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INFLUENCE OF ADHESIVES AND TIME LAG BETWEEN SEED TREATMENT AND SOWING ON SURVIVAL OF RHIZOBIA AND CROP RESPONSE TO INOCULATION

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ABSTRACT

Seed bacterisation is a common practice. Rice *kanji* is advocated to coat seeds with biofertilizers. Compared to water, rice *kanji* showed ten fold increase in the number of rhizobial cells adhering to red gram seed. Among the five adhesives tested for survival of inoculated rhizobia on red gram seed surface, gum arabic (40%) was the best followed by jaggery (25%), carboxymethylcellulose (1.5%), rice *kanji* and water. With rice *kanji* as adhesive on cowpea seed the inoculum load of 1.4 x 10^4 rhizobial cells per seed decreased to 2.2 x 10^2 cells after 1 h. There was no significant difference among the number of rhizobia on inoculated seeds between the time lag of 1 and 24 h. In unsterilised soil, compared to uninoculated control, significantly higher nodule dry weight and plant dry weight were observed with cowpea plants raised immediately after seed bacterisation. Nodule dry weight and plant dry weight of plants raised after a time lag of 1 to 24 h after seed inoculation were found to be on par with those of uninoculated control. Thus the crop response to rhizobial inoculation could be realised in unsterilised soil with a inoculum of 10^4 cells per seed but not with 10^2 cells per seed.

KEY WORDS : Rhizobial Inoculation Time Lag, Rhizobial Inoculant Adhesives

The objective of legume seed inoculation is to introduce specific *Rhizobium* into soil at the time of sowing so that the inoculated bacteria remain viable until the host seedling is capable of accepting infection and compete with the native rhizobia. In order to increase the quantity of inoculant adhering on seed and to protect the inoculated rhizobial cells from desiccation and death, it is suggested to apply the inoculant with any one of the adhesives like carboxymethylcellulose (CMC), gum arabic or household sugar (Brockwell, 1962). In Tamil Nadu, the use of adhesives is practised to a little extent due to its difficulty in its preparation at farm level. Therefore, the decanted gruel from cooked rice(rice *kanji*), a complex carbohydrate material readily available in every household, is recommended as a suspending medium for rhizobial inoculation (Balasubramanian and Prabakaran, 1979; Murugesan *et al.*, 1986). At present it is advised to sow the inoculated seeds within 24 h after inoculation. When rice *kanji* was used as suspending solution, rhizobial strains of proven efficiency at laboratory experiments have failed to perform well in many of our field experiments. The frequent failures of more number of rhizobial strains might be due to increased time lag between seed treatment and sowing. In the present study the effect of different adhesives and time lag between inoculation and sowing was investigated for the survival of rhizobia on seed surface and crop response to inoculation.

MATERIALS AND METHODS

Water,rice *kanji*, CMC (1.5%), jaggery (25%), and gum arabic (40%) were used as suspending solutions. Red gram (*Cajanus cajan*) seeds (100 g) of Co.5 cultivar without of any surface sterilization or fungicide treatment were treated with a peat based inoculum (2.5 g) of a rhizobial strain Co.R1 (cell load 3 x 10^7 g^{-1} dry weight) using different suspending solutions (2.5 ml). The seeds after treatment were shade dried for 30 min and stored in paper bags at room temperature (28 + 2°C). Samples were drawn at 0, 1, 6, 12 and 24 h intervals and thoroughly suspended in sterile saline by shaking (150 rpm) in a rotary shaker and serially diluted. The viable rhizobia were enumerated by plating appropriate dilution in yeast extract Congo red mannitol agar. Similarly, cowpea (*Vigna unguiculata*) seeds were also inoculated using rice *kanji* as suspending solution and the survival of rhizobia on seed surface was studied in the same manner as described above. At each time interval, cowpea seeds were also sown in pots of 15 cm dia

containing 1.5 kg of unsterilised soil. Three replications with three plants in each pot were maintained along with uninoculated control. The nodule number, nodule dry weight and plant dry matter were observed after 30 d.

RESULTS AND DISCUSSION

Among the five suspending solutions tested for survival of inoculated rhizobia on red gram seed surface, gum arabic (40%) was the best, followed by jaggery (25%), CMC (1.5%), rice *kanji* and water (Fig 1.) Enventhough maximum number of rhizobial cells was observed initially with CMC solution, it declined drastically (25 fold) after 1 h of seed inoculation. On the other hand, the decline in the number of rhizobial cells after 1 h of seed inoculation was found to be less than two fold in the cases of jaggery and gum arabic. With rice *kanji*, initial number of rhizobial cells adhering to redgram seed was found to be 10 fold more than that of water and 10 fold less than that of CMC and the decline in the number of rhizobial cells after 1 h of seed inoculation was found to be about 100 fold. This indicates that compared to gum arabic and jaggery, rice *kanji* is not good in conferring protection against the decline of inoculated rhizobia on seed surface. The loss of viability of rhizobial cells on the seed surface due to rapid death owing to desiccation is a common phenomenon. Burton (1967) also observed the highest death of cells in the first hour after inoculation. Vincent, Thompson and Donovan (1962) suggested that the decline of inoculated rhizobia on the seed surface was influenced greatly by the nature of suspending medium and the loss of viability during the first few hours is likely to be severe. An initial inoculum of 1000 cells per seed could fail to yield any survivor after 6 h, on the other hand gum arabic providing protection against desiccation could result in 500 survivors.

Table 1. Effect of time lag between rhizobial inoculation and sowing on nodulation and plant growth in cowpea

Time interval (h)	Rhizobial CFU per seed	Nodule number per plant	Nodule dry weight (mg per plant)	Plant dry weight (mg per plant)
0	14814	21.0	32.3	787
1	222	15.9	16.3	584
6	38	13.1	11.9	618
12	100	18.4	10.5	572
24	37	22.4	18.5	569
control	--	18.9	16.4	612
CRD SE	220.9	3.91	3.04	29.6
CD (5%)	680.8	N.S.	9.37	91.2

In the present investigation, when peat based rhizobial culture suspended in rice *kanji* was inoculated, cowpea seeds exhibited 14814 cells per seed just after inoculation which decreased several folds within 1 h. After 1 h rhizobial cell count of 222 per seed was observed. After 6 h rhizobia declined further to 38 cells per seed. Subsequent incubations showed fluctuation in cell number of rhizobia per seed. However, there was no significant difference among the cell numbers of rhizobia between 1 and 24 h. When the seeds were sown at different time interval after seed inoculation, significantly higher nodule dry weight and plant dry weight were observed only with plants raised from seeds sown with out any time lag after inoculation. Nodule dry weight and plant dry weight of plants raised after a time lag of 1 to 24 h after seed inoculation were found to be on par with those of uninoculated control (Table 1). This indicated that the crop response to rhizobial inoculation could be realised in unsterilised soil with a inoculum of 10^4 cells per seed but not with 10^2 cells per seed. For securing assured nodulation, a minimum rhizobial cell load of 10^3- 10^5 per seed at the time of sowing is recommended (Date and Roughley, 1977; Subba Rao, 1986a). As per the limits prescribed by the Bureau of Indian Standards, a rhizobial inoculant shall contain 10^7 - 10^8 cells per g dry weight of carrier (Subba Rao, 1986b). In TamilNadu, 200 g of inoculant is recommended per acre seed of pulses (about 10 kg) which will contain approximately 100 to 200 thousand seeds. Both the quality and quantity of the inoculant used in this study were well within these limits. At this recommended rate, with rice *kanji* as suspending solution which showed about 100 fold decline in the number of rhizobial cells after 1 hour of seed inoculation, the scope of securing adequate response to rhizobial inoculation is limited unless sown immediately after seed inocultion. Therefore, to enhance better nodulation and crop response, the

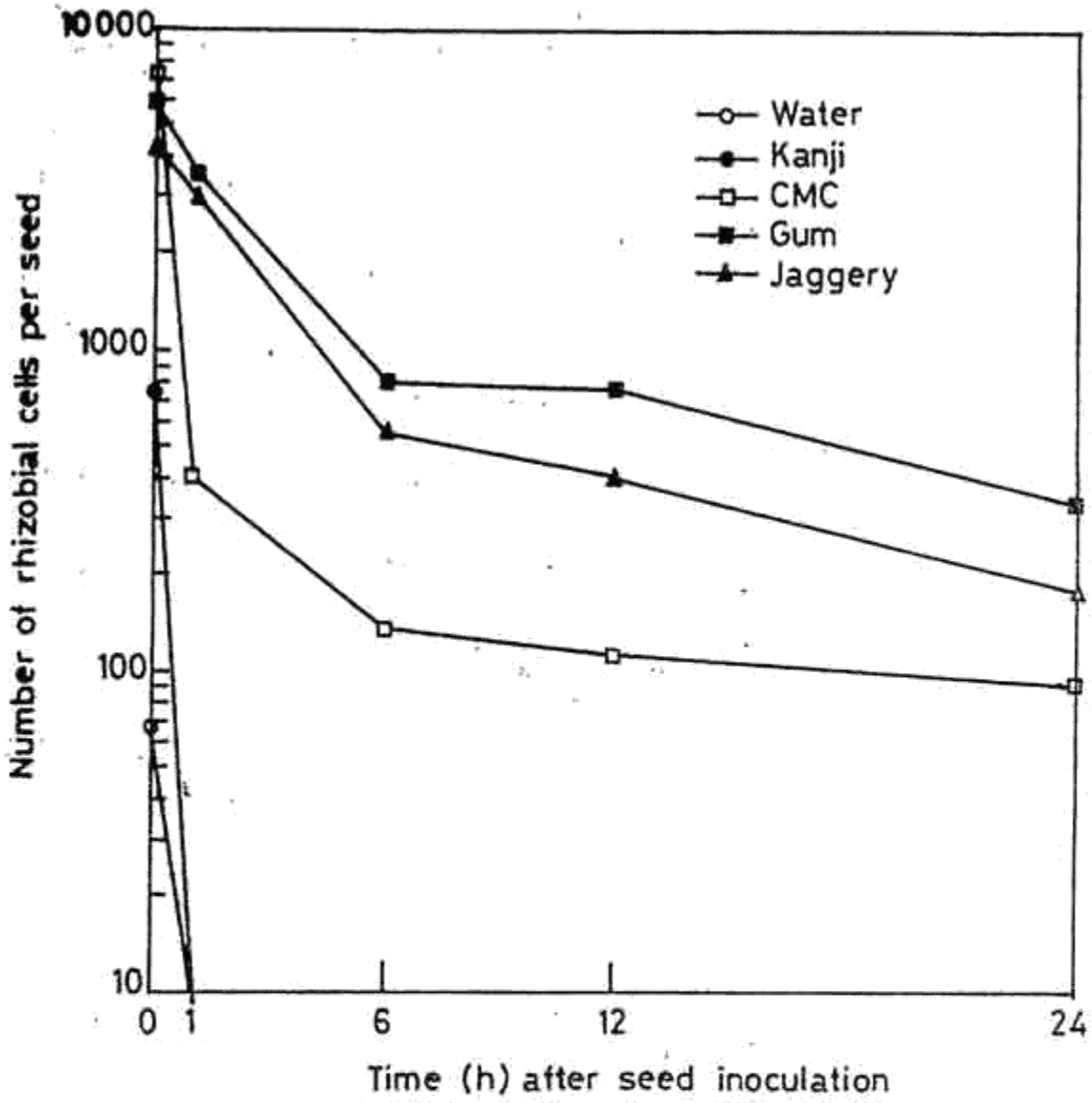

Fig.1. Effect of different suspending solutions on survival of rhizobia

rhizobial seed inoculation technology need to be further improved to facilitate the higher number of survivors.

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