Immobilization of beneficial microbe Methylobacteriumaminovorans in electrospun nanofibre as potential seed coatings for improving germination and growth of groundnut Arachis hypogaea

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Abstract

Seed inoculation with microbial cells is one of the potential invigouration techniques for enhancing the emergence and growth of plants. Herein, we approached a new localized delivery of beneficial microbial cells (*Methylobacterium*) by invigorating seeds with electrospun Polyvinyl alcohol (PVA) nanofibre contains microbial cells. *Methylobacterium is* a growth promoting bacteria recently draws attention in agriculture particularly for drought management. PVA was used in this research because of its electrospinnability and biodegradability. Encapsulation study shows effective immobilization of bacteria cells (*Methylorubrum aminovorans*) in PVA nanofibre, and SEM and TEM characterization further confirmed the entrapment of microbial cells. The microbial plating enumeration reveals 6.6 x 10^5 CFU g^{-1} of nanofibre to the initial loading population of 1 × 10^8 CFU. Viability of nanofibre encapsulated bacterial cells under ambient environment found 1.85 × 10^5 CFU g^{-1}, 2.2 × 10^4 CFU g^{-1} and 1.2 × 10^4 CFU g^{-1} on 10, 20 and 30 days after storage. *In vitro* bio-efficacy study exhibits that the seeds coated by PVA nanofibres containing *M. aminovorans* recorded higher germination, root & shoot length, seedling vigour, drymatter production, plant biomass, plant root volume, nodule numbers and fresh weight of nodules. The data of this study concludes that microbial cells could be immobilized in electrospun nanofibre for extended shelf-life of microbial cells and as effective seed coating for localized delivery.

Introduction

Plant growth promoting beneficial soil bacteria that cause a positive effect on plants through direct and indirect mechanisms. The beneficial effect of bacteria to increase plant nutrient uptake, nitrogen fixation and siderophore production. Inoculants can be applied to seeds with plant growth promoting microbes is viable tool to deliver of beneficial microorganisms to the soil where emerging plant roots may be colonized by them and to increase plant development. Nanofibers technology are currently practiced for seed treatment with chemical/biological substances/microbes encapsulation that improve seedling establishment and promote plant growth. Electrospinning is an advanced technology wherein liquid polymer is converted as fibre ranges from few nano to micro meter in diameter with high surface area when exposed to high voltage current (Li and Xia 2004; Bhardwaj and Kundu 2010; Sullivan et al. 2014). The high surface area to volume ratio and easy incorporation of active molecules has impelled researchers to investigate using electro spun nanofibers in agricultural application such as nano sensors for pesticide residues detection (Ding et al. 2009), protective clothing for farm labourers and agrochemical inputs (Lee and Lee 2012). Recently, seed invigoration with nanofibres developed from various biodegradable polymers was explored as an alternate approach for smart delivery of inputs (Krishnamoorthy et al. 2016; Krishnamoorthy and Rajiv 2017; Hussain et al. 2019). Further, e-spun fibre has been investigated as effective seed coating for localized and targeted delivery of inputs for improving germination and seedling growth of rice (Castaneda et al. 2014), black gram (Raja et al. 2020) and groundnut (Raja et al. 2020; Raja et al. 2017), and also for pathogen control in soyabean (Farias et al. 2019). The formation of fibre from polymer in electrospinning depends on various properties on various properties like solution parameters, solvent composition, and processing conditions (Yadav et al. 2019)

and there are many biodegradable polymers tested for production of fibres among which Polyvinyl alcohol (PVA) is one which has excellent physical and chemical properties (Damasceno et al. 2013). PVA is a semi-crystalline polymer that is biocompatible with high thermal and chemical stability (Mojaveri et al. 2020).

Generally, the growth promoting bacteria are inoculated in plants either by direct application of liquid or solid based formulation to soil or coating over seeds. The bacterial cells is applied over seeds through carrier based commercial formulations reduce the viability of organism due to toxin of the exudates of seed coat, increased environmental temperatures during inoculation and sowing. This focuses to develop an alternative, viable and cost effective technique. Encapsulation of microbes in biodegradable polymer e-spun nanofibre improves the shelf- life, and has been proposed as an alternative emerging and viable technique over the last half decades. Encapsulation of microbial cells in PVA nanofiber, would be an innovative one that could protect the microbial cells by providing favourable environment. Preserving, storing, and maintaining the biologically active materials in the fiber offer various advantages in the agricultural research. Encapsulation of bacteria in electrospun PVA nanofibre extended the shelf life period and survivability for three months without further loss (Semnani et al. 2018). Damasceno et al. (2013) demonstrated the successful rhizobial encapsulation in PVA electrospun nanofibre to protect the microbes and increase the shelf-life besides improved germination, seedling emergence, number of root nodules, plant biomass and growth in soybean seeds invigorated with rhizobium loaded nanofibres. Herein, we focused to entrap Methylotrophs, they are gram negative bacteria belonging to the genus *Methylobacterium* capable of colonize nodules and other plant tissue by using their ability to utilize single carbon substrates as a competitive advantage (Sy et al. 2005), fixing nitrogen, improving germination and growth (Meenakumari and Shehkar, 2012) by producing plant growth regulators like zeatin, cytokinins and auxins (Ivanova et al. 2005), seed inoculation with this microbial cells improves germination and growth even under limited water availability. Considering the potentiality of e-spun nanofibre, the present investigation was hypothesized with entrapping bacterial cells (*Methylorubrum aminovorans*) in electrospun PVA polymer matrix for extending the viability, survivability and as effective seed coating in groundnut for enhanced germination, seedling and growth eventually with increased productivity.

Materials And Methods

Material

Genetically and physically pure seeds of groundnut variety TMV13, Polyvinyl alcohol (PVA) having molecular weight of 1,15,000 g/mole purchased from M/s. Sigma Aldrich Ltd, and *Methylorubrum aminovorans* were the experimental materials

Development and characterization of PVA nanofibre for effective microbial immobilization

Polyvinyl alcohol (PVA) prepared at various concentrations *viz.*, 4, 5, 6, 7, 8 and 9 % (w/v), subjected for electrospinning at constant voltage of 15 kV and flow rate of 0.6 ml per hour with constant distance

between collector and tip of the needle. The fibres were characterized for morphological feature using Scanning Electron Microscope (SEM-Quanta 250, FEI, Netherlands).

Optimization of microbial cell concentration for nanofibre encapsulation

Microbial cell concentration for nanofibre encapsulation was standardized by adding the microbial cells obtained from cell shrinking technique and PVA polymer at 7 %. For this, PVA of 14 % solution was prepared and blended with equal volume of microbial inoculums contains 10^8 CFU mL^{-1} cells as detailed below and the blend was run for electrospinning and microbial cell concentration was optimized by observing the bacteria growth in electrospun nanofibre through imprinting method (Table 1.).

Immobilization of *Methylorubrum aminovorans* in electrospun PVA nanofibre and characterization

E-spin mixture prepared by blending optimized concentration of PVA and *Methylorubrum aminovorans* cells, loaded in to the syringe and electrospun of 15 kV and flow rate of 0.6 mL per hour with constant distance between collector and tip of the needle (monoaxial loading: It is type of electrospinning wherein the sheath (polymer) and the core (microbial cells) materials mixed together to form a single solution for electrospinning to form fibres). The Pink-Pigmented Facultative Methylotrophs (PPFMs) loaded fibres were characterized for morphology by SEM and TEM (TEM FEI TECHNAI SPRIT). PPFMs population encapsulated in PVA electrospun nanofibre was assessed by microbial plating method (James 1958).

Assessment of nanofibre encapsulated microbial viability

E-spin mixture (Microbial inoculums & PVA polymer blend) of 10 ml containing microbial load of 10^8 CFU mL^{-1} was prepared and immobilized in PVA nanofibre as detailed earlier. The microbial cells loaded nanofibre was divided into equal strips of 1 x 1 cm size each and calculated the initial microbial cells loading (James 1958). Then a total of 30 strips were selected randomly and stored under ambient conditions. The viability of bacteria was assessed by counting the microbial cells on 10, 20 and 30 days after inoculation.

PPFMs (*Methylorubrum aminovorans)* immobilized nanofibre seed invigouration on germination and seedling growth

The pure seeds of groundnut var. TMV 13 with initial viability 74 % surface sterilized with 0.2 % mercury chloride, and invigorated with *Methylorubrum aminovorans* (T_2), Polyvinyl alcohol nanofibre (T_3) and *Methylorubrum aminovorans* immobilized PVA nanofibre (T_4). The E- spun microbial cells encapsulated nanofibres along with PVA fibres (without bacteria cells) were applied electrostatically to the seed surface in electrospinning unit at the flow rate 0.6 mL/h and voltage of 15 kV with constant distance between needle tip and collector plate. The nanofibre invigorated seeds along with microbial cells alone (T_2) and uncoated seeds (T_1) seeds were evaluated for germination and seedling growth (IRST 2013), vigor index (Abdul-Baki and Anderson 1973) and drymatter production (Gupta 1993) under controlled laboratory conditions.

Effect of PPFMs (*Methylorubrum aminovorans)* immobilized nanofibre seed invigoration on plant growth parameters under pot culture

Nanofibre and microbial cells invigorated along with untreated seeds evaluated for its impact on plant growth parameters under pot culture experiment *in vitro* conditions. Pots with 30 × 30 cm size filled with field soil, and seeds were sown in equal distance and depth of 2.5 cm, and watered as per requirement. Observation on emergence, seedling length, plant growth, root volume, root nodules number and fresh weight and plant biomass was recorded.

Statistical analysis

The data obtained from various experiments were analyzed statistically by adopting the techniques (Panse and Sukhatme 1967). The critical differences (CD) were calculated at 5 % probability level. The data recorded in percentage were transformed into angular values (Arcsine transformation) wherever needed.

Results And Discussion

Synthetic hydrophilic polymer PVA was used to develop nanofibre with optimum size for encapsulating the beneficial microorganism *Methylorubrumaminovorans*. The results of fibre production revealed that quality fibres were developed with diameter size of 93.3 to 166.1, 164.8 to 218.2 and 194.4 to 303.2 nm at 7, 8 and 9 % respectively without formation of beads (Fig.1a-c). Due to higher viscosity which prohibits the beads formation and droplets than fibres at low concentrations (5 and 6 %) which had poor quality with more of beads because of low viscosity that persuaded the surface tension, which causes the breaking of entangled polymer chain in to fragments that leads to beads formation or beaded nanofibres. During electrospinning, a solution with low viscosity possesses a low viscoelastic force, which is not able to match the electrostatic and columbic repulsion forces that stretch the electrospinning jet. Under the effect of surface tension, the high numbers of free solvent molecules in the solution come together into a spherical shape causing formation of beads. Higher PVA concentration had increased the viscosity, which resulted increase in the chain entanglement that overcome the surface tension and ultimately results in beadles and uniform electrospun nanofibers development (Deitzel et al. 2001). Solution viscosity is major parameter in determining the fiber size and structure (Korycka et al. 2018). At higher viscosity, the developed fibers were free from beads while at lower viscosity the number of beads appeared to be more due to the influence of high surface tension, low charge density of the polymer resulting in formation of droplets or beads (Bhagure and Rao 2020). Higher concentration of polymer, overlapping of the polymer chains favours entanglement, which gives rise to a much stronger interaction and also leads to smooth fibres rather than particles (Yu et al. 2006).

In the microbial cells optimization study, the results of imprinting plating method showed the growth of microbial cells in all the blending proportions of PVA and microbial broth but the significant bacterial cells growth was observed at blending of 5 mL of microbial broth with 5 ml of 14 % polyvinyl alcohol (Fig. 2). Topography of electrospun fibre of microbial (*Methylorubrum aminovorans*) cells

immobilized revealed that the diameter of fibre was increased due to encapsulation of microbial cells. Polyvinyl alcohol (PVA) at 7 % found to produce electrospun fibres with diameter ranges from 93.30 nm to 166.1 nm (Fig. 1a). The diameter of bacteria cells encapsulated electrospun fibre measured from 379.9 nm to 845.5 nm (Fig. 3). The TEM image further confirmed the loading of bacteria cells depicting rod shaped morphology with size ranges from 267.7 nm to 466.0 nm (Fig.4). This finding derive support from the report (Salalha et al. 2006) there in *E. coli* bacteria had encapsulated in PVA nanofibre where the SEM and TEM images proved the immobilization of microbial cells in polymer matrix. Gensheimer et al. (2011) had encapsulated the bacteria *Micrococcus luteus* in polylactic acid and polyvinyl alcohol electrospun nanofibre. The SEM morphology of microbial cells immobilized nanofibre confirmed the bacteria cells loading by showing increased size of the fibre. The *Rhizobium* bacterium was entrapped successfully in PVA polymeric nanofibre and the loading was recognized in SEM morphology (De Gregorio et al 2017). According to Theron et al. (2001) the SEM morphology of polyacrylonitrile nano-fibres was altered due to fortification of eugenol as the average diameter of fibre increased from 127 ± 21 nm to 212 ± 29 nm after loading.

The enumeration of PPFMs (*Methylorubrum aminovorans*) population immobilized in polyvinyl alcohol electrospun nanofibre exhibited that a total of 2640 single colonies were observed per 0.004 g of nanofibre, and computed value showed that a total of 6.6 x 10^5 CFU g^{-1} were observed out of 1 × 10^8 CFU initially loaded (Fig. 5). De Gregorio et al. (2017) found that a total of 2.25 × 10^5 CFU of *Bradyrhizobium japonicum* was enumerated in PVA nanofibre to the total of 1 × 10^8 CFU added initially in the blend of polymer and microbial cells solutions. In the viability test of *Methylorubrum aminovorans* cells entrapped in PVA nanofibre stored under normal room temperature, microbial cells viability decreased with advance of storage time. There was a total of 1.85 × 10^5 CFU g^{-1}, 2.2 × 10^4 CFU g^{-1} and 1.2 × 10^4 CFU g^{-1} observed on 10, 20 and 30 days after storage, respectively (Fig.6).

Over all, the results indicated that viability of *Methylorubrum aminovorans* cells could be protected for more than 30 days under ambient environment when they are immobilized in polymeric electrospun nanofibre, and this might be due to polymeric matrix which acts as protective shell against environmental stress and dehydration of microbial cells. The microbes *viz*., *E. coli*, *Zymomonas* and *Pseudomonas* encapsulated in poly ethylene oxide (PEO) electrospun nanofibre of 100 to 300 nm prolonged the cell viability of microbes and precisely delivered at targeted site (Theron et al. 2001). Similarly, the cell viability of *Escherichia coli*, *Staphylococcus albus* and bacteriophage (Salalla et al. 2006), *Lactobasillus acidophilus* (Nagy et al. 2014), *Bradyrhizobium japonicum* (Damasceno et al. 2013), *L. rhamnosus* (Vejan et al. 2016), and *Pantoea agglomerans* (De Gregorio et al. 2017) immobilized in PVA nanofibre found to be prolonged while stored under ambient environment.

Microbial cells immobilized nanofibre seed invigoration on germination, seedling vigor and plant growth under *in vitro* conditions

The surface morphology of electrospun nano fibre coated seeds was depicted in figure 7a and 7b. In this study, the seeds inoculated with *Methylorubrum aminovorans* PVA electrospun nanofibres recorded

higher germination (84 %), root (11.4 cm) & shoot length (16.3 cm), seedling vigor (2322) (figure 8) and dry matter production (3.67 g per 10 seedlings) while tested under *in vitro* conditions. The increase was 10 % in germination (Fig. 9), 15.1 % in root length, 12.4 % in shoot length, 28.8 % in vigor (Fig. 10) and 13.6 % in dry matter production over untreated control. The improved germination and seedling vigour might be attributed to secretion of phytohormones (Auxins, Gibberellins, Cytokinins and IAA) by the *Methylobacterium*. Moreover, the hydrophilic effect of polyvinyl alcohol that increases rate of water uptake and maintain higher moisture content around the germinating seeds resulting in improved germination and seedling growth. In addition, the nutrient property of PVA triggers the metabolic events that results enhanced germination and seedling growth. Damasceno et al. (2013), they demonstrated the seed coating with rhizobia loaded electrospun polyvinyl alcohol (PVA) nanofibres improving germination and seedling growth in soybean.

The pot culture study expressed that the seeds inoculated with microbial cells immobilized nanofibre registered higher seedling emergence (83 %), seedling root growth (9.60 cm), seedling shoot growth (18.08 cm) and seedling vigor (2294). There was 9.0 % increase in seedling emergence 15.1 % in seedling root growth, 26.6 % increase in seedling shoot growth and 36.3 % increase in seedling vigor noted at the initial growth (Table 2). The higher emergence and potential seedling growth in microbial cells encapsulated nanofibre coated seeds is due to the combined effect of PPFMs and polymer matrix. Methylotrophs excrete phytohormones (IAA & cytokinin) and mobilize the nutrient from the seed on germination that contributed to the enhanced seedling emergence and growth. The hydrophilic nature and nutrient content of PVA also helped in improved seedling growth under potculture. De Gregorio et al (2017) observed that seeds invigorated with rhizobia entrapped polyvinyl alcohol (PVA) nanofibres promoted seedling growth in soybean.

The growth parameters *viz*., plant height, plant biomass, root volume, nodules number and fresh weight of nodules were observed on 25 and 45 days after sowing. The outcome of this experiment exhibited that the microbial cells encapsulated nanofibre coated seeds have expedited the plant growth as it recorded higher plant height & plant biomass (Table 3), root volume (Table 4 and Fig.11), root nodules number and fresh weight (Table 5; Fig.12a&b) at 25 and 45 days after sowing as compared to the control. The positive impact of bacteria cells loaded nanofibre invigorated seeds are ascribed to contribution of effective microbial colonization in the roots (Fig. 13), which positively promoted the plant growth under potculture. This result undoubtedly proved the potentiality of electrospun nanofibre to encapsulate the beneficial microorganisms for targeted delivery. Damasceno et al (2013) demonstrated the seed coating with rhizobia loaded electrospun polyvinyl alcohol (PVA) nanofibres in improving germination, seedling growth, number of nodules and plant biomass of soybean. Further, seed coating with PVA nanofiber-immobilized rhizobacteria *P. agglomerans* ISIB55 and *B. caribensis* ISIB40 contributed to the successful colonization of both bacteria on the plant root resulting in increased germination, seedling length & dry weight of root and leaf number in soybean (De Gregorio et al. 2017).

Conclusions

The study clearly evidenced the successful encapsulation of pink pigmented facultative methylotrophs (*Methylorubrum aminovorans*) in electrospun nanofibre for extended shelf-life of microbes besides applied to the seeds for an effective and targeted delivery of microbes, which enhanced microbial colonization in the roots, resulted in improved germination, seedling vigor and plant growth. This is a preliminary nanotechnological intervention to evolve innovative seed invigoration technique for improving seed quality hence, it needs further fine tuning, large scale testing and to be compared with the existing recommended seed treatment(s) for groundnut prior to reaching the farm gate.

Declarations

Conflict of interest

All the authors declare that they have no confict of interest.

Author Contribution

Chinna Mukiri- Investigation; **K. Raja**- Conceptualization; Writing - Original Draft; Supervision; **M. Senthilkumar**- Resources; Validation; **K.S. Subramanian**- Writing - Review & Editing; **K. Govindaraju**- Resources; Data Curation; Writing – Review;
D. Pradeep- Data Curation; Conceptualization; **Syndhiya Ranjan**- Investigation

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Tables

Table 1. Electrospinning and microbial cell concentration was optimized through imprinting method

S. No.	Quantity of microbial broth added (mL)	Quantity of 14 % PVA added (mL)
1	1.0	1.0
2	2.0	2.0
3	3.0	3.0
4	4.0	4.0
5	5.0	5.0

Table 2. Effect of PPFMs (*Methylorubrum aminovorans)* immobilized nanofibre seed invigoration on seedling emergence, growth and vigour index of groundnut var. TMV 13 under pot culture experiment

Treatments	Seedling emergence (%)	Root length (cm)	Shoot length (cm)	Vigor index
T_1	74 ± 3.18 (59.31)	8.34 ± 0.36	14.28 ± 0.61	1683 ± 98.13
T_2	78 ± 2.81 (62.02)	9.70 ± 0.35	15.42 ± 0.56	1959 ±127.71
T_3	76 ± 2.40 (60.66)	9.22 ± 0.29	15.08 ± 0.67	1826 ± 93.11
T_4	83 ± 212 (64.89)	9.60 ± 024	18.08 ± 0.64	2294 ± 81.11
CD (P=0.05)	2.907	0.558	0.391	121.369

(Figure in parenthesis are arcsine values)

T_1 – Untreated seeds
T_2 – PPFMs (*Methylorubrum aminovorans*) inoculated seeds
T_3 – Polyvinyl alcohol nanofibre coated seeds
T_4 - Polyvinyl alcohol (PVA) E-Spun nanofibre immobilized with PPFMs

Table 3. Effect of PPFMs (*Methylorubrum aminovorans)* immobilized nanofibre seed invigoration on plant growth and plant biomass of groundnut var. TMV 13 under pot culture experiment

Treatments	Plant height (cm)		Plant biomass (mg / plant)	
	25 DAS	45 DAS	25 DAS	45 DAS
T_1	22.85 ± 1.33	35.43 ± 2.07	555.16 ± 23.88	597.97 ± 25.72
T_2	28.34 ± 1.85	37.81 ± 2.46	565.58 ± 20.39	608.55 ± 21.94
T_3	27.45 ± 1.40	36.43 ± 1.86	561.73 ± 17.76	600.21 ± 18.98
T_4	35.10 ± 1.24	41.53 ± 1.47	615.22 ± 15.69	642.02 ± 16.37
CD (P=0.05)	1.108	1.395	5.353	29.866

T_1 – Untreated seeds

T_2 – PPFMs (*Methylorubrum aminovorans*) inoculated seeds
T_3 – Polyvinyl alcohol nanofibre coated seeds
T_4 - Polyvinyl alcohol (PVA) E-Spun nanofibre immobilized with PPFMs

Table 4. Effect of PPFMs (*Methylorubrum aminovorans)* immobilized nanofibre seed invigoration on plant root volume of groundnut var. TMV 13 under pot culture experiment

Treatments	Plant root volume (cc / plant)	
	25 DAS	45 DAS
T_1	180.0 ± 2.85	183.4 ± 2.90
T_2	182.3 ± 1.82	184.3 ± 1.84
T_3	181.6 ± 4.63	184.3 ± 2.80
T_4	181.8 ± 2.87	186.1 ± 2.90
CD (P=0.05)	0.821	0.932

T_1 – Untreated seeds
T_2 – PPFMs (*Methylorubrum aminovorans*) inoculated seeds
T_3 – Polyvinyl alcohol nanofibre coated seeds
T_4 - Polyvinyl alcohol (PVA) E-Spun nanofibre immobilized with PPFMs

Table 5. Effect of PPFMs (*Methylorubrum aminovorans)* immobilized nanofibre seed invigoration on root nodules number and nodules fresh weight of groundnut var. TMV 13

Treatments	Nodules number / plant		Nodules fresh weight (mg/ plant)	
	25 DAS	45 DAS	25 DAS	45 DAS
T_1	3.9 ± 0.17	12.6 ± 0.54	46.60 ± 2.00	91.13 ± 1.44
T_2	7.3 ± 0.26	17.6 ± 0.63	62.92 ± 2.27	119.92 ± 1.20
T_3	4.3 ± 0.19	13.2 ± 0.59	47.80 ± 2.14	99.28 ± 1.51
T_4	7.5 ± 0.27	20.5 ± 0.72	64.18 ± 2.27	131.55 ± 2.08
CD (P=0.05)	1.035	1.045	4.357	5.657

T_1 – Untreated seeds
T_2 – PPFMs (*Methylorubrum aminovorans*) inoculated seeds
T_3 – Polyvinyl alcohol nanofibre coated seeds
T_4 - Polyvinyl alcohol (PVA) E-Spun nanofibre immobilized with PPFMs

Figures

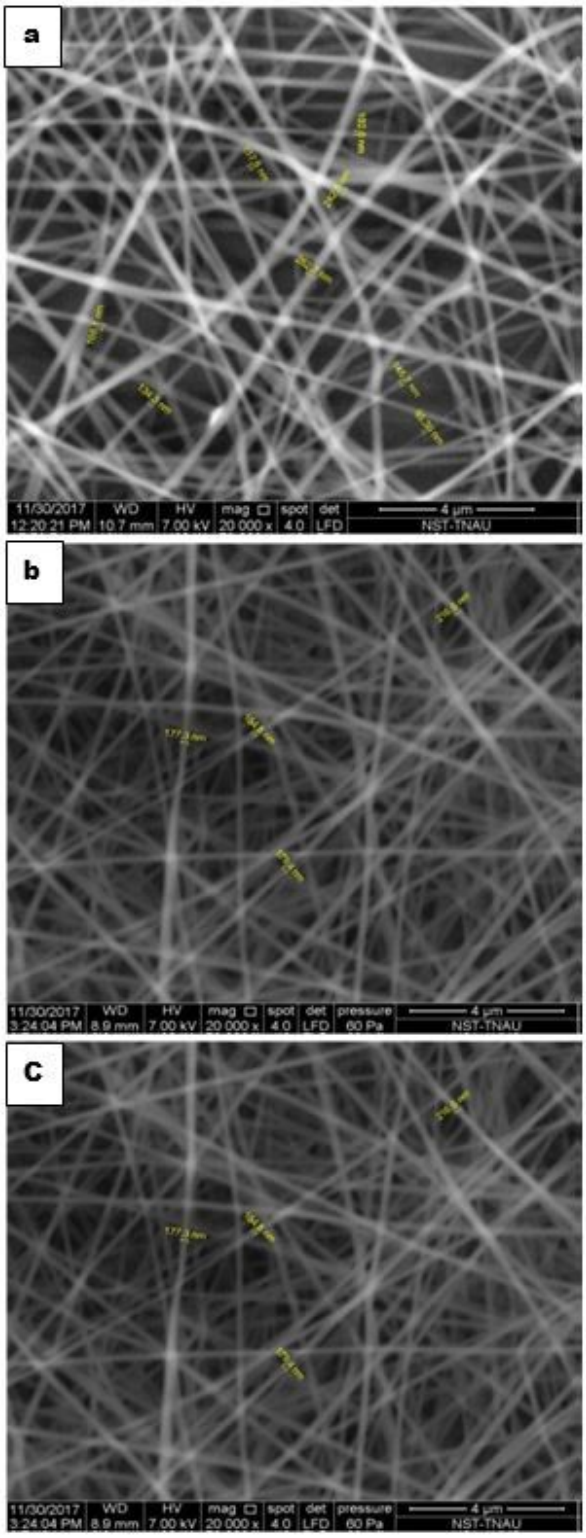

Figure 1

SEM morphology of PVA nanofibre a) 7 %, b) 8% and c) 9 %

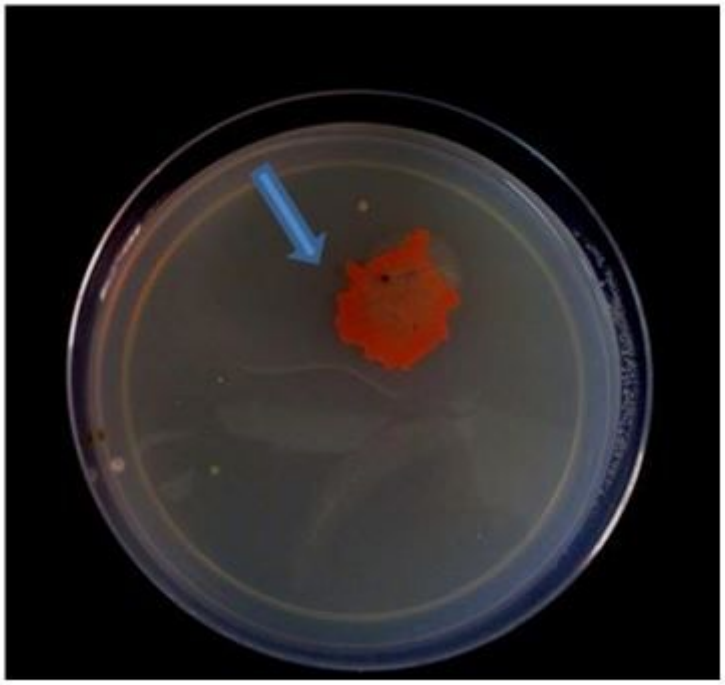

Figure 2

Methylorubrum aminovorans cells growth – imprinting plating method

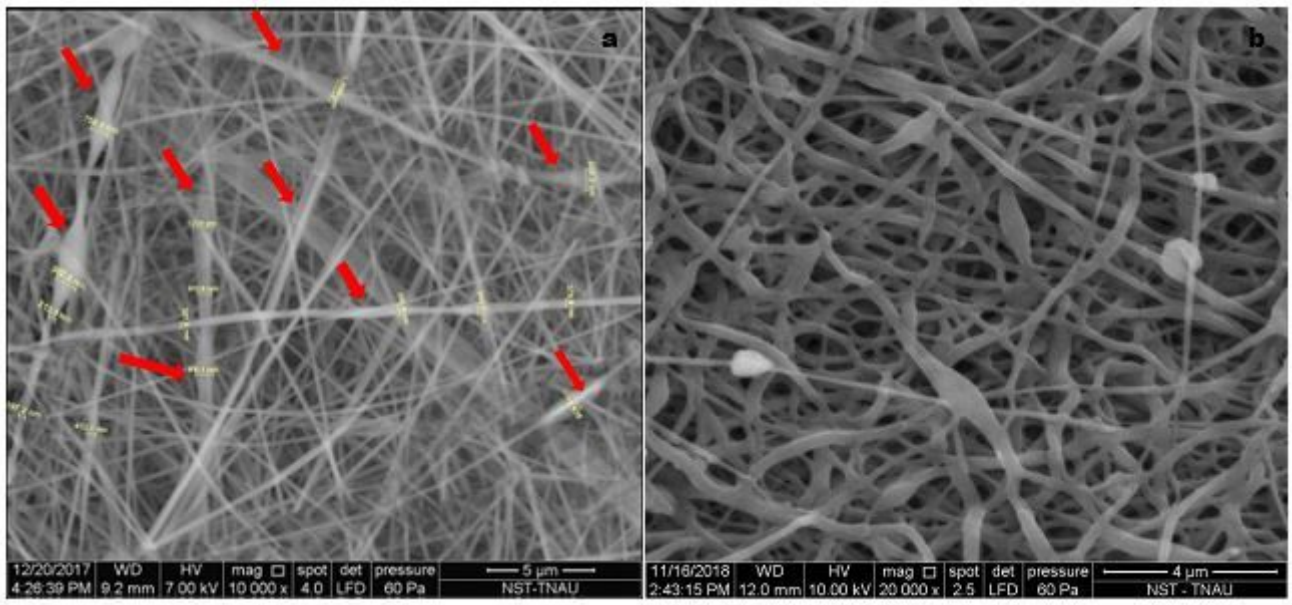

Figure 3

SEM morphology of Methylorubrm aminovorans immobilized PVA nanofibre

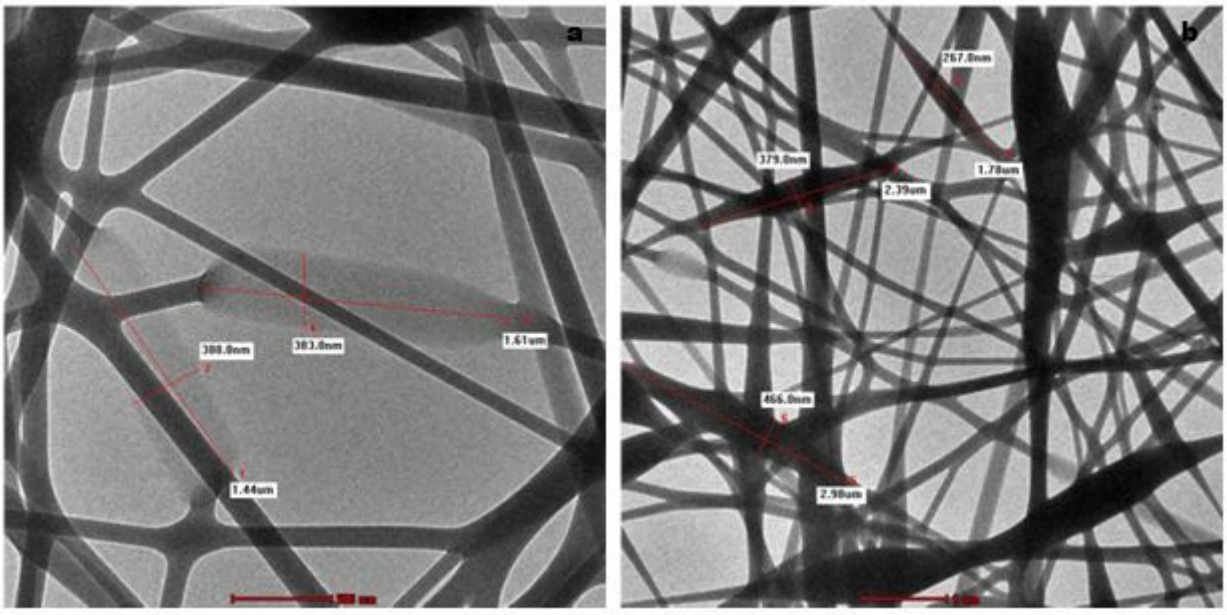

Figure 4

TEM morphology of Methylorubrm aminovorans immobilized PVA nanofibre

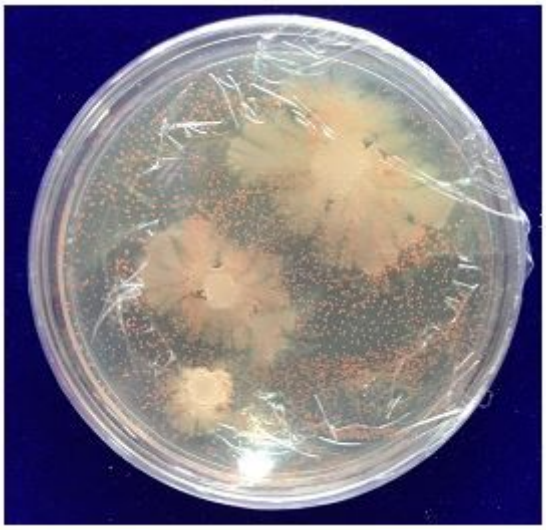

Figure 5

Enumeration of Methylorubrum aminovorans population in polyvinyl alcohol nanofibre

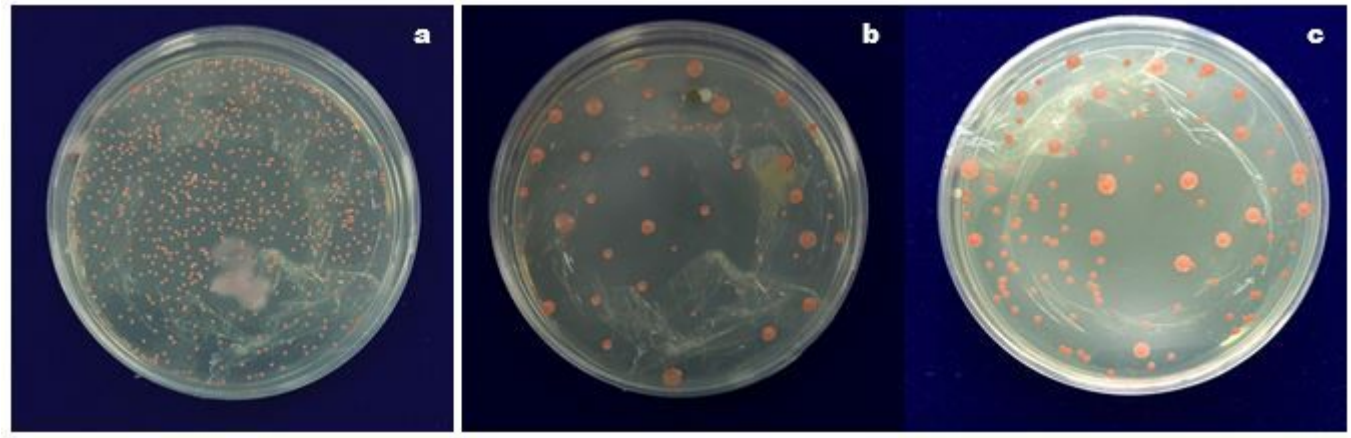

Figure 6

Methylorubrum aminovorans cells viability – nanofibre entrapped microbial cells stored under ambient condition a) control, b) 10th day after bacteria loaded, c) 20th day after bacteria loaded, d) 30th day after bacteria loaded

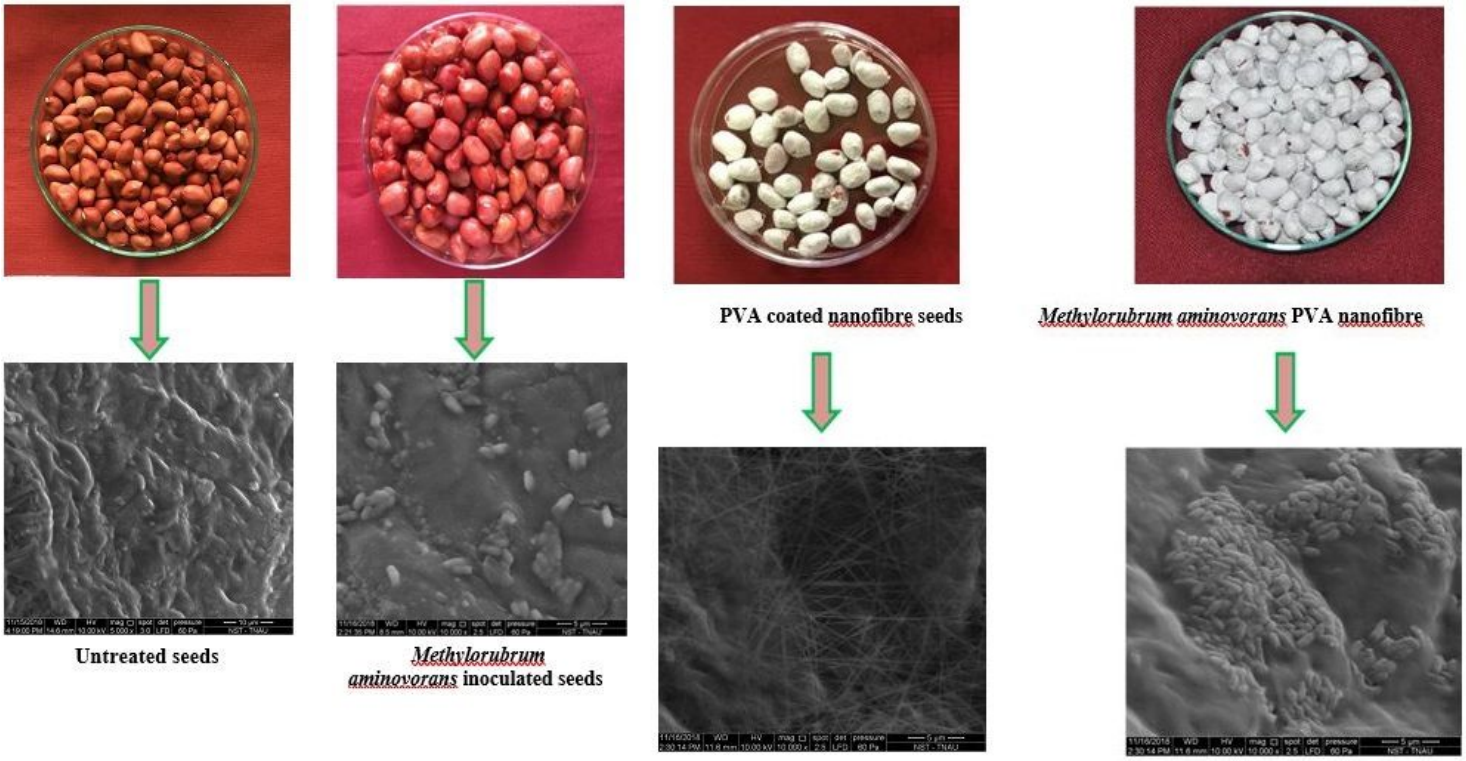

Figure 7

Figure 7a: SEM surface morphology of Methylorubrum aminovorans immobilized nanofibre coated seeds of groundnut var. TMV 13. Figure 7b: SEM surface morphology of Methylorubrum aminovorans immobilized nanofibre coated seeds of groundnut var. TMV 13

Figure 8

Methylorubrum aminovorans immobilized PVA nanofibre seed invigoration on germination and seedling growth

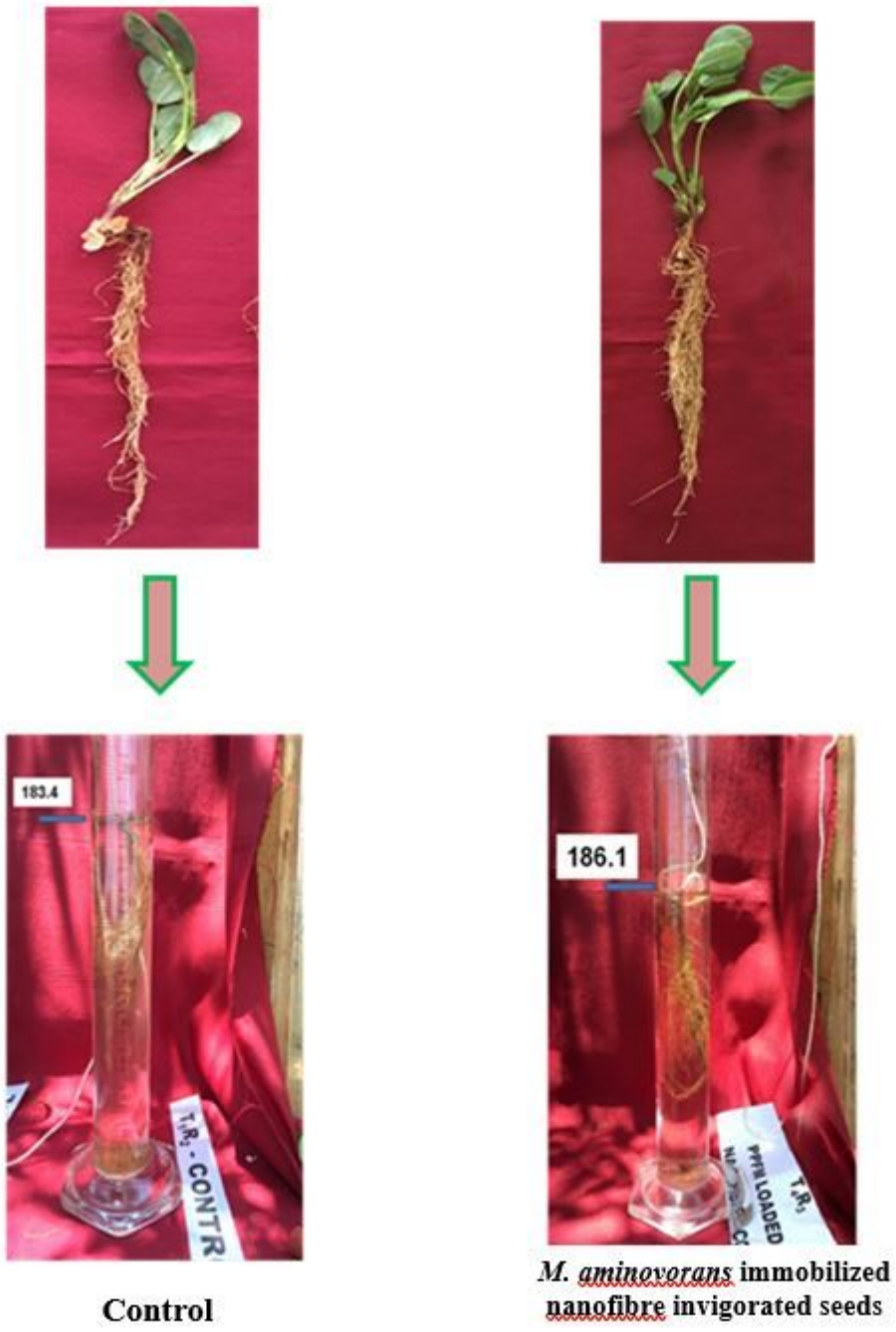

Figure 9

Methylorubrum aminovorans entrapped nanofibre coated seeds on root volume at 45 days after sowing under potculture

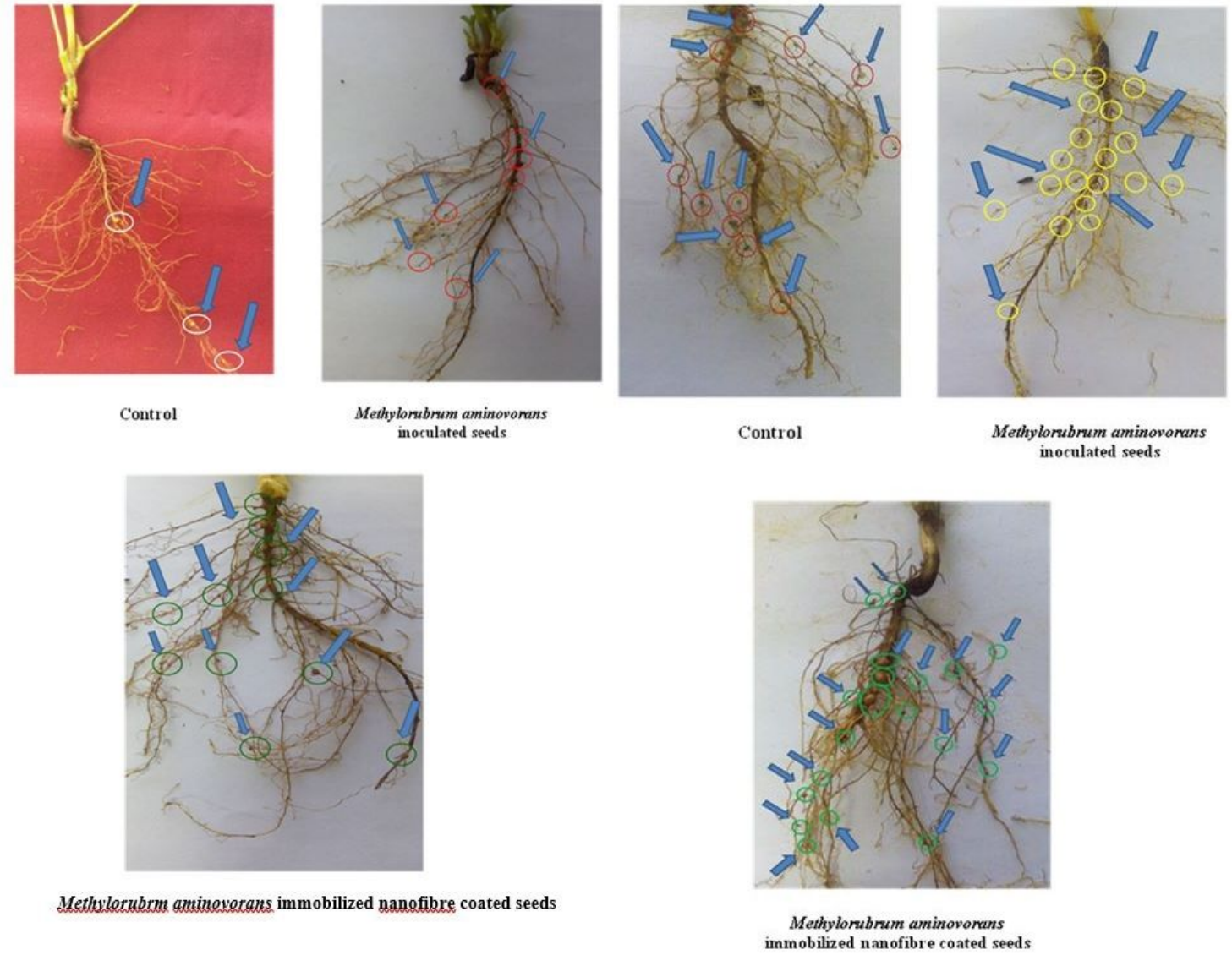

Figure 10

Figure 10a. Methylorubrum aminovorans entrapped nanofibre coated seeds on root nodules at 25 days after sowing under pot culture. Figure 10b. Methylorubrum aminovorans entrapped nanofibre coated seeds on root nodules at 25 days after sowing under pot culture

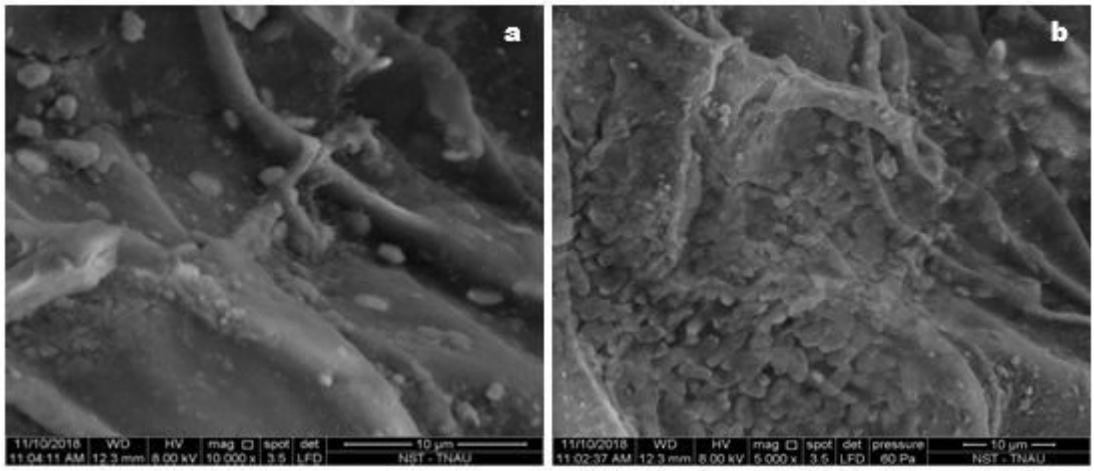

Figure 11

Methylorubrum aminovorans colonization at roots of groundnut after 25 days after sowing under pot culture a) Control b) Methylorubrum aminovorans immobilized nanofibre coated seeds