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Response of Amended Melamine Phosphate with Different Levels of Fertilizers on *Rhizobium* Population and Soil Microbial Activity

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The experiment was conducted under field conditions at Instructional cum Research Farm, College of Agriculture, Raipur (Chhattisgarh) during *kharif* season 2011-12. The data revealed that at 45 and 60 DAS treatment T2 (11.01 and 7.16 x10^3) was recorded significantly highest *rhizobium* population which was at par with T9 (10.6 and 6.86 x10^3) and T7 (9.99 and 6.24 x10^3). However at harvest stages Treatment T2 (4.66) was observe significantly highest *rhizobium* population among all the treatments but it was recorded significantly at par with T9 (4.35 x10^3), whereas lowest *rhizobium* population was recorded under treatment T1(Absolut control) at all the growth stages. Result recorded that Response of amended melamine phosphate on rhizosphere soil respiration was found significantly higher in treatment T9 among all the treatments, but it was found significantly at par with treatment T2 (0.367 CO2/hr/100g soil) and T7 (0.356 CO_2/hr/100g soil), however lowest rhizosphere soil respiration was recorded in treatment T1 (0.183 CO2/hr/100g soil) (Absolute control) at 45 DAS. At 60, 90 and at harvest stages treatment T2 (0.523,0.467 and 0.423 CO2/hr/100g soil) was recorded significantly highest among all the treatment in soybean field. Response of amended melamine phosphate on rhizosphere soil dehydrogenase activity at different stages recorded significantly higher at 45 DAS in T9 (22.0 μg/TPF/h/g soil) followed by T2 (20.6 μg/TPF/h/g soil) but treatment T2 was recorded significantly at par with T7 (19.9 μg/TPF/h/g soil). At 60 and at harvest stage treatment T2 (45.2, 28.3 μg/TPF/h/g soil) was recorded significantly highest dehydrogenase activity among all the treatments followed by treatment T9 (44.4, 26.5 μg/TPF/h/g soil), however, at 90 DAS dehydrogenase activity T2 (42.4μg/TPF/h/g soil) was recorded significantly highest among all the treatments.

Key words: Amended melamine phosphate, *rhizobium* population, Dehydrogenase activity and soil respiration.

Soybean originated in China and was introduced to India centuries ago through the Himalayan routes and also via. Burma (now Myanmar) by traders from Indonesia. As a result, soybean has been traditionally grown on a small scale in Himachal Pradesh, the Kumaon Hills of Uttaranchal, eastern Bengal, the Khasi Hills, Manipur, the Naga Hills, and parts of central India. Because of its high protein and oil content and other attributes such as its beneficial effects on soil fertility, several attempts are being made to popularize soybean cultivation in India.

The importance of soybean as a source of oil and protein and its ability to grow symbiotically on low-N soils. Improvements in biological nitrogen fixation can help to enhance soybean productivity per unit area. In Chhattisgarh, the area, production and productivity

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of soybean are 1, 33,060 ha, 1, 48,228.84 tones and 1.114 tones ha^{-1}, respectively, while at national level the area, production and productivity are 9.95 million hectares, 12.57 million tones and 1.264 tones ha^{-1}, respectively (Anonymous, 2010). From nutritional point of view, it is called as miracle bean (Anonymous, 2001). It contains about 40% protein, well balanced amino acids, 20% oil rich with poly unsaturated fatty acids (PUFA) specially Omega 6 and Omega 3 fatty acids, 6-7% total mineral, 5-6% crude fiber and 17-19% carbohydrates (Chauhan *et al.*, 1988).

Rhizobium can apply to seed because this is an easy, convenient way to establish the bacteria in the root zone of the developing seedling. The laborious task of spreading tons of soil to provide a few rhizobia could thus be by passed (Peppler and Perlman, 1979).

At flowering stage of crop, microbial population was increased considerably as compared to vegetative stage of crop due to higher root activities *i.e.* greater rhizosphere effect (Shetty and Rangaswami, 1969).

Louw and Webley (1959) reported that count of bacteria in the root region increased during the growing period of the plant. According to Nutman (1975) stimulation of the rhizobia is greatest at places where lateral roots emerge and generally extends to 10-20 mm from the root surface into the soil. Increase growth of *Rhizobium* in the rhizosphere is a response to excretion of energy source, amino acids, growth factors, especially B group vitamins and enzymes by plant roots. The rhizosphere stimulation is a response to a complex mixture of substances.

The use of high-quality input including slow releasing source of nutrient like melamine phosphate with Mo, Fe and S and study about their benefits can still make a significant contribution in many soybean growing countries to increase biological nitrogen fixation as well as soybean productivity per unit area.

MATERIALS AND METHODS

The experiment was conducted under field conditions at Instructional cum Research Farm, College of Agriculture, Raipur (Chhattisgarh) during *kharif* season 2011-12. The crop was sown on 5th July, 2011, by the seed drill @ 80 kg ha^{-1} .Spacing between plant to plant and row to row was 10 x 30 cm. Melamine phosphate amended with Mo, Fe, S was applied as basal soil application @ 5kg ha^{-1} and foliar application @ 3gl^{-1} at 20 and 45 DAS. Nitrogen, Phosphorus and Potassium @ 20:60:20kg ha^{-1} (100% RDF) and 15:45:15 kg ha^{-1} (75% RDF) was applied as basal through urea, single super phosphate and murate of potash. The experiment was laid out in Randomized Block Design (RBD) with three replications. The treatments comprised of 9 nutritional schedules. This study was planned with different treatments (T1: Absolute Control *i.e.* no fertilizers and no rhizobial inoculation, T2:Inoculation of Rhizobium +100% RDF, T3: Inoculation of Rhizobium + 75% RDF, T4: Inoculation of Rhizobium + Soil application of amended melamine phosphate, T5: Inoculation of Rhizobium + Foliar application of amended melamine phosphate, T6: Inoculation of Rhizobium +75% RDF + Soil application of amended melamine phosphate, T7: Inoculation of Rhizobium +75%RDF+ Foliar application of amended melamine phosphate, T8: Inoculation of Rhizobium + Soil and foliar application of amended melamine phosphate and T9: Inoculation of Rhizobium +75% RDF+ Soil and foliar application of amended melamine phosphate). Healthy soybean seed was treated before sowing with thiram @ 3g kg^{-1} seed. After fungicidal treatment, soybean (JS-335) seed was inoculated with homologous effective local culture of *Rhizobium* @ 5 g kg^{-1} seed. Neutralized gum arabic and lignite were used as sticking and wetting agent. Amount of matured YEM- *Rhizobium* suspension was fixed to ensure at least 10^5 viable cells were received by every seed (Nambiar *et al.*, 1984 and Nambiar, 1985).The inoculated seeds were kept in cool and dry shed before sowing.

Microbial analysis of soil and YEM broth

Microbial analysis of soil and YEM broth was done by serial dilution plating method (Subba Rao, 1988). Soil samples up to 5-10 cm depth were drawn out with the help of sterilized spoon from each plot at different stages of the crop growth. The sampling of soil was done at 45, 60 DAS and at Harvest. Soon after sampling, the soil samples were kept in polythene bags to prevent the moisture loss and properly tagged, sealed and stored in refrigerator for quantitative estimation of *Rhizobium*. Microbiological estimations with

respect to rhizobial count in the soil and YEM broth samples were done by dilution plate method (Subba Rao, 1988). For rhizobial counting, serial dilutions of the samples were done by taking 1 gm of soil sample in 9 ml sterilized water in a dilution tube (Tuladhar, 1983) and it was shaken on a shaker for 30 minutes. After shaking, the dilution tube (No.1) was kept for 30 minutes to allow the soil particles to settle down, in this way 10^1 dilution of the soil sample was obtained. Now 1.0 ml of the rhizobial suspension from dilution tube No.1 was drawn out with the help of auto-pipette and transferred to another dilution tube No. 2 containing 9 ml sterilized water resulting 10^2 dilution. It was again kept on rotary shaker for 5 minutes. Again 1.0 ml suspension was drawn from dilution tube No. 2 for 10^3 dilutions and this way serial dilution of a soil sample was carried out up to desirable dilution and finally a complete set of desirable dilutions of each sample was obtained. Similarly, population density of *Rhizobium* in mature YEM broth was also determined.

Composition of the medium Yeast Extract Mannitol Agar media (YEMA) for *rhizobium*

Mannitol	10.0g
K_2 HPO_4	0.5g
$MgSO_4$ $7H_2O$	0.2g
NaCl	0.1g
Yeast Extract	1.0g
Agar	15.0g
Distilled water	1000.0ml
Congo red solution (1%)	2.5ml
pH	7.0

(Subba Rao, 1988)

About 20 ml of the appropriate sterilized and partially cooled agar media was poured in to the sterilized Petri plates containing 1 ml aliquot of appropriate dilution at the bottom which was drawn out from the dilution tube with the help of sterilized tips of auto-pipette and the plates were incubated at 28°C in the incubator. Counting of rhizobial colonies was started after 24 hours of incubation. Counted colonies were marked with the instant marker to avoid the repetition in counting and the process of counting was continued up to 7 days of incubation. Colony counting was done on colony counter.

Plating of each samples was done in duplicate and mean values were worked out for each samples. One control was also incorporated with each set of plating. After counting of colonies, rhizobial population was calculated on the basis of per gm of dry soil using following formula (Schmidt and Caldwell, 1967.). Rhizobial population density in the YEM broth was also estimated by using the same formula.

Number of rhizobia per gm of oven dry soil

$$= \frac{\text{No. of colony forming units (CFU) x dilution}}{\text{Dry weight of one gm moist soil sample x aliquot taken}}$$

Number of rhizobia/ml of matured YEM broth

$$= \frac{\text{No of colony forming units (CFU) x dilution}}{\text{Aliquot taken}}$$

The operation of making serial dilutions, setting of agar plates, inoculation with appropriate media, was done in sterilized atmosphere of laminar flow. Characterization study for confirmation of *Rhizobium* isolates was completed by using plant infection test, Gram's staining technique, microscopy through phase contrast microscope Leica DMRBE etc. before using the selected isolate of *Rhizobium.*

Basal Soil Respiration study

This study was conducted to know the respiration rate of micro-flora present in the soybean rhizosphere soil. Basal soil respiration was conducted by measuring the CO_2 evolution rates (Anderson, 1982). 100 g soil (oven dry basis) was taken in 1L conical flask. The water is added to bring its moisture content to field capacity. 20 ml of 0.5N NaOH was taken in test tubes. The tubes were hanged with the help of thread inside the conical flask without touching the soil and kept the flasks air tight by rubber stoppers and note down the time. The flasks were kept in an incubator at 28 °C for about 20 hrs. After incubation test tubes were took out from the flask and incubation period was recorded. Immediately transfer the 0.5N NaOH solution from the test tube to a 150 ml conical flask. 5 ml of 3N $BaCl_2$ solution and few drop of phenolphthalein indicator were added. Titrated the content with standard 0.5N H_2SO_4 slowly until the pink colour just disappears. After getting the end point recorded the exact amount of acid required for titration.

Soil respiration (mg of CO2/h/100g soil) = (B-V) NE/ hours of incubation

Where,

B = Volume of acid (ml) needed for the blank.
V = Volume of acid (ml) needed for the NaOH exposed to soil.
N = Normality of acid
E = Equivalent weight, i.e. 22.

Dehydrogenase activity

The procedure to evaluate the dehydrogenase activity of soil described by Lenhard (1956), in which 1gm air dried soil sample was taken in a 15 ml airtight screw capped test tube. 0.2 ml 3% TTC solution was added in each tube to saturate the soil and 0.5 ml distilled water was also added in each tube. Gently tap the bottom of the tube to driven out all trapped oxygen so that a water seal was formed above the soil. No air bubbles were formed that was ensured**.** The tubes were incubated at 37°C for 24 hrs. Then 10 ml of methanol was added. Shake it vigorously and allowed to stand for 6 hrs. Clear pink coloured supernatant was withdrawn and readings were taken with a spectrophotometer. The amount of TPF formed was calculated from the standard curve drawn in the range of 10 mg to 90µg TPF/ml.

RESULTS AND DISCUSSION

Table1 reveales that at 45 and 60 DAS treatment T2 (11.01and 7.16 x10^3) was recorded significantly highest *rhizobium* population which was at par with T9 and T7. However, at harvest stages treatment T2 was observed significantly highest rhizobium population among all the treatments, but it was recorded significantly at par with T9, whereas, *rhizobium* population was recorded significantly lowest under treatment T1 at all the growth stages. Similar type of results were also reported by Chowdhury, 1991; Katre *et al*., 1997. They reported that rhizobial population increased considerably up to flowering stage of crop growth due to higher degree of rhizosphere effect. These observations are also in close agreement with Shetty and Rangaswami (1969), Rao, (1980), Gupta *et al.* (1988), Gupta *et al.*(1992).

Table2 shows that rhizosphere soil respiration was found significantly highest under treatment T9 (0.375 CO_2/hr/100g soil) among all the treatments, but it was found significantly at par with treatment T2 (0.367 CO_2/hr/100g soil) and T7 (0.356 CO_2/hr/100g soil), however lowest rhizosphere soil respiration was recorded in treatment T1 (Absolute control) at 45 DAS. At 60, 90 DAS and at harvest stages treatment T2 (0.523,0.467 and 0.423 CO_2/hr/100g soil) was recorded significantly highest rhizosphere soil respiration among all the treatment in soybean field. This finding was similar as reported by Roger, P.A. *et al*. (1994), who stated that pesticides application on soil at recommended dose rarely had a detrimental effect on microbial population or their activities.

Table3 shows that rhizosphere soil dehydrogenase activity at different stages were recorded. Significantly higher rhizosphere microbial dehydrogenase activity was recorded at 45 DAS in treatment T9 (22.0 µg/TPF/h/g soil) followed by T2 (20.6 µg/TPF/h/g soil) but treatment T2 was recorded significantly at par with T7 (19.9 µg/TPF/h/g soil). At 60 DAS and at harvest stage

Table 1. Response of amended melamine phosphate on *Rhizobium* population at different stages of field grown soybean

Treatment	Rhizobial population per gm soil (x10^3) at45 DAS	Rhizobial population per gm soil (x10^3) at60 DAS	Rhizobial population per gm soil (x10^3) at harvest
T1	6.76	4.25	1.46
T2	11.01	7.16	4.66
T3	9.47	5.96	2.97
T4	7.86	5.16	2.46
T5	8.39	5.64	2.79
T6	9.53	5.93	2.93
T7	9.99	6.24	3.19
T8	8.5	5.75	2.85
T9	10.6	6.86	4.35
CD(0.05)	1.16	0.97	0.60

Table 2. Response of amended melamine phosphate on rhizosphere soil respiration at different stages of field grown soybean

Treatment	45 DAS (CO2/hr/100g soil)	60 DAS (CO2/hr/100g soil)	90 DAS (CO2/hr/100g soil)	At harvest (CO2/hr/100g soil)
T1	0.183	0.230	0.212	0.192
T2	0.367	0.523	0.467	0.423
T3	0.300	0.383	0.358	0.321
T4	0.218	0.275	0.242	0.221
T5	0.235	0.307	0.268	0.242
T6	0.322	0.413	0.379	0.333
T7	0.356	0.454	0.412	0.365
T8	0.265	0.364	0.321	0.263
T9	0.375	0.490	0.439	0.392
CD(0.05)	0.02	0.01	0.02	0.02

Table 3. Response of melamine phosphate on rhizosphere soil dehydrogenase activity at different stages of field grown soybean

Treatment	45 DAS (µg/TPF/h/g soil)	60 DAS (µg/TPF/h/g soil)	90 DAS (µg/TPF/h/g soil)	At harvest (µg/TPF/h/g soil)
T1	13.3	29.3	26.9	17.6
T2	20.6	45.2	42.4	28.3
T3	16.3	36.3	34.2	21.9
T4	14.3	31.5	28.7	19.2
T5	15.1	33.2	29.6	20.4
T6	17.5	38.8	36.2	23.2
T7	19.9	41.7	37.4	24.1
T8	15.7	34.6	32.9	21.2
T9	22.0	44.4	39.8	26.5
CD(0.05)	1.19	2.71	2.53	2.24

treatment T2 (45.2, 28.3 µg/TPF/h/g soil) was recorded significantly highest dehydrogenase activity among all the treatments followed by treatment T9 (44.4, 26.5 µg/TPF/h/g soil). Whereas, at 90 DAS dehydrogenase activity T2 (42.4µg/TPF/h/g soil) was recorded significantly highest among all the treatments. These observations are in lines with that of Chendrayan et al. (1980) who also gave similar finding that the increase in dehydrogenase activity was mainly due to the higher microbial population. Rate of fertilizer utilization by microbes can improve soil enzyme activities.

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