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Effect of Multinutrients Loaded Electrospun PVA Nanofibre on Germination and its Growth Parameters of Green Gram [*Vigna radiata* (L.) Wilczek]

J. Mohanraj[1], K.S. Subramanian[2], K. Raja[1]

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ABSTRACT

Background: In the present study, redundant blanket applications of chemical fertilizer application to the soil set foot into the aquatic environment and cause eutrophication. Besides it cause low nutrient efficiencies, low organic matter, imbalanced fertilization and low fertilizer response influence to decrease crop yield. To solve this serious problem localized delivery of multi nutrients loaded with electrospun nanofibre through the seed coating approach. The nanofibre regulates the nutrient release at the targeted site (rhizospheric zone) in a sustained pattern of using e-spin technology.

Methods: The electrospun carrier was developed from PVA at 10%, diameter of fibre ranges form (260 nm to 540 nm) was used as nanocarrier for encapsulate the multi nutrient at different concentration of 25, 50, 75, 100%. A field experiment was conducted during 2019-2020, to study the impact of crop response of MNC loaded nanofibre coated green gram seed.

Result: It conclude that the novel nanofibre technology improve the higher plant height (47.38 cm) at maturity stage, total leaf area (1093.95), net assimilation rate (2.92 mg cm^{-2} day^{-1}) at flowering stage, seed yield (883.41 kg ha^{-1}) as compared to absolute control approaches. This nanofibre technology will give novel idea for the fertilizer industry owing to its unique larger surface area and controlled release kinetics.

Key words: Electrospinning, Multi nutrient composite, Nanofibres, Seed coating, Slow-release.

INTRODUCTION

The ineffective application of Agri input in the agricultural system is imperiled to food safety and security leading to cause serious environmental impacts. Moreover,it has been reported that most of the applied fertilizers were lost due to evaporation, degradation, leaching (Benzon *et al*., 2015). This issue causing ground water pollution and eutrophication in the aquatic system (Subramanian *et al*., 2015). On the other hand a current estimate by FAO reported that food grain production needs to increase by 70-100% by 2050 to feed the burgeoning population in the globe and achieving this level of productivity is one of the biggest challenges due to changing climate and the constant nutrient use efficiencies of conventional fertilizer.

For the past 60 years, the nutrient use efficiency in conventional fertilizers reports the range of 30-35%, 18-20% and 35-40% for N, P and K, respectively (Subramanian and Thirunavukkarasu, 2017). The inefficiency of the conventional fertilization method need the development of an alternative efficient nutrient delivery system. Biodegradable nano-fibre based multi nutrient delivery is one such novel system to deliver the nutrients at the exact target site and crop demand. (*i.e.*, right time, right place, right dose (Ks, 2019) Among nanotechnological approaches, electrospun nanofibre is one such effective technique for précised delivery of inputs in the targeted area.

Whereas the word electrospinning emanate from electrostatic spinning was used from 1994, A polymer solution contains polymer with active compounds was subjected into the electric field. The nutrients encapsulated nanofibres were formed from the emulsion, between opposite charged two electrodes. One electrode connected into the solution and another electrode was connected into collector placed with seeds. Once polymer ejected from small hole, the charged solution jets when solvent get evaporated to become non-woven nano fibers which were deposited over the collector. Hence, the present study hypothesized to develop nanofibre technique to enhance the nutrient use efficiency and overcome the multi nutrient deficiency through the seed coating, which can play an important role in curtail the nutrient losses, regulate the nutrient release at the zone of seed and improve the plant growth habit and yield.

[1]Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[2]Directorate of Research Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

Corresponding Author: K.S. Subramanian, Directorate of Research Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India. Email: kss@tnau.ac.in

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MATERIALS AND METHODS

Field experiments were conducted at field no 11, Department of pulses, Tamil Nadu Agricultural University, Coimbatore, India. During *kharif* season 2019-2020. Multi nutrient solution mixture 100% concentrated has been specially formulated for loading on to the electrospun nanofibre were depicted in Table 2. The five treatments were selected with four replication and each consisted of absolute control ($T_0R_{1-}R_5$), recommended dose of fertilizer (RDF-$T_1R_{1-}R_5$) and 25% MNC+NF ($T_2R_{1-}R_5$), 50% MNC+NF ($T_3R_{1-}R_5$), 75% MNC+NF (T_4 $R_{1-}R_5$), 100% MNC+NF ($T_5R_{1-}R_5$). In this connection,the different concentration of multi nutrient solution of 100 ml of 25, 50, 75% multi nutrient solution was prepared by 25, 50, 75 ml of multi nutrient solution diluted with 75, 50, 25 ml of deionized water respected with each concentration. Were as in 100% MNC concentration, raw multi nutrient solution was taken as polymer blending. After prepared a multinutrient solution 100 ml of 10% PVA contains multinutrient solution was prepared for electrospinning. This solution contains (PVA+NF) were transferred to dispovan syringe and developed the electrospun nanofibre. The each treatment of concentration was splitted into 4 replications (experimental design was presented in (Table 1). All the agronomic practices were carried out uniformly for raising the crop and the design of experiments was chosen to be randomized block design (RBD) (Thilagavathi *et al.*, 2007). Plant samples were collected for chemical analysis of multinutrients such as nitrogen was estimated by micro Kjeldahal method (Piper, 1966). P in the extract was determined by Colorimetric ($HClO_4$: HNO_3 extract) method (Jackson *et al.*, 1973). K in the extract was determined flame photometry ($HClO_4$: HNO_3 extract) by (Jackson *et al.*, 1973). S of the plant extract was estimated by turbidimetric method (Chesnin and Yien, 1951). Ca and Mg is estimated versenate method by using EDTA (Cheng and Bray, 1951). Estimate the total micronutrient of the clear extract analyzed by using atomic absorption spectroscopy (AAS). The results obtained from the field trial and nutrient analysis were interpreted using IBM SPSS statistics ver. 25 (Haswell, 1991). The graphs and representations were prepared using originlab 8.5.

The crop growth parameter observed manually on five randomly selected typical plants from each concrete ring of representative plot furthermore yield and yield attributing feature were recorded as per the standard protocol. Yield attributes phenomena of crop also reveal the physiological improvement. The seed and straw yield was recorded from net plot area of each treatment. Analysis of variance obtained from various characters under study described by (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Characterization of nanofibres

SEM Topography of multinutrients loaded PVA electrospun fiber expose the fibre diameter was enlarging on account of encapsulates the multinutrients to nanofibre (Fig 1b and d) starting from 260 nm to 540 nm. According to (Biswal and Subramanian, 2019) the SEM morphology of PVA nanofibres was enlarged due to fortification of hexanal molecule from 150±57 nm to 250±59 nm after loading. Similarly, TEM internal structure of MNC loaded electrospun nanofibre (Fig 1a and c) shows the nanofibre diameter increases due to impregnation of multinutrients ions to that. The similar result were obtained from the author (Mukiri *et al.*, 2021).

SEM-EDAX analysis of multi nutrient presents in nanofibre

The adsorption of multinutrient ions 100% concentrated loaded on to the electrospun nanofibre was confirmed by TEM EDAX analysis (Fig 2). The result confirmed the composition of multinutrient ions present in electrospun nanofibre it reveals that the N, P, K, Mg, Ca , S was loaded with electrospun nanofibre.

Effect of MNC Loaded electrospun nanofibre on growth parameters

Plant height (cm)

The MNC loaded nanofibre treatment record plant height of 47.38 cm while absolute control registered lowest plant height of 33.08 cm at maturity stage (Table 4). Other treatments also exhibited comparable plant heights. These results are coincide with conformity of Farias *et al.*, (2019).

Table 1: Design of experiments for green gram field trial.

Treat-ments	Treatment expansion	Sub-replication (r)	
T_0	Absolute control	R_1	R_2
		R_3	R_4
T_1	Recommended dose of fertilizer (RDF)	R_1	R_2
		R_3	R_4
T_2	25% MNC+PVA Electrospun nanofibre	R_1	R_2
		R_3	R_4
T_3	50% MNC+PVA Electrospun nanofibre	R_1	R_2
		R_3	R_4
T_4	75% MNC+PVA Electrospun nanofibre	R_1	R_2
		R_3	R_4
T_5	100% MNC+PVA Electrospun nanofibre	R_1	R_2
		R_3	R_4

Table 2: Nutrient content in multinutrient solution.

Nutrients	Concentration (g/l)
KNO_3	202
$Ca(NO_3)_2$	236
Iron chelate	15
$MgSO_4$	240
NH_4NO_3	80
H_3BO_3	2.86
$MnCl_2$	1.81
$ZnSO_4$	0.22
$CuSO_4$	0.051
H_2MoO_4	0.09
Na_2MoO_4	0.12
KH_2PO_4	136

Table 3: Initial experimental soil analysis.

Parameters	Units	Values
pH	-	8.18
EC	µS cm^{-1}	0.23
N	Kg ha^{-1}	178.6
P	Kg ha^{-1}	11.1
K	Kg ha^{-1}	794.7
S	Mg kg^{-1}	9.3
Zn	Mg kg^{-1}	2.58
Mn	Mg kg^{-1}	2.74
Cu	Mg kg^{-1}	4.20
Fe	Mg kg^{-1}	2.74

Table 4: Effect of MNC loaded electrospun nanofibre on growth parameters.

Treatments	Plant height (cm)		
	At 30 DAS	At flowering	At maturity
T_0	13.46	26.70	33.08
T_1	15.20	35.29	42.99
T_2	14.42	31.52	37.03
T_3	14.46	32.61	40.96
T_4	15.34	33.99	44.84
T_5	15.61	36.11	47.38
SE (d)	0.269	0.688	0.710
CD (0.05)	0.579	1.479	1.527

Crop growth rate (CGR) (g m^{-2} day^{-1})

Crop growth rate recorded between vegetative to flowering stage (15~30 DAS), flowering to pod filling stage (30~45 DAS) and pod filling to harvest stage (45~75 DAS) is conferred in (Table 5). The crop growth rate varied from 0.96 to 1.57, 2.92 to 3.91 and 12.02 to 21.24 g m^{-2} day^{-1} at 15~30, 30~45 and 45~75 DAS, respectively. The treatment (100% MNC loaded NF) registered the maximum CGR (1.57, 3.91, 21.24 and 9.52 g m^{-2} day^{-1}) at all growth. There was no significant variation among the treatments at harvest stage.

Net assimilation rate (NAR) (mg cm^{-2} day^{-1})

The electrospun nanofibre registered the higher NAR values at all the stages of crop period *viz*., 30~45, 45~60, 60~75 DAS. The trend of NAR observed highest in 2.18 to 2.98 mg cm^{-2} day^{-1} in flowering stage followed by 1.32 to 1.93 mg cm^{-2} day^{-1} in harvest stage and lowest trend was observed in 0.47 to 0.56 mg cm^{-2} day^{-1} at 30 DAS. As compared to nanofibre coating method conventional fertilizer applied plots and absolute control method registered the lowest NAR trend were presented in the (Table 5).

Effect of MNC loaded electrospun nanofibre on yield and yield attributes and quality

The result on number of nodules $plant^{-1}$ was significantly differed in all the treatments (Fig 3A). Among the treatments, T_5 (100%) MNC Loaded NF showed highest nodules $plant^{-1}$

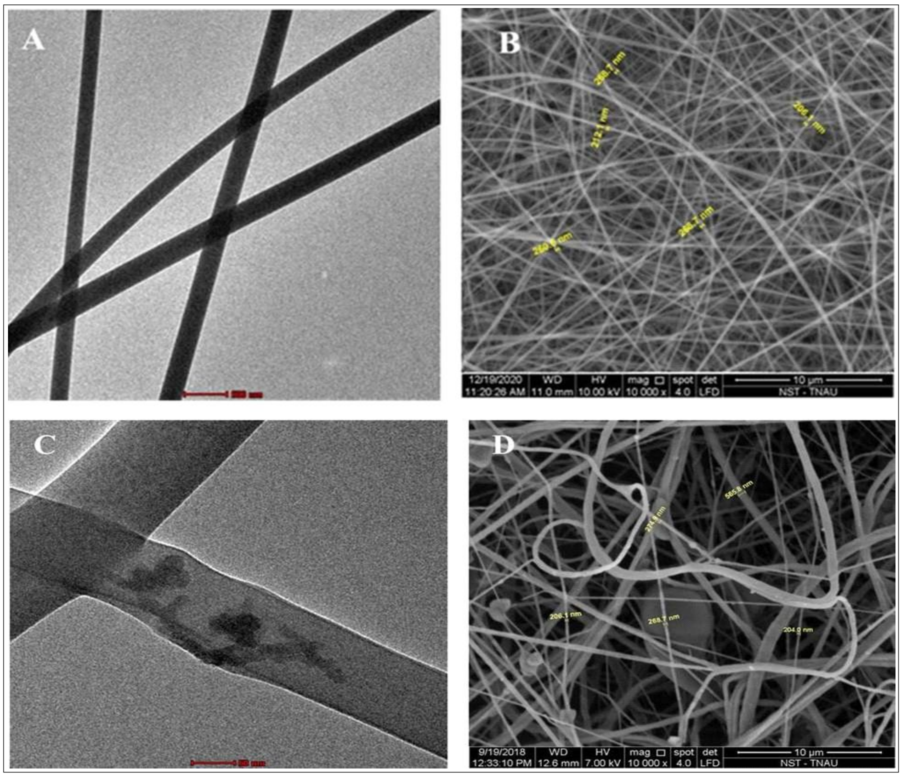

Fig 1: a) TEM micrographs of pure PVA nanofibre; c) TEM micrographs of MNC+NF; b) SEM micrographs of pure PVA nanofibre; d) SEM micrographs of MNC+NF.

(19.91 no's) at flowering stage, which was followed by T_1, T_4, T_3 and T_2 were the lowest nodules plant^{-1} were observed in T_0- absolute control 17.20 at flowering stage.

The yield attributes *viz.*, number of pods plant^{-1} and number of seeds pod^{-1} varied significantly with different concentration of multinutrients loaded nanofibre coated seeds (Fig 3B and C) Were as 100% concentrated multi nutrients loaded nanofibre coated seeds recorded higher number of pods plant $^{-1}$ (22.87) and higher number of seeds pod^{-1} (11.89) and was at par with same treatment. This may due to favorable steady release of minimal concentration of complete set of nutriments supply from nanofibre to seed surface. Similarly nanofibre capable to hold soil moisture helped to develop agreeable environment for crop growth which resulted in progressive yield parameters (Sun *et al.*, 2010).

Test weight

The nanofibre coating technology also influenced the increase the 100 seed weight maximum 3.84 g and minimum as 3.39 g observed represented in (Fig 3D).This could be attributed the combination of multinutrient nutrients through electrospun nanofibre leading to regulate the nutrients steady state manner at seed coat were its resulting in higher uptake of nutrients. This could have to increase flower production and eventually pod emergence and yield characteristics. The increased 100 seed weight due to regulated moment of multinutrients to the

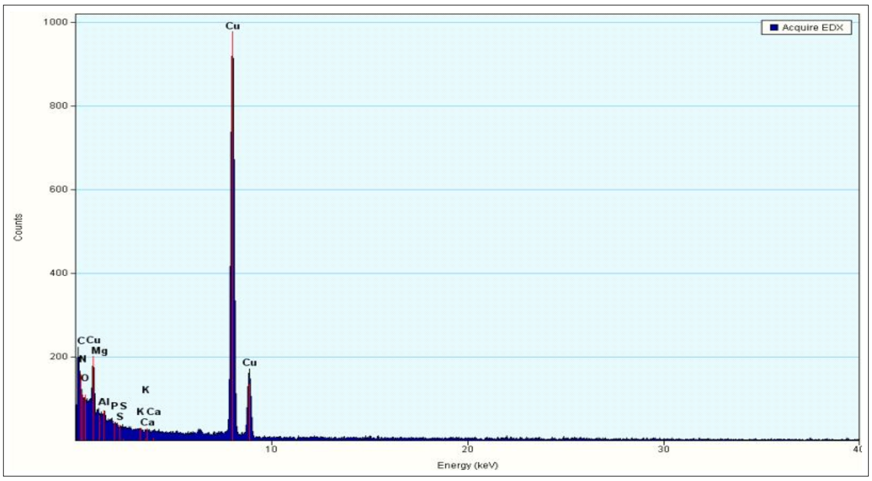

Fig 2: TEM–EDAX analysis of Multi nutrient confirmation onto the nanofibre.

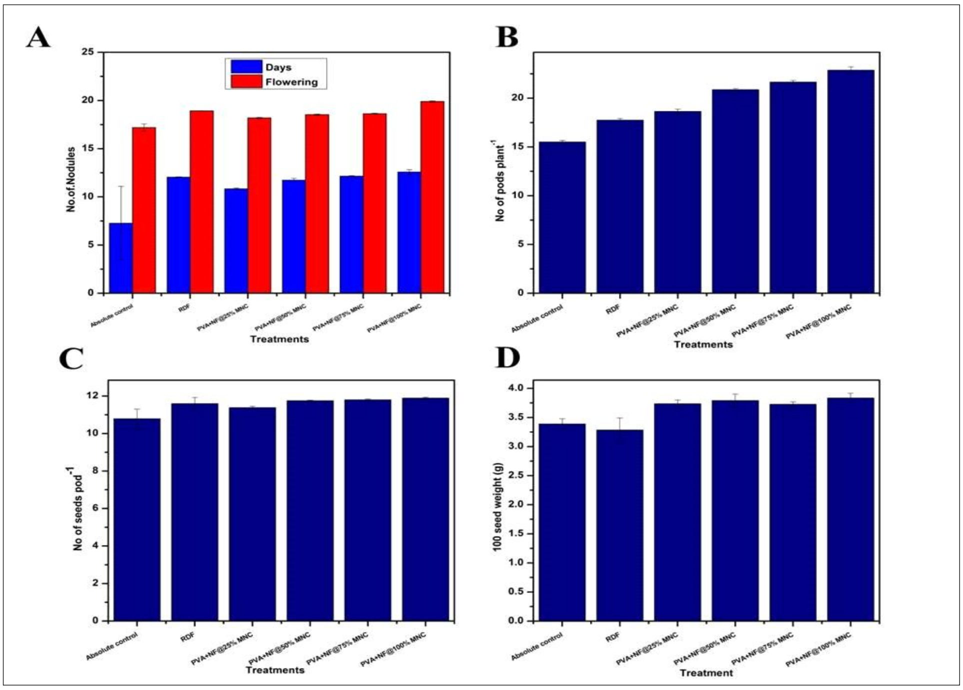

Fig 3: Effect of MNC on yield attributes.

reproductive sinks. Similar findings were reported by (Kampeerapappun and Phanomkate, 2013).

Grain yield

Grain yield showed that interaction between MNC loaded electrospun nanofibre over surface of the seed coat significantly influenced grain yield (Fig 4A). Application of 100% MNC loaded electrospun nanofibre coated green gram seeds exhibit (883.41 kg ha^{-1}) was found to be relatively on par with 100 per cent RDF application (797.50 kg ha^{-1}) and found to be significantly superior over other treatment *viz.*, 75% (583.02 kg ha^{-1}), 50% (725.59 kg ha^{-1}) and 25% (679.91 kg ha^{-1}) followed by T_0 (409.23kg ha^{-1}). The positive impact of multi nutrients loaded nanofibre coated seeds promoted the seed yield under concrete ring experiment studies. This result undeniably proved the electrospun nanofibre loaded multinutrients improves the uptake of multinutrients by plants. According to author (Damasceno *et al.*, 2013) the *via*bility of *Rhizobium* bacterial cells infused along with fertilizer in PVA nanofibres increases the plant biomass and yield in soybean seeds.

Haulm yield

100% MNC loaded PVA electrospun nanofibre resulted in the increase of haulm yield by 41% over absolute control (Fig 4B). The haulm yield improvement due to the adoption of different concentration of MNC loaded onto nanofibre influenced as haulm yield ranged from 1907.70 to 2479.05 kg ha^{-1} over control. This might be due to regulated supply of nutrients which could have controlled by crop demand as a result increase the leaf area and dry matter resulting in higher haulm yield. Similar result of nanofibre coated seed increase haulm yield observed previously by (Mahajan *et al.*, 2011).

Nitrogen uptake (%)

In 100% MNC loaded electrospun nanofibre coated seeds have influenced to increase nitrogen contents in vegetative, flowering, grain filling and harvest stage (Fig 5A). The highest N uptake was registered in grain filling as (2.98%) while lowest uptake of N were observed in absolute control plants had 2.63%. On the other hand, conventional fertilized plants had the highest N content of 2.92% which is on par with 100% MNC loaded electrospun nanofibre coated seeds. Current finding of nitrogen uptake confirmed by (Mohanraj, 2013).

Phosphorous uptake (%)

The trend of P uptake were increases by influence of 100% MNC coated nanofibre coated in vegetative, flowering, grain filling and harvest stage (Fig 5B). The highest P uptake was observed in grain filling stage (2.82%) while lowest uptake of P was observed in absolute control plants had 2.63%. On the other hand, conventional fertilized plants had the highest P uptake of 2.98% which is on par with 100% MNC

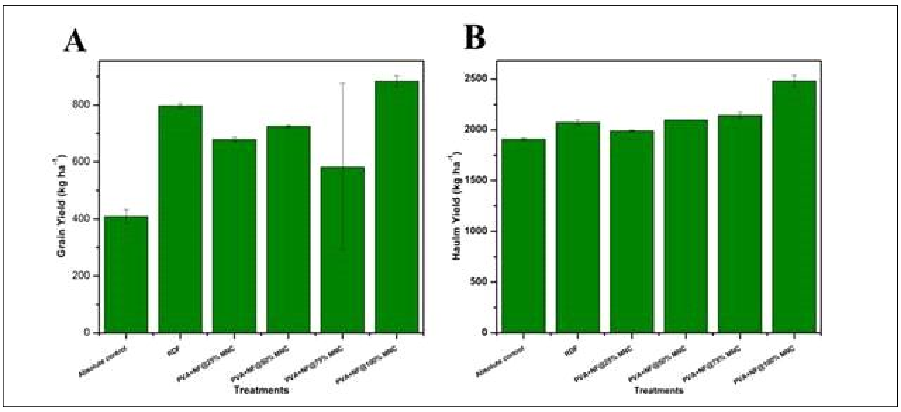

Fig 4: Effect of MNC on grain and haulm yield.

Table 5: Effect of MNC Loaded electrospun nanofibre on physiological parameters.

Treatments	Crop growth parameter (g m^{-2} d^{-1})				Net assimilation rate (mg cm^{-2} day^{-1})		
	15-30 DAS	30-45 DAS	45-60 DAS	60-75 DAS	At 30 DAS	At flowering	At maturity
T_0	0.96	2.92	12.02	6.88	0.47	2.18	1.32
T_1	1.50	3.42	20.87	9.46	0.54	2.86	1.86
T_2	1.12	3.02	16.55	7.91	0.49	2.73	1.59
T_3	1.47	3.04	17.65	8.72	0.53	2.76	1.82
T_4	1.51	3.30	18.67	9.29	0.54	2.88	1.84
T_5	1.57	3.91	21.24	9.52	0.56	2.92	1.93
SE (d)	0.015	0.298	0.406	0.038	0.008	0.009	0.012
CD (0.05)	0.032	0.641	0.874	0.081	0.018	0.020	0.026

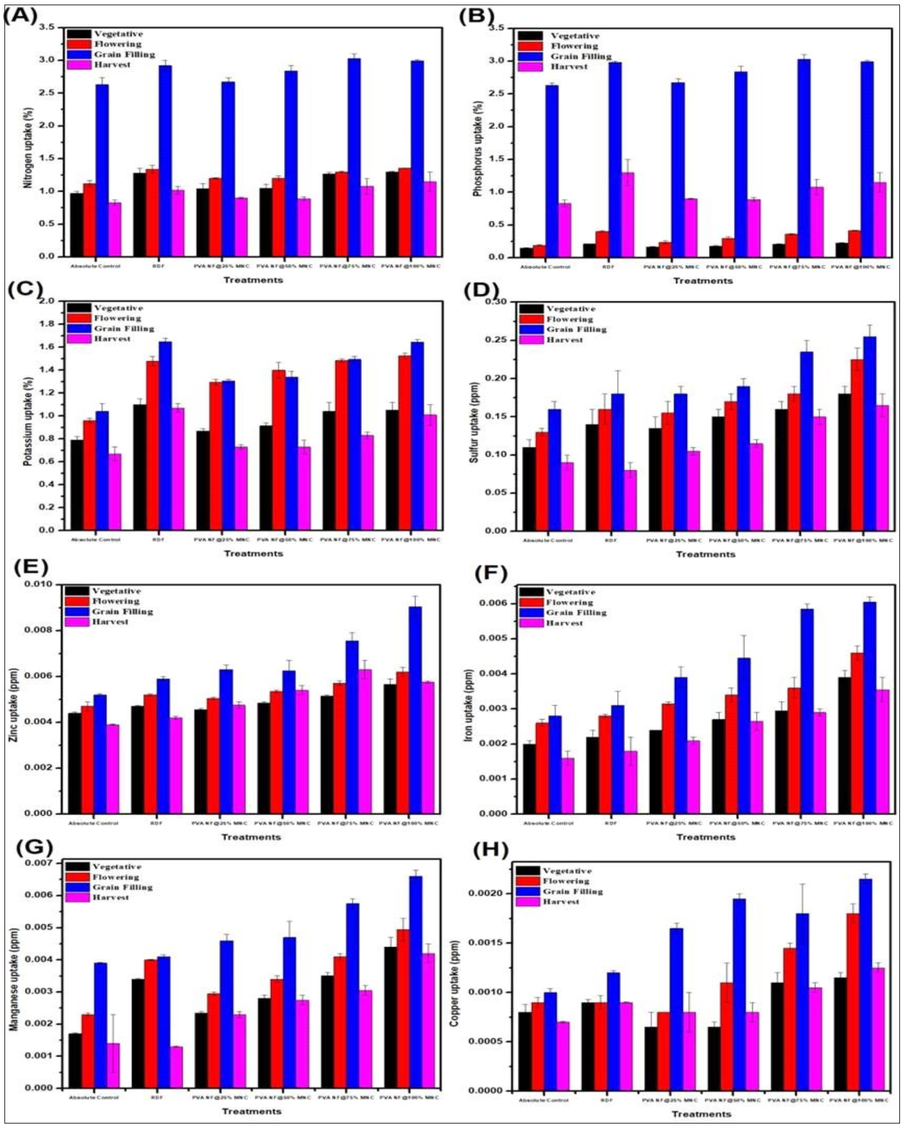

Fig 5: Effect of MNC on nutrient uptake.

loaded electrospun nanofibre coated seeds. The result of P uptake was coincide with the (Raliya and Tarafdar, 2013) by using ZnO based nanofertilizer to promote the P mobilization in cluster bean.

Potassium uptake (%)

The K uptake by the green gram maximum recorded in grain filling stage from 1.65 to 1.67% while lowest uptake of K was observed in absolute control plants (Fig 5C) had 1.04% This increase in K uptake may be ascribed to higher grain and straw production and K content in green gram due to regulated release of nutrients from the PVA electrospun nanofibre. Current findings were coincided with result previously done by (Kubavat *et al.*, 2020) chitosan based nanofertilizer were improve potassium uptake in *Zea mays* (L.).

Micronutrients uptake (ppm)

In green gram micronutrient uptake was measured at various stages of crop period vegetative, flowering, grain filling and harvest stage (Fig 5D), In case of higher uptake of micro nutrients were observed in grain filling stage from the analysis S from 0.27 ppm; Zn from 0.95 ppm; Fe from 0.62 ppm; Fe from 0.062 ppm; Mn from 0.062 ppm; and Cu (0.0010 ppm). The control gave the minimum mean uptake of all nutrients. Application of 100% MNC loaded electrospun nanofibre influences maximum plant uptake of all nutrients it might all the nutrient were steadily released from hydrophilic nanofibre. The same results was pertaining from the author (Ashfaq *et al.*, 2017).

CONCLUSION

In summary, the world inhabitants look forward to conquer 9.5 billion by 2050. In this kind of unavoidable situation a great need to bring out mass-produce of food grains. Researchers are conducting a variety of research into food grain production to meet this global demand. This study clearly demonstrated that encapsulation of multinutrients in PVA electrospun nanofibre as an aid to improve the green gram growth and yield by conveyance the required amount of nutrients at the target site and full fill the crop demand. The result of MNC loaded nanofibre seed coating is improves the plant growth and yield. However, this nanofibre based nutrient delivery system in agriculture is new thought and further it needs fine tuning, exhausting trial and replicating the investigation before foregoing to reach the farmers hand.

Conflict of interest: None.

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