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DEVELOPMENT OF SEED TREATMENTS BIOFERTILIZER AND NUTRIENT UPTAKE STUDY USING TRACER TECHNIQUE FOR OKRA PLANTS

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ABSTRACT

Development of biofertilizer seed treatments for okra seeds were carried out by mixing phosphate solubilising bacteria (AP 3) and plant growth promoter (AP 2) with adhesives. The seeds were coated with inoculums and four types of adhesives namely, Gum Arabic; Polyethylene Glycol (PEG); Sodium Alginate and Methycellulose respectively. From eight seed treatments, all seed treatments significantly increased seed germinations except treatment T4 (Gum Arabic and AP3). In general, maximum germination rates and log of viable cells were observed when treated with polyethylene glycol 4000 (PEG) mixed with AP2 (T7) and AP3 (T8). These results show that using PEG as adhesive enhanced the germination rates and log of viable cells of AP2 and AP3. Thus, PEG could be a good adhesive for seed treatment. In greenhouse experiment, okra seeds treatment with AP2 and PEG (T1) showed the highest dry weight compared to other treatments. Seeds treatment with AP3 and PEG (T2) showed higher contribution of N compare to seeds treatment (T1). There were no significant different within seed treatments and urea treatment in okra yield. All treatments significantly increased yields compared with control.

Keywords: biofertilizer, phosphate solubilising bacteria (AP 3), seed treatments.

INTRODUCTION

Plant growth promoter, phosphate solubilising bacteria or nitrogen fixing bacteria were well known to increase growth and yield of many crops (Rodriguez and Fraga, 1999; Wu *et al*., 2005; Nelson, 2007 and Linu *et al*., 2009). These beneficial microorganisms were applied as soil drench, mixed with carrier such as peat, vermiculite, compost and soil. Most research has shown application of inoculums to the seeds enhanced plant growth and yield. Inoculants were mixed with seeds just prior of sowing or coating to seeds (Jahuri, 2001; Zarrin *et al*., 2006). Adhesive such as Gum Arabic (Shahnaz*et al*., 2008; Gholami *et al*., 2009); Polyethylene Glycol (PEG) (Bishnol *et al*., 2010); Sodium Alginate (Kwang *et al.*, 2008), Methycellulose (Phua *et al*., 2004) or rice gruel (Gomathy *et al*., 2008)was used to improve stickiness. These adhesive may increase or decrease seed germination and growth of inoculants. Hence, development of good quality adhesive inoculants for seeds treatments is needed. In view of this, the objectives were framed to evaluate the effect of adhesive and viability of inoculants as seed treatment and to evaluate their effectiveness on nutrient uptake in greenhouse experiment.

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MATERIALS AND METHODS

(1) Development of seed treatments for okra seeds

Phosphate solubilising bacteria (AP3) and plant growth promoter (AP2) isolated from compost samples were used as seed treatments. Okra seeds were sterilised using 0.25 % sodium hypochlorite for 5 minutes and rinsed thoroughly in sterile distilled water. The seeds were coated with inoculums (approximately 10^8 cfu/ml) and Gum Arabic (20%); Polyethylene Glycol 4000 (PEG) (400 g L-1 water); Sodium Alginate 3% and Methycellulose (1.5%) as adhesive respectively. Seeds treated with sterile distilled water served as control (Table 1). Seeds were placed in petri dishes with two layers of filter papers moistened by adding 2.5 ml sterile distilled water (moisture content was 25%). Seeds were incubated at 28 ± 2^0C and germination rates were recorded daily. Data was transformed into arc-sine values and analyzed by ANOVA with separated by Duncan's test ($P \leq 0.05$). Log of viable cells were determined by plate counts. Data was analyzed by ANOVA with separated by Duncan's test ($P \leq 0.05$).

(2) Nutrient uptake study

In greenhouse experiment, two sets of experiments were carried out. Plant growth promoter (AP2) and phosphate solubilising bacteria (AP3) were mixed with PEG 4000 as seed treatments, namely T1 and T2 respectively. Chemical fertilizer was used as treatments T3 and non-treated T4 as control. Two-week-old seedlings were transplanted into pots that contained 1 kg of soil mixture consisting of soil, peat and sand in the ratio of 2:1:1. A week before transplanting 0.1 g of ^{15}N labeled ammonium sulfate (10.18 % atom excess) were mixed with 1 kg soil. Non-treated plants (T4) were used as control. In experiment I, crops were harvested after two months. Dry weights were measured. N_2-fixing activity was carried out by ^{15}N dilution method. ^{15}N abundance in samples was determined by emission spectrometry after Kjeldahl digestion and titration of digests. Percentage of N derived from labeled fertilizer (%Ndff), atmosphere (%Ndfa) and soil (%Ndfs) were calculated by using the following equations:

$$\% \text{ Ndff} = \{^{15}\text{nae}/^{15}\text{N } (10.18)\} \times 100 \%$$
$$\% \text{ Ndfa} = \{1 - (\text{ndff treatment} / \text{ndff control})\} \times 100\%$$
$$\% \text{ Ndfs} = 100 - \% \text{ ndff} - \% \text{ ndfa}$$

In experiment II, yields were harvested two seasons and fresh weight was measured. Data were analyzed by ANOVA with the means separated by Duncan's test ($P \leq 0.05$).

Table1: Seed treatments for Okra Seeds

T1 - sodium alginate + AP2
T2 - sodium alginate + AP3
T3 - gum arabic + AP2
T4 - gum arabic + AP3
T5 - methycellulose + AP2
T6 - methycellulose + AP3
T7 - polyethylene glycol 4000 (PEG) + AP2
T8 - polyethylene glycol 4000 (PEG) + AP3
T9 - control

RESULTS AND DISCUSSION

(1) Development of seed treatments for okra seeds

Eight seed treatments of phosphate solubilising bacteria and plant growth promoter mixed with different adhesives were treated on Okra seeds. Seed treatments on the germination rates of Okra seeds are shown in Figure 1. All seed treatments significantly increased seed germinations except treatment of Gum Arabic mixed with AP3 (T4). With this view, the result showed that seed treatments with adhesive may increased seed germinations. This finding was supported by Zarrin *et al*., (2006) reported that soybean seeds inoculated with Rhizobium strains as seed coating just before sowing and mixture of phosphate solubilising bacteria were increase growth in root or shoot. The improvement in seed germination by seed treatment with adhesive was also found in work with Gholami *et al*., (2009) that inoculation maize seeds with plant growth-promoting rhizobacteria coated with gum Arabic enhanced seed germination and seedling vigour. Shahnaz *et al*, (2008) also reported germination and growth on okra and sunflower plants were significantly enhanced by increasing seed coating materials (sugar, molasses, glucose and gum Arabic) and *Trichoderma harzinum*. These finding may be due to the increased synthesis of hormones like gibberellins, which would have triggered the activity of specific enzymes that promoted early germination, such as a-amylase, which, have brought an increase in availability of starch assimilation. Beside, significant increase in seedling vigour would have occurred by better synthesis of auxins (Bharathi *et al*., 2004).

Figure 1 also shows maximum germination rates were observed when treated with polyethylene glycol 4000 (PEG) mixed with AP2 (T7) and AP3 (T8). Germination rates for T7 and T8 were 97%. Treatment with Methycellulose mixed with AP3 (T6) also showed high germination rates, which was 90%. Szopirinska and Tylkowska, (2009) indicated that *Zinnia Elegans* seeds osmoprimed with PEG improved seed vigour. Joyce and Melissa, (2008) also showed seed priming with PEG resulted in significantly higher germination percentage than untreated control tomato seeds. Tomato seeds bio-primed with plant growth promoter and phosphate solubilising bacteria mixed with Methycellulose enhanced seed germination rates (Phua *et al*., 2004).

Figure 2 illustrates the maximum log of viable cells when treated with PEG mixed with AP2 (T7) and AP3 (T8). Viable cells coated for a seed was 9.33 X 10^9 and 6.61 X 10^9cfu per seed. Treatment with Methylcellulose and AP2 (T5) also showed significant higher viable cells as compared to other treatment except T7 and T8. Crisphead lettuce seeds germinated from alginate coated with *Pseudomonas aeruginosa* LY-11 maintain at least 10^4 cfu per gram of plant tissues in plant rhizosphere and reduced damping-off (Kwang *et al.*, 2008).Gomathy *et al*., (2008) showed that survival of phosphobacteria applied as seed inoculation on black gram, soybean and maize with rice gruel adhesive performed better than without adhesive. These could be due to adhesive increased stickiness of the inoculants.

Therefore, using PEG as adhesive enhanced the germination rates and log of viable cells of AP2 and AP3. PEG could be a good adhesive for seed treatment. Methylcellulose could be second choice of adhesive for seed treatment.

(2) Nutrient uptake study

Greenhouse experiments revealed that okra seeds treatment with AP2 and PEG (T1) resulted the highest dry weight (9.16 g) compared to other treatments (Figure 3).Figure 4 shows urea treatment (T3) had the highest contribution of N while seeds treated with AP3 and PEG (T2) had higher contribution of N (34.84%) compared to seeds treatment (T1) with 11.83% of N. There was no significant difference within seed treatments (45.83 g for T1 and 49.59 g for T2) and urea treatment

T3 (61.72g) in okra yields (Figure 5). All treatments significantly increased yields compared with controlT4 (22.14g). Similarly, seeds inoculation with plant growth promoter or phosphate solubilising microorganism increased plant growth or yield were reported by other workers (Phua *et al.*, 2004 and Zarrin *et al.*, 2006). Gholami*et al.*, (2009) reported increasing germination, seedling growth and yield of maize when inoculated seeds with plant growth promoting rhizobacteria. Seeds dressing with *Trichoderma harzainum*, *Bacillus thuringiensis*, *Rhizobium meliloti* and *Aspergillus niger* increased germination, shoot length, shoot weight, root length and root weight for okra and sunflower (Shahnaz *et al.*, 2008).Seed inoculation of cowpea by phosphate solubilises improved nodulation, root and shoot biomass, straw and grain yield and phosphorus and nitrogen uptake of the crop (Linu *et al.*, 2009). Thus, seed treatments with plant growth promoter or phosphate solubilising bacteria can be used as an alternate or as supplement to chemical fertilizer to increase agricultural production with less input capital and energy.

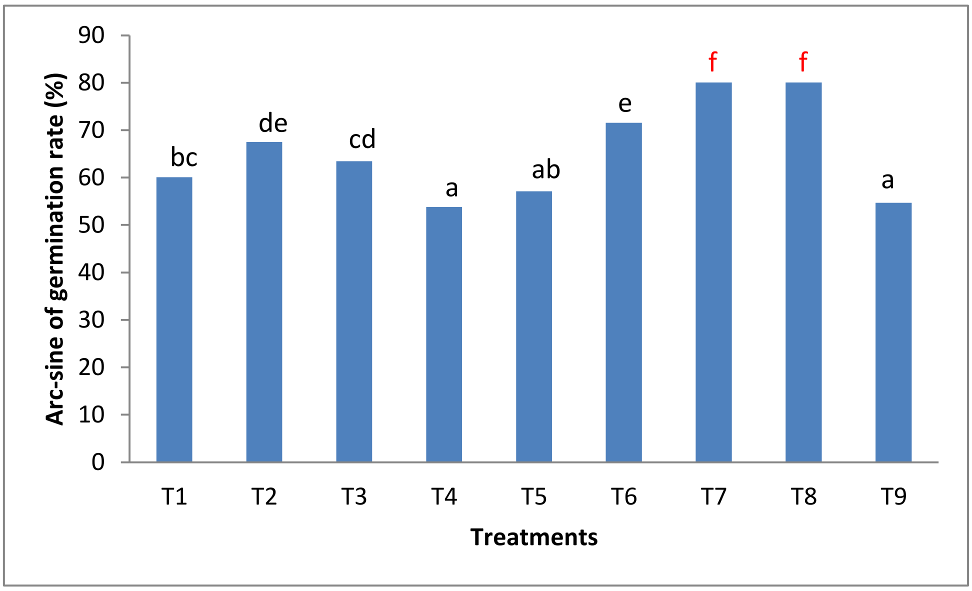

Fig. 1: Germination rates of seed treatments on Okra seeds

Key:

T1 - sodium alginate + AP2
T2 - sodium alginate + AP3
T3 - gum arabic + AP2
T4 - gum arabic + AP3
T5 - methycellulos + AP2
T6 - methycellulose + AP3
T7 - polyethylene glycol 4000 (PEG) + AP2
T8 - polyethylene glycol 4000 (PEG) + AP3
T9 - control

Note: All values are means of four replications with fifteen seeds per replication. Means followed by the same letter among are not significantly different from each other ($p \leq 0.05$) as determined by Duncan's test.

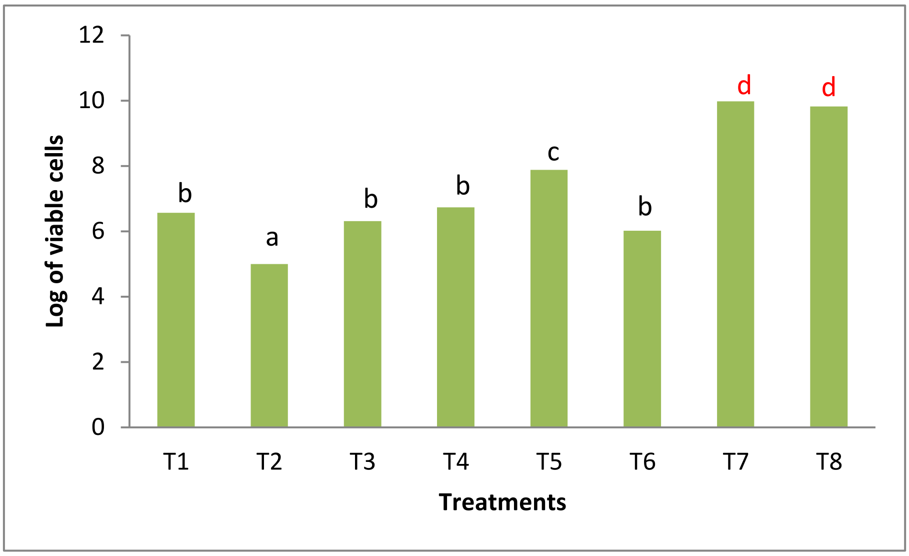

Fig. 2: Log of viable cells of seed treatments on Okra seeds

Key:

T1 - sodium alginate + AP2	T5 - methycellulose+ AP2
T2 - sodium alginate + AP3	T6 - methycellulose+ AP3
T3 - gum arabic + AP2	T7 - polyethylene glycol 4000 (PEG)+ AP2
T4 - gum arabic + AP3	T8 - polyethylene glycol 4000 (PEG)+ AP3
T9 - control	

Note: All values are means of four replications with fifteen seeds per replication. Means followed by the same letter among are not significantly different from each other ($p \leq 0.05$) as determined by Duncan's test.

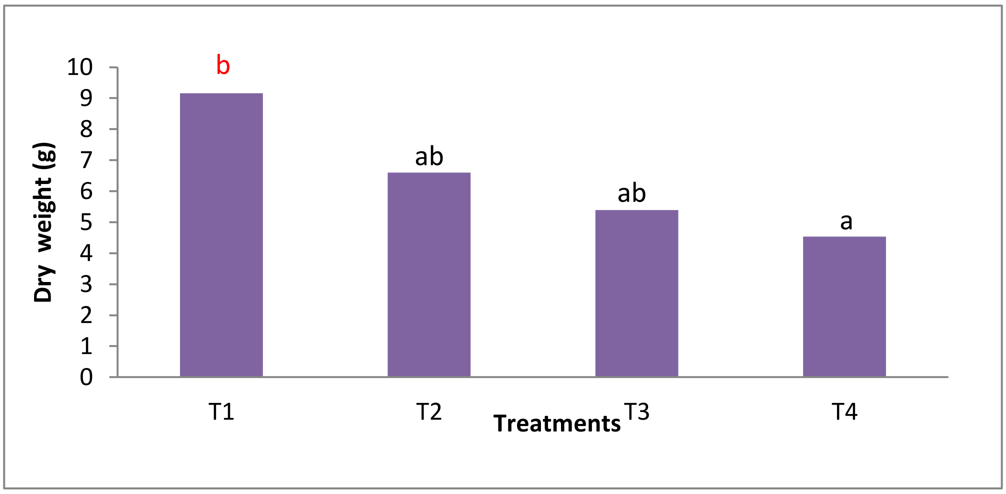

Fig. 3: Dry weight (g) of seed treatments on Okra plants.

Key:

T1 - PEG + AP2	T3 - Urea
T2 - PEG + AP3	T4 - Control

Note: All values are means of three replications with four plants per replication. Plants were harvested after two months of planting. Means followed by the same letter among are not significantly different from each other ($p \leq 0.05$) as determined by Duncan's test.

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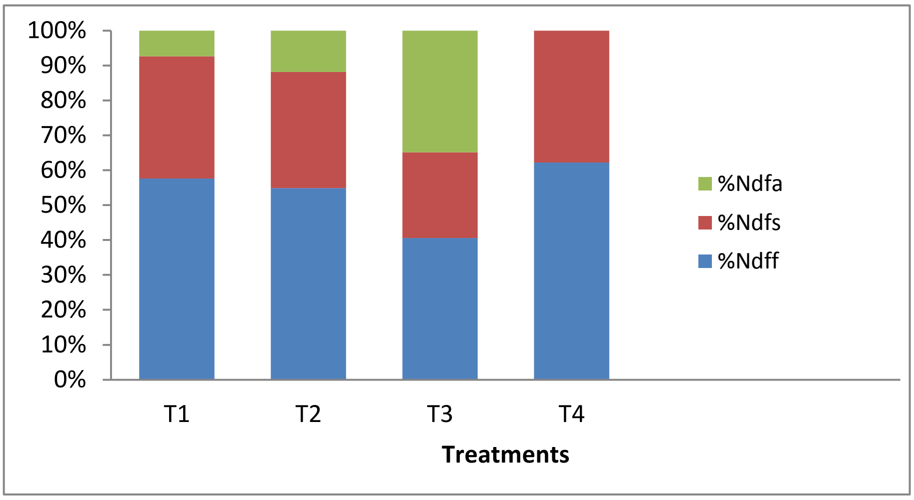

Fig. 4: Effects of seed treatments in contributing N_2 to Okra plants by using ^{15}N isotopic tracer

Key:
% Ndff = % N derived from labelled fertilizer
% Ndfs = % N derived from soil
% Ndfa = % N derived from treatments or atmosphere

Note: All values are means of three replications with four plants per replication. Plants were harvested after two months of planting.

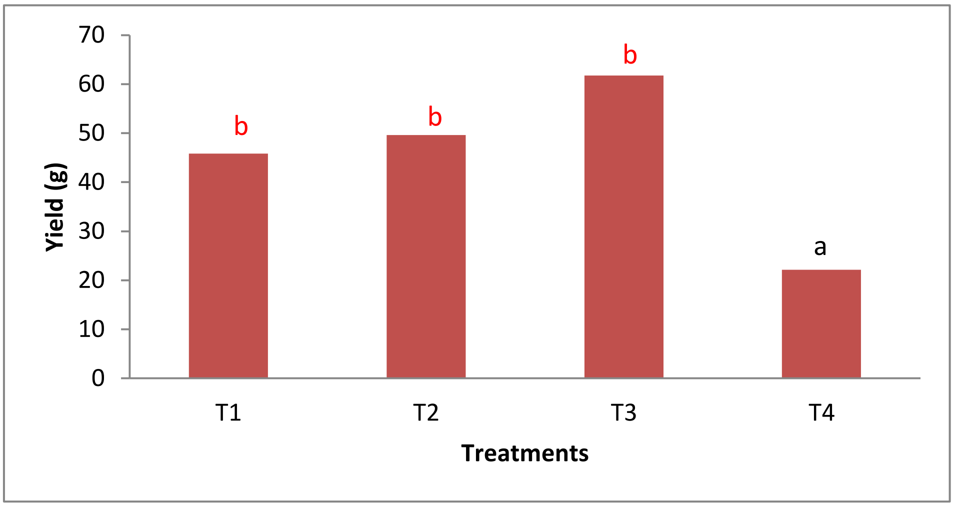

Fig. 5: Effects of seed treatments on Okra yields (g).

Key:
T1 - PEG + AP2 T3 - Urea
T2 - PEG + AP3 T4 - Control

Note: All values are means of three replications with four plants per replication. Yields were harvested after three months of planting. Means followed by the same letter among are not significantly different from each other ($p \leq 0.05$) as determined by Duncan's test.

CONCLUSIONS

Eight seed treatments of phosphate solubilising bacteria and plant growth promoter mixed with different adhesives were treated on Okra seeds. Maximum germination rates and log of viable cells were observed when treated with polyethylene glycol 4000 (PEG) mixed with AP2 (T7) and AP3 (T8). Thus, PEG could be a good adhesive for seed treatment. Seed treatments with plant growth promoter (T1) and phosphate solubilising bacteria (T2) increased dry weights, yields and N uptakes compared to control. Seed treatments could be alternative for chemical fertilizers.

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