Influence of Pesticide-Treated Seeds on Survival of *Mesorhizobium* sp. *Cicer*, Symbiotic Efficiency and Yield in Chickpea

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Abstract

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Chemical seed protectants are used to reduce the adverse effects of seedling fungal pathogens or insect attack on legume pastures and crops. Chickpea seeds are also frequently treated with *Mesorhizobium* sp. *Cicer* inoculant to promote effective symbiotic nitrogen fixation (SNF), which seems to be a cost effective measure. The population of viable *Mesorhizobium* sp. *Cicer* on seeds of chickpea declined with time of storage (4°C) in pesticide treated and untreated chickpea seeds *in vitro*. A significant reduction in chickpea rhizobia was observed in seed treatment with Captan followed by Endosulfan and Chlorpyrifos. In a field experiment during the winter season 2006–2008, no difference in the emergence count of chickpea plants was observed. Treatments inoculated with *Mesorhizobium* sp. *Cicer* alone or along with Captan, Chlorpyrifos or Endosulfan showed improved plant growth and symbiotic parameters (plant height, nodulation, leghaemoglobin content, and nitrogen content) in comparison with the uninoculated control treatment. Significantly higher grain yield (9.6%) was observed in the treatment inoculated with *Mesorhizobium* sp. *Cicer* alone as compared to the uninoculated control. A non-significant difference in grain yield among treatments where *Mesorhizobium* sp. *Cicer* along with a mixture of fungicide and insecticides was applied was observed in contrast to the *Mesorhizobium* sp. *Cicer* treatment. In conclusion, the recommended rates of fungicide and insecticides as seed treatment were not detrimental to chickpea-*Mesorhizobium* sp. *Cicer* symbiosis, hence they can be safely used to obtain higher productivity.

Keyword: gram; *Mesorhizobium*; captan; chlorpyrifos; endosulfan; SNF

Chickpea (*Cicer arietinum* L.) is the major winter season pulse crop of India and it was grown on an area of 7.63 million hectares with the average yield of 782 kg/ha (Singh 2007). In Punjab, it was grown on an area of 3.8 thousand hectares with the average yield of 1010 kg/ha in the years 2006–2007 (Anonymous 2007). Being a leguminous crop, chickpea improves physical, chemical, and biological properties of soil through symbiotic nitrogen fixation (SNF) and can fix up to 141 kg N/ha/year through a symbiotic association with an effective strain of *Mesorhizobium* sp. *Cicer*, reducing its dependence on soil nitrogen (Sharma & Sharma 2008). Inoculation is more helpful when local and resident soil rhizobial populations are either absent or very low (Catroux *et al.* 2001). The main purpose of inoculation is to bring the rhizobia near to the seed during germination for effective nodulation and SNF. *Mesorhizobium* sp. *Cicer* inoculation improves growth and yield of chickpea, which seems to be a cost effective measure (Pareek & Chandra 2005). The success of inoculation is often limited by several factors including environmental conditions, the number of infective cells applied and agrochemicals (Kyei-Boahen *et al.* 2002).

Root rot diseases are a major factor to obtain maximal legume production. The diseases depress seedling germination and cause post-emergence

damping off, resulting in poor crop stand and low yields. The causal agents of the disease are seed borne but most farmers often use seeds saved from previous harvest, a practice that negates the principle of sanitary practices (Buruchara 1990). Chemical seed treatment before planting is a common practice to prevent seed and seedling rots, damping off and other fungal diseases. However, the problems arise when the chemical seed treatments are to be used in conjunction with rhizobial inoculants.

Chickpea seeds and seedlings are known to be susceptible to various fungal pathogens and insect pests. Ascochyta blight and *Botrytis* are the major fungal pathogens of chickpea that result in huge losses in the production of chickpea. The application of the fungicide Captan (à 3 g/kg seed) has been recommended in chickpea seed before sowing for the control of fungal diseases. Termites also affect the seedlings of chickpea at the time of germination and lower plant productivity. Insecticides like Chlorpyrifos 20EC or Endosulfan 35EC (à 10 ml/kg seed each) have also been used for seed treatment for the control of termites in chickpea (Anonymous 2007). It is likely that the inhibition of rhizobia on seeds by toxic pesticides, followed by slow establishment of surviving rhizobia in the rhizosphere, can lead to reduced nodulation by seeds applied *Rhizobium*. The effect of pesticides on the viability of *Mesorhizobium* also depends upon the species and strain of the bacterium (Mallik & Tesfai 1983; Kyei-Bohaen *et al.* 2001). For the last three decades, studies on compatibility of rhizobial strain with fungicides have been controversial. Therefore, the present study was conducted to examine the effect of fungicide and insecticide on the survival of inoculated culture of *Mesorhizobium* sp. *Cicer* on chickpea seeds *in vitro* and symbiotic efficiency of chickpea *in vivo*.

MATERIAL AND METHODS

The survival of a recommended strain of *Mesorhizobium* sp. *Cicer* (LGR 33) on chickpea seeds *in vitro* treated with recommended rates of fungicide (Captan) and insecticides (Chlorpyrifos and Endosulfan) was studied according to Curley and Burton (1975) (Table 1). An *in vitro* experiment was conducted with four treatments: *Mesorhizobium* sp. *Cicer* (M) alone, Captan+M, Chlorpyrifos+M and Endosulfan+M in a complete randomised block design (CRBD). In *Mesorhizobium* sp. *Cicer* alone treatment, 1×10^7 cells/g of charcoal based inoculant were applied to sterilised chickpea seeds (Vincent 1970). The survival of *Mesorhizobium* sp. *Cicer* was assessed in stored seeds at 4°C after 0, 4, 8, 12, 16, 20 and 24 h of inoculation. Seeds were picked randomly from each treatment and divided into four subsamples of 10 seeds each. Each subsample was transferred into test tubes containing 10 ml sterile water and shaken vigorously for 30 s to wash off the inoculum from seeds. One ml of resultant suspension from each treatment was diluted serially and 0.1 ml of each dilution was plated on yeast extract mannitol agar (YEMA) medium. *Mesorhizobium* sp. *Cicer* colonies were counted after incubation at 28 ± 2°C for 7 days.

Field experiments were conducted in the winter season (2006–2008) on the Pulses Research Farm, Punjab Agricultural University, Ludhiana in chickpea (PBG 1). The soil was loamy sand in texture with pH 8.1, low in organic carbon 0.30% and available nitrogen (N) 0.5 kg/ha but medium in available phosphorus (P) 18 kg/ha. The maximum and minimum temperature during the crop growth period (2006–2008) was 27.6°C and 11.2°C, respectively. The experiment was laid out in the plots of net plot size 4.0 m × 2.4 m (total plot size 4.0 m × 3.0 m) following the completely randomised block design (CRBD) in three replications. Seven treatments were applied, viz control, *Mesorhizobium* sp. *Cicer* (M), Chlorpyrifos+M, Endosulfan+M, Captan+M, Captan+Endosulfan+M and Captan+Chlorpyrifos+M. Chickpea seeds were inoculated with charcoal based recommended cultures of *Mesorhizobium* sp. *Cicer* (LGR 33) as per treatment à 20 g/kg seed (the number of

Table 1. Composition of pesticides

Type	Trade name	Chemical name	Recommended dose/kg seed
Fungicide	Captan	*N*-(trichloromethylthio)cyclohex-4ene-1,2-dicarboximide	3 g
Insecticide	Chlorpyrifos	*O*,*O*-diethyl-*O*-3,5,6-trichloro-2-pyridylphosphorothioate	10 ml
	Endosulfan	1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylenebismethylene)sulfite	10 ml

rhizobia per seed was 10^7 CFU/ml). The chickpea crop was grown for the first time in the field where the fodder crop was grown for the last 15 years and the number of native rhizobia before sowing was 10^2 cells/g of soil. In fungicide treatment (à 3 g/kg seed), Captan was applied at first and then seeds were inoculated with *Mesorhizobium* sp. *Cicer*. In seed treatments with a combination of the fungicide Captan (à 3 g/kg seed) applied at first and insecticides (à 10 ml/kg seed), Chlorpyrifos or Endosulfan and then *Mesorhizobium* inoculant followed. Seeds without *Mesorhizobium*, insecticides and fungicide served as control. Before sowing, inoculated seeds were dried at room temperature under shade and sown immediately. The crop was raised following the recommended agronomic practices. Five plants from each plot were randomly uprooted along with the soil core at 55 daya and 90 days after sowing (DAS). Roots were washed off to remove the adhering soil and then data on plant height, nodule number and dry weight of nodules were recorded. At flowering stage the leghaemoglobin content of fresh, bold and pink nodules was determined with Drabkin's solution (Wilson & Reisenauer 1963) and nitrogen content of straw was assessed according to McKenzie and Wallace (1954). Grain yield from each plot having 40 plants (g/plot) was recorded and expressed in kg/ha at harvesting stage (166 DAS). All the data were subjected to analysis of variance as per standard statistical procedure CPCS 1 software developed by Department of Mathematics, Statistics and Physics, Punjab Agricultural University, Ludhiana.

RESULTS AND DISCUSSION

In vitro studies revealed that the seeds treated with Captan, Chlorpyrifos and Endosulfan along with *Mesorhizobium* sp. *Cicer* significantly decreased the viable number of rhizobial cells on chickpea seeds on storage at 4°C as compared to *Mesorhizobium* sp. *Cicer* treatment (Figure 1). After 4 h of storage (4°C) 13% of rhizobia died in the seed treatment inoculated with *Mesorhizobium* sp. *Cicer* alone. This loss is normal as reported by Curley and Burton (1975). This loss of rhizobia could be due to dehydration and presence of toxic seed coat substances (Revellin *et al.* 1993). The toxicity of Captan (38%) to viable rhizobial cells was higher as compared to Chlorpyrifos (25%) and Endosulfan (30%). The number of viable rhizobial cells was reduced significantly after the initial 4-h contact with fungicide and insecticides and *Mesorhizobium* sp. *Cicer* and it further decreased during 8-h to 16-h contact. However, these viable rhizobial cells of control treatment remained constant for 16 h to 24 h storage period. The high recovery of viable rhizobial cells with insecticide could be due to the presence of some additional adhesives in liquid formulation (Kyei-Boahen *et al.* 2001). Similarly, the mixture of Lindane and Chlorpyrifos had no deleterious effect on the survival of *B. japonicum* after 24-h contact with soybean seed (Revellin *et al.* 1992). However, a drastic reduction in growth was reported by Tu (1971) as in our studies the prolonged storage reduced the number of rhizobia. The rapid loss of rhizobia viability due to the deleterious effect

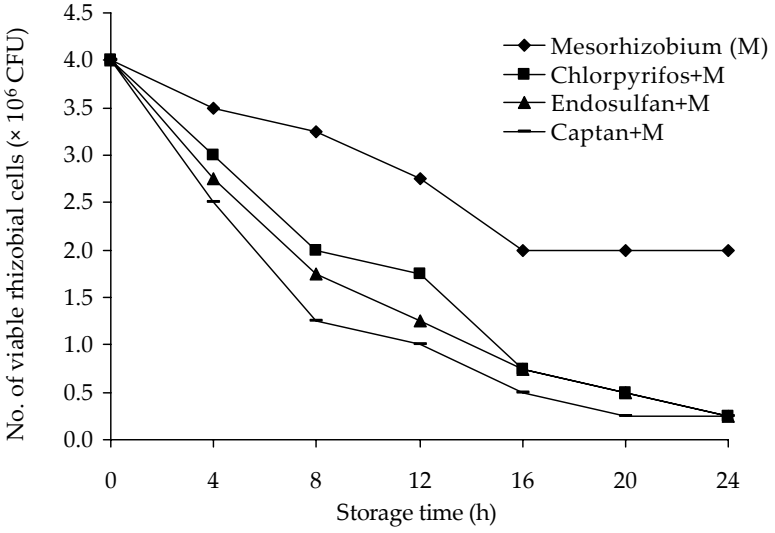

Figure 1. Effect of storage on the survival of *Mesorhizobium* sp. *Cicer* (× 10^6 CFU) on seeds treated with fungicide and insecticides separately in desi chickpea

Table 2. Effect of seed treatment with Captan, Chlorpyrifos and Endosulfan along with *Mesorhizobium* sp. *Cicer* on growth and symbiotic parameters in desi chickpea

Treatments	Emergence count (% germination)	Plant height (cm)		Number of nodules/plant		Dry weight of nodules/plant (mg)	
		55 DAS	90 DAS	55 DAS	90 DAS	55 DAS	90 DAS
Control	86.5	13.7	35.7	11.3	17.8	31.9	84.6
M	88.9	14.6	39.9	16.7	26.6	44.1	108.6
Chl+M	88.2	14.3	37.5	15.2	26.0	38.8	99.7
Endo+M	88.7	14.3	36.6	14.0	24.5	40.1	101.6
Cap+M	89.1	14.3	37.7	14.9	25.5	38.2	102.2
Cap+Chl+M	87.7	14.3	36.6	14.7	26.0	40.2	103.1
Cap+Endo+M	88.5	14.3	37.9	14.4	24.7	40.8	103.5
CD 5%	ns	ns	4.0	3.0	3.8	2.5	3.2

M – *Mesorhizobium* sp. *Cicer*; Chl – Chlorpyrifos; Endo – Endosulfan; Cap – Captan; CD – coefficient of dispersion (variation?); ns – not significant

of Captan is in close agreement with previous reports on *B. japonicum* (Annapurna *et al.* 2000), *R. phaseoli* (Graham *et al.* 1980) and *R. ciceri* (Kyei-Boahen *et al.* 2001).

In the field experiment, data on emergence count (pooled mean) showed non-significant differences among different treatments. Germination in different treatments was quite good and it varied from 86.5% to 89.1% (Table 2). The seed treatment of chickpea with fungicide and insecticides along with *Mesorhizobium* sp. *Cicer* had no adverse effect on germination count. Similarly, the pesticides applied to seed had no significant effect on seedling emergence in soybean (Revellin *et al.* 1992), lentil, pea and faba bean (Rennie *et al.* 1985). At both vegetative and flowering stages, the increased plant height was observed in *Mesorhizobium* sp. *Cicer* inoculation alone as compared to the other treatments (Table 2). The seed treatment of chickpea with fungicide and insecticides along with *Mesorhizobium* sp. *Cicer* had no adverse effect on plant height at both stages. The results were confirmed by the results of Togay *et al.* (2008) and Kabi and Behari (1990) in chickpea. Pooled nodulation data at vegetative (55 DAS) and flowering stages (90 DAS) for two successive years (2006–2008) are shown in Table 2. All the treatments showed significantly higher nodulation as compared to the control. This might be due to the availability of effective and infective rhizobia in the rhizosphere of chickpea (Khurana & Sharma 1998). The treatments with Captan, Chlorpyrifos and Endosulfan in combination with *Mesorhizobium* sp. *Cicer* inoculant showed a non-significant difference in nodulation compared to *Mesorhizobium* sp. *Cicer* treatment. Similarly, no deleterious effect on nodulation was observed in Chlorpyrifos-treated soybean seeds (Annapurna *et al.* 2000) but Captan reduced the nodulation in pea (Fritz & Rosen 1991). At 55 DAS significantly higher dry weight

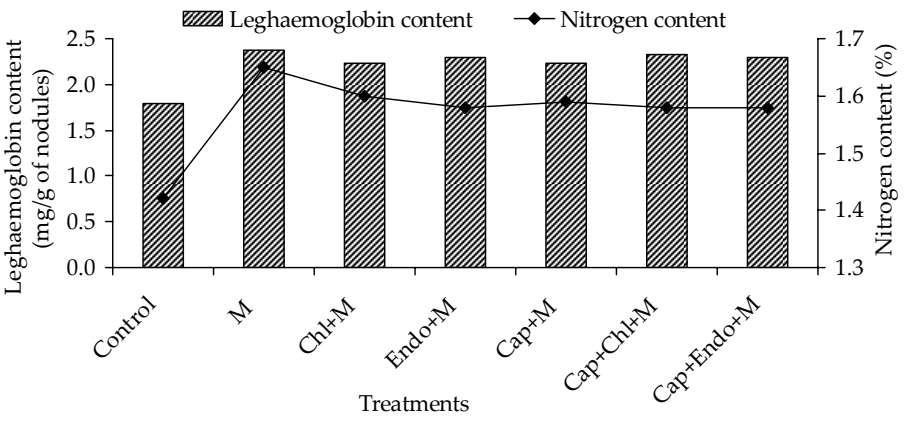

Figure 2. Effect of seed treatment with Captan, Chlorpyrifos, and Endosulfan along with *Mesorhizobium* sp. *Cicer* on leghaemoglobin content in desi chickpea at 90 DAS (M – *Mesorhizobium* sp. *Cicer*; Chl – Chlorpyrifos; Endo – Endosulfan; Cap – Captan)

of nodules in *Mesorhizobium* sp. *Cicer* inoculated treatment (44.1 mg/plant) was observed as compared to the uninoculated control (31.9 mg/plant) (Table 2). Whereas, at 90 DAS, a 28.3% increase in dry weight of nodules was observed in the treatment with *Mesorhizobium* sp. *Cicer* alone over the uninoculated control. Dry weight of nodules was on par in treatments Captan+Chlorpyrifos+M and Captan+Endosulfan+M whereas *Mesorhizobium* sp. *Cicer* treatment showed significantly higher dry weight of nodules than these treatments at both stages. Fungicide (Captan) and insecticides (Chlorpyrifos and Endosulfan) reduced the viable counts of rhizobia on chickpea seeds but these seed protectants did not show any deleterious effect on nodulation under field conditions. These results are consistent with Annapurna *et al.* (2000) in soybean and Kyei-Boahen *et al.* (2001) in chickpea where also a low relationship between viable counts on seed treated with chemical protectants and performance in fields was reported.

All the treatments with *Mesorhizobium* sp. *Cicer* inoculation improved the leghaemoglobin content significantly compared to the uninoculated control (Figure 2). Significantly higher leghaemoglobin content of nodules was recorded in *Mesorhizobium* sp. *Cicer* treatment (2.37 mg/g of nodules) followed by Captan+Chlorpyrifos+M (2.32 mg/g of nodules) and Captan+Endosulfan+M (2.30 mg/g of nodules) in pooled mean data. The increased leghaemoglobin content in *Mesorhizobium* sp. *Cicer* treatments might be due to effective nodulation and symbiotic nitrogen fixation. When the nodules from treatments where *Mesorhizobium* sp. *Cicer* was applied along with fungicide and insecticide alone or as a mixture were cut and opened, all of them were pink in colour not indicating a high reduction in leghaemoglobin content. Similarly, the soil insecticides viz Carbofuran, Thimet, Dasanit and heptachlor had no apparent effect on the leghaemoglobin content of groundnut nodules as reported by Kulkarni *et al.* (1974). A significant increase in the nitrogen content of straw was observed in *Mesorhizobium* sp. *Cicer* treatment (1.65 %) over the uninoculated control (1.42 %). The increase in nitrogen content (1.58 %) was on par in treatments with Captan+M, Endosulfan+M, Captan+Chlorpyrifos+M and Captan+Endosulfan+M in pooled mean data (Figure 3). These results were similar to those observed by Ogutcu *et al.* (2008) in chickpea. Chickpea seeds treated with pesticides (Captan, Chlorpyrifos/Endosulfan) along with *Mesorhizobium* sp. *Cicer* showed a non-significant difference in nitrogen content in comparison with *Mesorhizobium* sp. *Cicer* treatment. Similarly, soybean seeds treated with pesticides like benomyl and oxamyl and inoculated with the respective resistant strains of *R. japonicum* showed an increased nitrogen content (Hossain & Alexander 1984).

On the basis of pooled mean data (2006–2007 and 2007–2008) the *Mesorhizobium* sp. *Cicer* inoculation showed a 9.6% increase in the grain yield that was significantly better than in the uninoculated control (Figure 4). In treatments where fungicide and insecticides were applied as seed protectants to chickpea seeds the grain yield improved numerically over the uninoculated control treatment. However, the difference in grain yield between Captan+Chlorpyrifos+M (1645 kg/ha) and Captan+Endosulfan+M (1652 kg/ha) was non-significant as compared to the treatment with *Mesorhizobium* sp. *Cicer* alone (1662 kg/ha). The increase in yield after *Mesorhizobium* sp. *Cicer* inoculation was due to its superiority over native rhizobia in chickpea. *Mesorhizobium* sp. *Cicer* inoculation gave a higher grain yield over

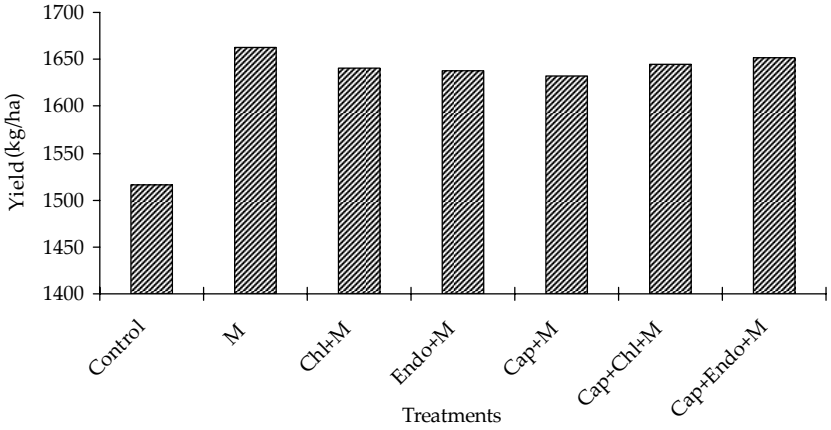

Figure 3. Effect of seed treatment with Captan, Chlorpyrifos and Endosulfan along with *Mesorhizobium* sp. *Cicer* on nitrogen content in desi chickpea at 90 DAS (M – *Mesorhizobium* sp. *Cicer*; Chl – Chlorpyrifos; Endo – Endosulfan; Cap – Captan)

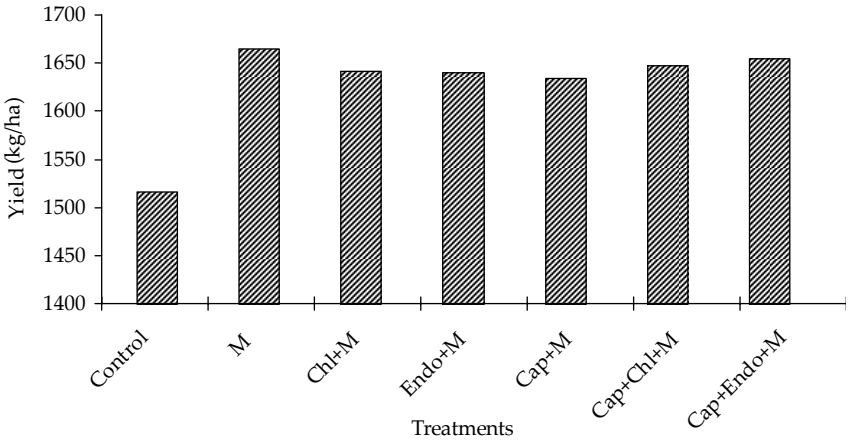

Figure 4. Effect of seed treatment with Captan, Chlorpyrifos, and Endosulfan along with *Mesorhizobium* sp. *Cicer* on grain yield in desi chickpea at 166 DAS (M – *Mesorhizobium* sp. *Cicer*; Chl – Chlorpyrifos; Endo – Endosulfan; Cap – Captan)

the uninoculated control. Improvement in grain yield due to *Mesorhizobium* sp. *Cicer* inoculation has been reported in chickpea (El Hadi & El Sheikh 1999; Elkoca *et al.* 2008; Togay *et al.* 2008). A non-significant difference in grain yield in *Mesorhizobium* sp. *Cicer* inoculated chickpea seeds treated with fungicide and insecticides was in agreement with Potter (2004), who reported no difference in grain yield in different fungicide and insecticide treatments of soybean. Good moisture conditions in the soil might have reduced the toxic effects by diluting the concentration of pesticides in soils (Fox *et al.* 2007). Moreover, the presence of low and inefficient rhizobia (10^2 CFU/g of soil) and a higher number of viable rhizobia on treated seeds (10^7 CFU/seed) sown immediately after inoculation also helped in the improvement of symbiotic parameters and yield in chickpea.

The inconsistency between the standard plate count and the field study reveals that a reliable measure of fungicide and/or insecticides and *Mesorhizobium* sp. *Cicer* compatibility must involve both the viability test and field study as the toxicity of pesticides increased with time. It shows that chemically treated seeds should always be sown as soon as possible after inoculation as the toxic effect of these pesticides reduced the survivability of rhizobia with prolonged storage. The seed treatment with recommended rates of fungicide (Captan) and/or insecticides (Chlorpyrifos or Endosulfan) as seed treatment along with *Mesorhizobium* sp. *Cicer* inoculant was not detrimental to the chickpea-*Mesorhizobium* sp. *Cicer* symbiosis when sown immediately after application. This study shows that fungicide (Captan) and insecticides (Chlorpyrifos or Endosulfan) at their recommended rates should safely be used along with *Mesorhizobium* sp. *Cicer* to obtain good plant stand, healthier plant growth and higher productivity in chickpea. A significant increase in symbiotic parameters and yield due to inoculation was observed as the population of native rhizobia at sowing was low and inefficient. Moreover, the presence of viable rhizobia on seeds after treatment before sowing led to better manifestation of symbiotic parameters and yield in chickpea regardless of the chemicals used for seed protection.

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