

Full Length Research Paper

Response of *Glycine max* in relation to nitrogen fixation as influenced by fungicide seed treatment

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***Glycine max* – *Rhizobium* interaction is a well known symbiotic association occurring in nature and responsible for biological nitrogen fixation. Thiram a well-known fungicide has been in practice as seed dressing in order to prevent fungal colonization. In the present study the effect of various thiram concentrations is investigated. Thiram concentration beyond 500 µg/ml was observed to be highly toxic with respect to plant growth factors and rhizobial infection to the *G. max*. The nodulation, nodule dry weight, nitrogenase activity were observed to be maximum at 100 µg/ml of thiram. The study is useful in determining the threshold concentration of fungicide for soybean seed dressing for effective nitrogen fixation and crop yield.**

Key words: Ascorbic acid, chlorophyll content, *Glycine max*, nodulation, nodule dry weight, nitrogenase activity, protein content, thiram.

INTRODUCTION

Most of the terrestrial plants live in symbiosis with root infecting microorganism. Colonization of roots with *Bradyrhizobium* species is beneficial because it provides N₂ in fixed form as to the host and to the soil system (Beijerick, 1888b; Saffald, 1888; Fred et al., 1932). Several microbial species interacts in both positive and negative ways among each other (Anderson, 1978, Gaur, 1980, Kundu and Trimohan, 1989). Intraspecific interaction prevails within the species of *Bradyrhizobium* and results into nodule occupancy (Burton, 1979) in competitive manner by specific strain (Rennie, 1986). Not only biological factors are involved in root nodulation and N₂ fixation (Garrett, 1963, Halverson and Stacey, 1986; Beijerick, 1888b; Saffald, 1888; Fred et al., 1932) but abiotic components such as soil profile and certain

synthetic chemicals including fungicides, biocides, insecticides and fertilizers also influence nodulation process of biological N₂ fixation (Anderson, 1978; Gaur, 1980; Kundu and Trimohan, 1989).

The fungicide applied to leguminous plants either as seeds dressing or soil drench reach the soil and may affect the symbiotic relationship. Further, fungicide applied to another crop may be sufficiently persistent to effect nitrogen level (Gaur, 1980). Apart from this, several plant pathogenic microbes affects soybean plant health at different growth stages. The uses of non-mercurial fungicide TMTD (Thiram) for seed dressing of *G. max* have been practiced in most of the agricultural operations.

Several deviating observation in relation to compatibility of *Rhizobial* strains with fungicides have been previously demonstrated (Afifi et al., 1969; Rivellin et al., 1993; Grahm et al., 1980; Guene et al., 2003). In the present study the effect of thiram on *Rhizobium* inoculants with respect to nodulation, N₂ fixation, plant factors such as chlorophyll content, ascorbic acid concentration and protein content was investigated. *G. max* is one of the important crops of Madhya Pradesh (India) and it becomes necessary to elucidate the role of

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Abbreviations: Chl, chlorophyll; N₂-ase, nitrogenase; TMTD, thiram; YEMA, yeast extract mannitol agar.

thiram in field conditions effecting productivity of *G. max* as well as enhancement of nitrogen content of soil in agricultural field.

MATERIAL AND METHODS

Glycine max (L) merril, variety Punjab-1, seeds from Plant Breeding Department, R.A.K. Agriculture College, Sehore, Madhya Pradesh, India, were after pre-sterilization with mercuric chloride solution (HgCl_2). Experiments were performed in black cotton soil obtained from Raisen District of Madhya Pradesh, India. Seeds were inoculated in both sterilized and unsterilized soil at pH 8.2. Apart from this, the *G. max* was also inoculated on agar media (Gibson, 1963; Thrompton, 1930). Pure culture of *Rhizobium japonicum* strain SB 119, obtained from IARI, New Delhi, India, were grown on YEMA (Vincent, 1970). Soybean seeds were pretreated with varying concentration of thiram ranging from 10 to 750 $\mu\text{g/ml}$. All experiments were performed for 75 days followed by removal of soybean plant for further estimation of nodule number, nodule dry weight and N_2 -ase activity (Hardy et al., 1973; Turner and Gibson, 1980; Subba Rao 1984).

The residual N_2 content of soil was estimated by Kjeldahl method (Ferrai, 1960; Subba Rao, 1979) from the soil obtained upto the depth of 6 cm. After 75 days of incubation of *G. max* chlorophyll content was measured by the method performed by Arnon (1949) and Witham et al. (1971). The ascorbic acid content was also measured by the method of Harris (1935) and Sadasivam et al. (1987), and observations were inferred with the help of standard curve of oxalic acid. The protein content of leaves was calculated by Lowry's method (Lowry et al., 1975).

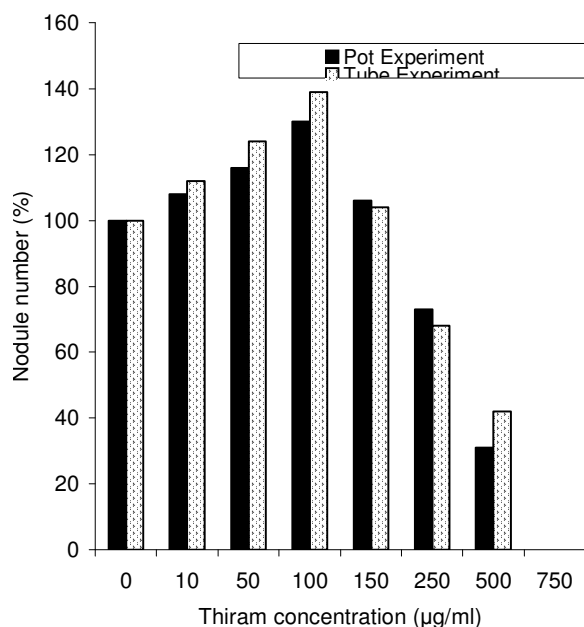


Figure 1a. Effect of different concentrations of thiram on nodule number of *G. max* inoculated with *R. japonicum* in pot and tube experiments.

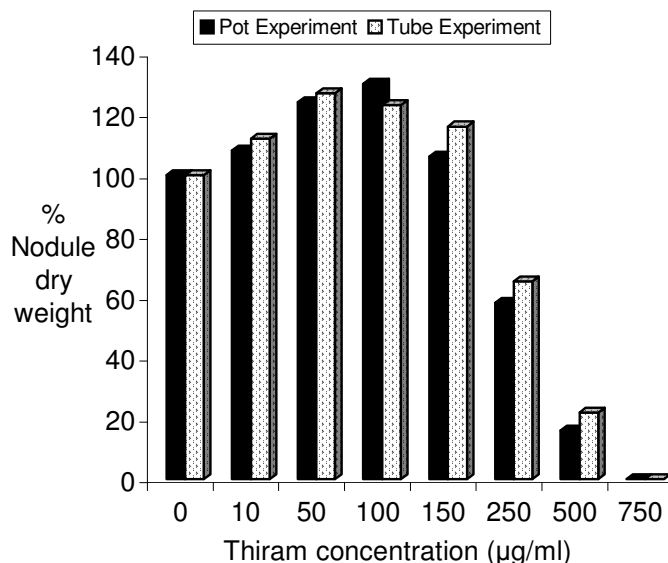


Figure 1b. Effect of different concentrations of thiram on nodule dry weight of *G. max* inoculated with *R. japonicum* in pot and tube experiments.

RESULTS

Nodulation and Nodule dry weight

The nodule number and dry weight in *G. max* was observed in thiram treated and untreated seed inoculum in soil and synthetic media. Thiram upto 100 $\mu\text{g/ml}$ was found to promote nodule number and dry weight which was reduced by further increase in thiram concentration and reached zero at 750 $\mu\text{g/ml}$ (Figure 1a). The present result is similar to earlier findings of Vyas et al. (1990). The increase in nodule number was found to be 6, 8, 19 and 33% in pot experiment and 4, 13, 27 and 36% in tube culture in the presence of 150, 100, 50 and 10 $\mu\text{g/ml}$ thiram, respectively. Similar trend of nodule dry weight was observed in *G. max* when seeds were pretreated with varying concentrations of thiram (Figure 1b). In case of synthetic media the nodule dry weight was found to increase to a maximum of 40% whereas, 46% enhancement was observed in soil condition at 100 $\mu\text{g/ml}$ of thiram. A fall in nodule dry weight was observed at 250 and 500 $\mu\text{g/ml}$ of thiram concentration, which was 30 and 70% in case of pot experiment, and 39 and 80% in case of tube experiment (Figure 1b).

Nitrogenase activity

There were no nodules appearing at 750 $\mu\text{g/ml}$ of thiram and also the N_2 -ase activity was observed only upto 500 $\mu\text{g/ml}$ thiram in both pot and tube experiments. Increase in N_2 -ase activity in pot experiment was 12, 10 and 8% and in tube experiment it was 25, 18 and 15% at thiram concentrations of 100, 50, 10 $\mu\text{g/ml}$,

respectively (Figure 2). After 100- $\mu\text{g/ml}$ thiram a sharp decline in nitrogenase activity occur and no activity was observed beyond 500 $\mu\text{g/ml}$ thiram. The result was consistent with the previous phenomenon of formation of nodule under similar conditions of thiram treatment in both pot and synthetic media.

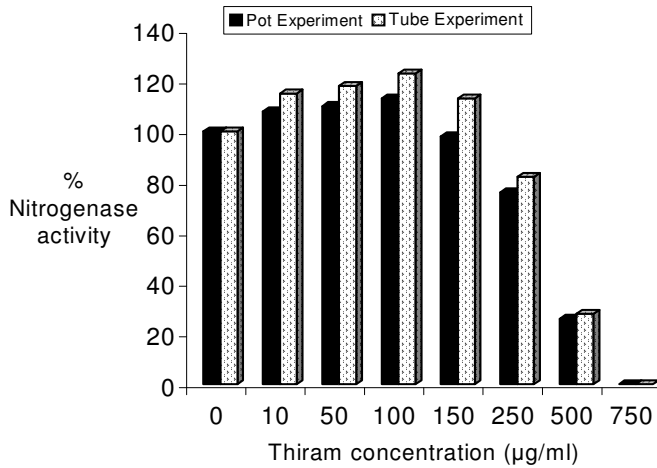


Figure 2. Effect of different concentrations of thiram on nitrogenase in root nodules of *G. max* inoculated with *R. japonicum* in pots and tube experiments.

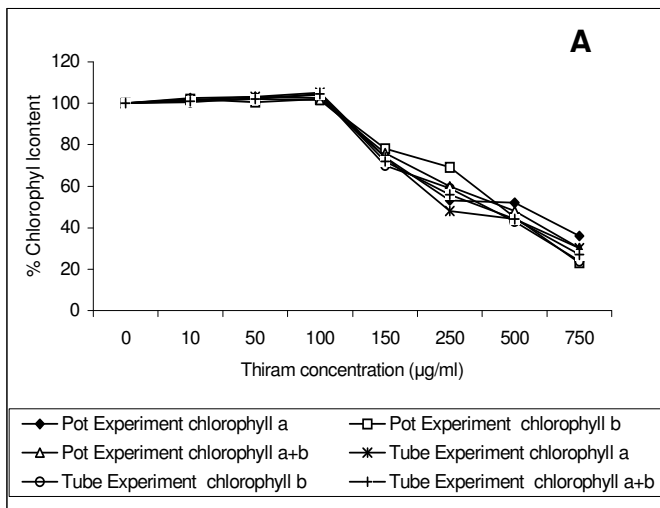


Figure 3a.

Effect of thiram on plant factors

Response of *G. max* in relation to plant factors were seen with respect to photosynthetic ability of plants considering chlorophyll content, ascorbic acid concentration and leaf protein content (Figures 3a,b,c).

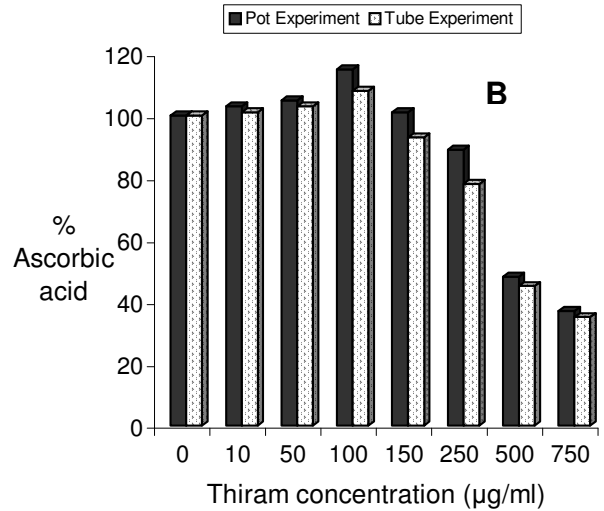


Figure 3b.

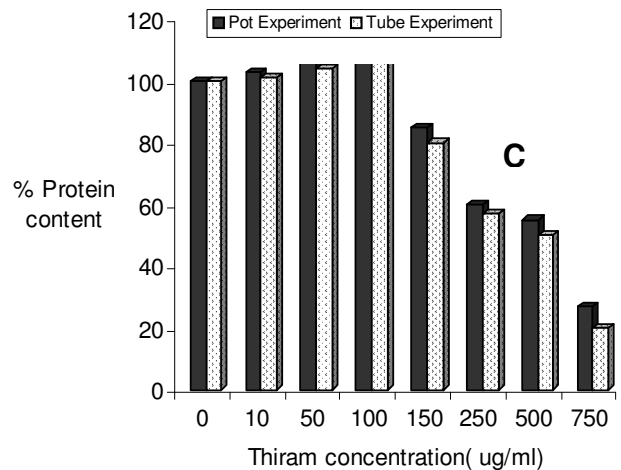


Figure 3c.

Figure 3. Effect of different concentrations of thiram on (a) chlorophyll, (b) ascorbic acid and (c) protein contents in leaves of *G. max* inoculated with *R. japonicum* in pot and tube experiments.

concentration and leaf protein content (Figures 3a,b,c). The increase in chl a with respect to control in pot experiment was 4, 3.46 and 2.466%, and in tube experiment it is 6, 4.28 and 1.26% at thiram concentrations of 100, 50 and 10 $\mu\text{g/ml}$, respectively. However reduction was noticed at thiram concentrations of 150 $\mu\text{g/ml}$ and higher. Increase in total chlorophyll content in both pot and tube experiments were 3.5, 2.0, and 1.28% in pot, and 5.5, 3.45 and 1.0% in tube experiments at thiram concentrations of 100, 50, and 10 $\mu\text{g/ml}$, respectively (Figure 3a).

Two sets of experiments performed by inoculating fungicide treatment seeds in pot and synthetic media to

study the ascorbic acid content of *G. max* (Figure 3b) showed significant negative correlation with increasing concentration of thiram. Increase in ascorbic acid concentration up to 100 µg/ml, and thereafter fell. Ultimately, the pattern of increase and decrease in protein content of soybean leaf in both experimental conditions reflected similar trends as compared to other factors such as ascorbic acid content and total chl content and N₂-ase activity (Figure 3c). Decrease in protein content was observed to be 14.0, 6.0, and 2.0%, and 12.0, 4.0, and 1.0% in pot and tube experiments, respectively, at 100, 50, and 10 µg/ml thiram concentrations.

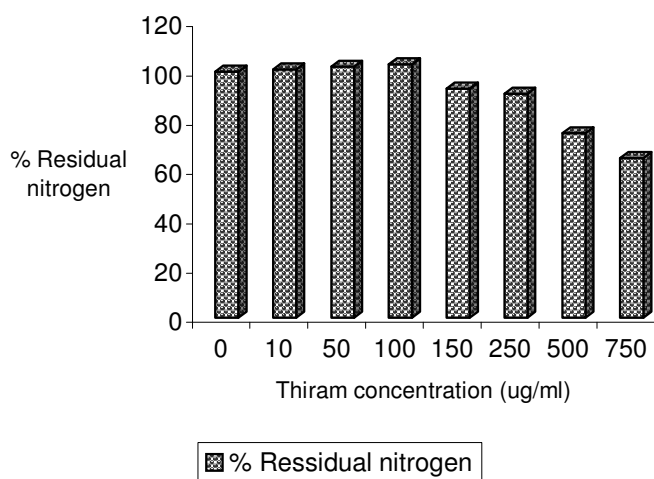


Figure 4. Effect of different concentrations of thiram on residual nitrogen content in pot experiments after harvesting of *G. max* nodulated by *R. japonicum*.

Residual N₂

Presently, pre treatment of seeds with fungicide was found to have positive role in amendment of biologically fixed N₂ in the soil up to concentration of 100 µg/ml thiram (Figure 4).

DISCUSSION

The increase in nodule dry weight in soil as compared to pure synthetic media might be due to lowering of thiram activity by soil parameters thereby making thiram less effective in pot experiment as well as field conditions (Balasundaram and Subba Rao, 1977). Introduction of *R. japonicum* significantly increases the nodulation and nodule dry weight (Balasundaram and Subba Rao, 1977). Absence of nodules at the 750 µg/ml of thiram was seen and the N₂-ase activity was found only upto 500 µg/ml thiram concentration in both pot and tube

experiments. After 100 µg/ml concentration of thiram a sharp decline in N₂-ase activity occurred and no activity was seen beyond 500 µg/ml concentration of thiram in both cases.

The pattern of chl b concentration followed similar trend as observed in case of for chl a content in thiram treated *G. max*. Ascorbic acid content of *G. max* showed significant negative correlation with increasing concentration of thiram. The increase and decrease pattern in protein content of the soybean plant also indicated negative significant correlation.

Nitrogen fixing contributes to fertility of soil resulting in increased production of subsequent crop. The observed 35% decrease in residual N₂ at 750 µg/ml thiram concentration was due to the fact that the N₂ available in the soil in the absence of *Rhizobium* activity was by the inoculated soybean for its initial growth and development. *Rhizobium* failed completely to form effective nodule with soybean in tube culture as well as in pot experiment at 750 µg/ml thiram concentration (Afifi et al., 1969).

The amount of chl, ascorbic acid and protein directly indicates the growth of plant in favorable and unfavorable conditions, which may be either due to fungicide or other chemicals or toxins. The above findings consistent to work done by Richards (1954), Zentmeyer (1995), Afifi, et al. (1969), Szkolink (1978), Sullia and Anusuya (1989), and Vyas et al. (1990). From the current study we conclude that soybean seeds either treated with thiram before or after the sowing do not make any difference in nodule number and nodule dry weight (Bollon, 1961; Domsch, 1964; Sullia and Anusuya, 1989; Vyas et al., 1990). The N₂-ase activity which was found to be maximum at thiram concentration of 100 µg/ml. This optimum concentration is effective with increase in nodule number, nodule dry weight and growth estimation factors such as chl, ascorbic acid and protein content of *G. max* thereby enhancing the total production.

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