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Influence of Amended Melamine Phosphate (AMP) at Different Levels of Fertilizer on Nitrogen Accumulation, Yield and Microbial Population on Soybean at Chhattisgarh Plain

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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 observed that treatment T2 (1.47%, and 65.42 kg ha^{-1}) was recorded significantly higher N content % and N uptake ha^{-1} in the straw, followed by T9 (1.40% and 58.34 kg ha^{-1}) and T7 (1.40% and 55.86 kg ha^{-1}).Whereas, N content % and N uptake in grain recorded significantly similar results in the treatment T9 (6.31% and 157.12 kg ha^{-1}) and T7 (6.29% and 153.04 kg ha^{-1}). However, total N uptake by soybean was recorded significantly highest under treatment T2 (235.18 kg ha^{-1}) followed by T9 (215.46 kg ha^{-1}).Number of nodulation plant^{-1} was recorded significantly higher in treatment T6 (80.00) which was at par with T2 (79.33), T9 (77.67) and T7 (71.67). Dry weight of nodule, N content of nodule, number of pods plant^{-1}, grain yield and straw yield was recorded significantly higher in treatment T2 (0.196 g plant^{-1},7.12%,34.12, 26.65 qha^{-1} and 44.51 qha^{-1}) which was at par with T9 (0.187 g plant^{-1},6.89%,32.14, 24.90 qha^{-1} and 41.09 qha^{-1}), T7 (0.181 g plant^{-1},6.82%,31.47 qha^{-1} and 24.33 qha^{-1}), T6 (0.178 g plant^{-1},6.75%,29.60 and 23.92 qha^{-1}) and T3 (0.170 g plant^{-1},6.73%,28.50 and 23.65 qha^{-1}). Response of amended melamine phosphate on Rhizobial population, gram^{-1} of soil at 45 DAS T2 (11.03 gm^{-1} soil) was recorded significantly higher among all the treatments which was at par with treatment T9 (10.6 gm^{-1} soil) and T7 (9.99 gm^{-1} soil). Soil respiration and soil dehydrogenase activity was found significantly higher in treatment T9 (0.375CO2/hr/100g soil, 22.0μg/TPF/h/g soil) which was at par with T2 (0.367 CO2/hr/100g soil) and T7 (0.356CO2/hr/100g soil) incase of soil respiration and T2 (20.6μg/TPF/h/g soil) was at par in soil dehydrogenase activity.

Key words: Amended melamine phosphate, N accumulation, *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. (Patel *et al* 2015).

The importance of soybean as a source of oil and protein and its ability to grow symbiotically on low-N soils. Improvements in

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biological nitrogen fixation can help to enhance soybean productivity per unit area. In Chhattisgarh state, the area, production and productivity 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. 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.

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.

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

Mannitol	10.0g
K_2HPO_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)

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 37ÚC 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

Response of amended melamine phosphate on nitrogen accumulation shown in Table 1. It is observed that treatment T2 was recorded significantly higher N content % and N uptake ha^{-1} in the straw and grain), followed by T9 and T7. Whereas, N content and N uptake in grain was recorded significantly similar results in the treatment T9 and T7. However, total N uptake by soybean was recorded significantly highest under treatment T2 followed by T9. Findings of the present investigation are close to observations of Miyan *et al.*, 1989; Mullar and Perira, 1995 and Gupta *et al.*, 2000. Further, this observation is also supported by Egamberdiyeva *et al.,* 2004; Gupta *et al.*, 2005; they reported that significantly increase in N accumulation was due to rhizobial inoculation with different levels of fertilizers. Tang *et al.* 1992 and Hara, 2001 also supported the positive influence of Mo, Fe and S amended melamine phosphate on soybean. In this connection, Hara (2001) mentioned that direct requirement in metabolism of rhizobia are carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, potassium, calcium, magnesium, iron, manganese, copper, zinc, molybdenum, nickel, cobalt and selenium for improving biological nitrogen fixation. Similar type of views were also given by several researchers on effectiveness of legume –*Rhizobium* symbiosis (Gupta and Gangwar, 2012).

Table 2 revealed that number of nodulation plant^{-1} was recorded significantly higher in treatment T6 which was at par with T2,

Table 1. Response of amended melamine phosphate on N accumulation by field grown soybean at harvest.

Treatment	Straw N- content (%)	Straw N-uptake (Kg ha^{-1})	Grain N-content (%)	Grain N-uptake (Kg ha^{-1})	Total N-uptake (Kg ha^{-1})
T1	1.14	26.04	5.61	88.36	114.40
T2	1.47	65.42	6.37	169.76	235.18
T3	1.37	52.16	6.23	147.34	119.50
T4	1.23	40.41	6.06	119.75	160.16
T5	1.25	42.35	6.08	121.60	163.95
T6	1.39	53.86	6.26	149.74	203.60
T7	1.40	55.86	6.29	153.04	208.90
T8	1.28	44.61	6.13	126.89	171.50
T9	1.40	58.34	6.31	157.12	215.46
CD(0.05)	0.06	5.69	0.28	16.87	19.66

T9 and T7 and lowest number of nodules was recorded in treatment T1 which was absolute control. Dry weight of nodule, N content of nodule, number of pods plant^{-1} and grain yield was recorded significantly higher in treatment T2 which was at par with T9, T7, T6 and T3. Incase of straw yield, treatment T2 was recorded statically maximum followed by treatment T9 and treatment T1 was recorded significantly lowest straw yield. Findings of the present investigation are close to observations of Zhang *et al.,* 1996, who reported that significantly increased N accumulation due to treatment with different fertilizers levels. Similar findings were also reported by Prasad and Ram (1986), Alagawadi *et al.* (1993) and Quasim *et al* (2001), they mentioned that number of nodules and biomass can be increased due to different levels of fertilizer and rhizobial inoculation. This observation is also supported by the findings of El-Din and Moawad, 1991; Paradkar and Deshmukh 2004; Azeez and Adetunji 2008. Further, Shindhe and Soni 1981; Hassan *et al*., 1994; Gupta *et al*., 1995; Sharma and Namdev 1999; Ganeshamurthy and Raddy 2000; Gupta *et al.,* 2000; Patil et al., 2002; Tomar *et al*., 2004, they mentioned significantly higher number of nodule, fresh and dry weight was due to treatment with different levels of fertilizers. Findings of the present investigation are also close to observations of Tomar *et al* 2004; Khutate *et al*., 2005, Tripathi *et al*.2008, and Tomer and Khajanji , 2009. They clearly mentioned that grain and straw yield of soybean significantly increased by use of different levels of fertilizer.

Response of amended melamine phosphate on Rhizobial population, Soil respiration, Soil dehydrogenase activity are present

Table 2. Response of amended melamine phosphate on number of nodulation, dry weight of nodule, N content of nodule, number of pods, grain yield and straw yield

Treatment	No. of nodulation plant^{-1}	Dry wt. of nodule (g plant^{-1})	N content of nodule (%)	No. of pods plant^{-1}	Grain yield (q/ha)	Straw yield (q/ha)
T1	29.67	0.115	5.25	22.52	15.75	22.84
T2	79.33	0.196	7.12	34.12	26.65	44.51
T3	70.33	0.170	6.73	28.50	23.65	38.08
T4	59.00	0.148	6.19	23.68	19.76	32.86
T5	55.33	0.154	6.30	25.50	20.00	33.88
T6	80.00	0.178	6.75	29.60	23.92	38.75
T7	71.67	0.181	6.82	31.47	24.33	39.90
T8	63.67	0.157	6.35	26.90	20.70	34.85
T9	77.67	0.187	6.89	32.14	24.90	41.09
CD(0.05)	8.43	0.03	0.63	5.03	3.32	3.56

Table 3. Response of amended melamine phosphate on Rhizobial population, Soil respiration, Soil dehydrogenase activity at 45 DAS.

Treatment	Rhizobial population per gm soil (x10^3) at45 DAS	Soil respiration (CO2/hr/ 100g soil) at 45 DAS	Soil dehydrogenase activity (µg/TPF/h/g soil) at 45 DAS
T1	6.76	0.183	13.3
T2	11.01	0.367	20.6
T3	9.47	0.300	16.3
T4	7.86	0.218	14.3
T5	8.39	0.235	15.1
T6	9.53	0.322	17.5
T7	9.99	0.356	19.9
T8	8.5	0.265	15.7
T9	10.6	0.375	22.0
CD(0.05)	1.16	0.02	1.19

in Table 3. Rhizobial population gram^{-1} of soil at 45 DAS was recorded significantly higher among all the treatments which was at par with treatment T9 and T7. 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).Soil respiration and soil dehydrogenase activity was found significantly higher in treatment T9 which was at par with T2 and T7 incase of soil respiration and T2 was at par in soil dehydrogenase activity. This finding was also 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. Patel *et al*. (2015), who reported that the increase in dehydrogenase activity was mainly due to the higher microbial population and rate of fertilizer utilization by microbes can improve soil enzyme activities.

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