublin T.R. and Vander Heijden M.G.A. (2006) *New Phytologist*, 172 (4), 732-738.
[5] Lisette J.C. Xavier J. and Germida J. (2003) *Biol. Fertil Soils.*, 37:261–267.
[6] Jain A.K., Kumar S. and Panwar J.D.S. (2007) *Adv. Plant Sci*., 20(2), 337-339.
[7] Rahman M.K., Kabir S.M., Mohsin G.M., Alam M.D. and Mandal R. (2008) *J. Phytol. Res*., 21(2), 247-251.
[8] Ray J.G. and Valsalakumar N. (2009) *J. Plant Nut*., 21, 3-4.
[9] Thenua O.V.S., Singh S.P. and Shivakumar B.G. (2010) *Ind. J. Crop Sci*., (12), 253-261.
[10] Philips J.M. and Hayman D.S. (1970) *Transactions British Mycological Society*, 55(1), 158-161.
[11] Albrecht C., Geurts T. and Bisseling R. (1999) *EMBO J*. 18, 281–288.
[12] Salih S.H., Hamd S.A.M., Dagash Y.M.I. (2015) *Universal Journal of Agricultural Research*, 3(1),11-14.
[13] Taiwo L.B. and Adegbite A.A. (2001) *Moor Journal of Agricultural Research*, 2, 110-118.
[14] Poi S.C., Ghosh G. and Kabi M. C. (1989) *Zentralbl Mikrobiolog* 144, 249–253.
[15] Singha F.M. and Sharma G.D. (2013) *Life Science*, 2 (6), 2277 – 8160.
[16] Bhat M.I., Rashid A., Faisul-urRasool, Mahdi S.S. Haq S.A. and Bhat R.A. (2010) *Research Journal of Agricultural Sciences*, 1(2), 113-118.
[17] Khan I.A., Ayub N., Mirza S.N., Nizam S.M. and Azam M. (2008) *Pak. J. Bot.*, 40(2), 939-945.
[18] Talaat N.B. and Abdallah A.M. (2008) *J. Applied Sci. Res*., 4(9),1092-1102.
[19] Murat E., Demir S., Tufenkci E.O.S., Oguz F. and Akkopru A. (2011) *Field Crops Res*., 122(1), 14-24.
[20] Sujatha B. and Sharma G.D. (2012) *Advances in Microbiology*, 2, 561-564.
[21] Yaseen T., Ali. K., Munsif F., Rab A., Ahmad M., Israr M. and Baraich A.K. (2016) *Pak.J.Bot*., 48(5), 2101-2107.
[22] Ruiz-Lozano J.M. and Azcon R. (1993) *Symbiosis*, 15, 217–226.
[23] Xavier L.J.C. and Germida J.J. (2002) *Soil Biol Biochem*., 34,181–188.
[24] Manjunath A., Bagyaraj D.J. and Gowda H.S.G. (1984) *Plant Soil,* 78, 445-448.
[25] Pacovsky R.S., Fuller G. and Stafford A.E. (1986) *Plant Soil*, 92, 37-45.
[26] Azcon R., Rubio R. and Barea J.M. (1991) *New Phytol*., 117, 399-404.
[27] Karim M.R. Islam F. Akkas Ali M. and Haque F. (2001) *Bangladesh Journal of Agricultural Research*, 26, 93-94.
[28] Lipman J. G. and Conybeare A.B. (1936) *New Jersey Agricultural Experiment Station Bulletin*, 607.
[29] Clark R. B. and Zeto S. K. (2000) *Journal of Plant Nutrition*, 23, 867-902.
[30] Tavasolee A., Aliasgharzad N., Salehijouzani G., Mardi M. and Asgharzadeh A. (2011) *African Journal of Microbiology*, 10, 7585-7591.