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CHEMICAL CONTROL OF COLLAR ROT DISEASE OF CHICKPEA

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A B S T R A C T

Collar rot caused by a fungus *Sclerotium rolfsii,* is a serious soil-borne disease of chickpea (*Cicer arietinum* L.). Present study was carried out to manage this disease by commercial fungicides under *in vitro* and *in vivo* conditions. *In vitro* bioassays were carried out using four fungicides namely Tegula (tebuconazole), Thiophanate Methyl, Ridomil Gold (metalaxyl + mancozeb) and Mancozeb at 50, 100, …, 250 ppm concentrations. All the concentrations of these fungicides significantly decreased radial growth of *S. rolfsii* over control. *In vivo* bioassays were carried out in plastic pots of 15-cm diameter and 20 cm deep with 1 kg soil in each pot. Treatments included a negative control, a positive control (with pathogen only), and two chemical fungicides viz. Thiophanate methyl and Mancozeb. There was 95% and 50% reduction in plant mortality due to Thiophanate methyl and Mancozeb over positive control, respectively, after 30 days of sowing.

Keywords: Chemical control, chickpea, collar rot, *Sclerotium rolfsii.*

INTRODUCTION

Chickpea (*Cicer arietinum* L.), family Fabaceae, is one of the most important leguminous crops grown all around the world (Knights *et al.,* 2007). Firstly, it was cultivated in south eastern areas of the world but now it is also cultivated in semi-arid regions (Agarwal *et al.,* 2012). It is not only a major source of dietary protein for human consumption but it also plays an important role in the management of soil fertility because of having the ability of nitrogen fixation in its root nodules (Hossain *et al.,* 2010). There is a growing demand of chickpea due to its nutritional value. It is the better source of carbohydrates and proteins as compared to other important pulses (Chibbar *et al.,* 2010). It is free of cholesterol and provides several vitamins and minerals (Wood and Grusak, 2007). Globally total chickpea cultivated area is 12.0 million ha, with 10.9 million MT production and an average yield of 913 kg ha^{-1} (Sheehy and Sharma, 2012). In Pakistan, its total production quantity was 496000 tones with average yield of 470.7 kg ha^{-1} and total cultivated area is 1053800 hectares (Newman *et al.,* 2012). Collars rot disease caused by *Sclerotium rolfsii* Sacc., is a serious threat to chickpea that may cause 55-95% mortality of the crop at seedling stage under favourable environmental conditions (Gurha and Dubey, 1982). Diseases caused due to *S. rolfsii* requires warm climates, occurs more frequently at high moistures and high temperatures (Al-Askar *et al.,* 2013). *S. rolfsii* control has met with very limited success. This may be due to the prolific growth, extensive host range of the pathogen and having the ability to produce large number of sclerotia that may persist in the soil for several years (Sennoi *et al.,* 2013). This pathogen causes many diseases like collar rot, sclerotium wilt, stem rot, charcoal rot, seeding blight, damping off, foot rot, stem blight, and root rot in many economically valuable crops (Gopalakrishnan *et al.*, 2005).

Mycelial growth of *S. rolfsii* or the sclerotia germination can be restricted by the use of several fungicides (Zamora *et al.,* 2008). Some fungicides like vitavax-thiram and vitavax-captan can successfully retard the *in vitro* growth of *S. rolfsii*. Other commonly used fungicides to control *S. rolfsii* in several crops are thiram, quitozene, captan, carbendazin, benomyl, oxicarboxin and triadimenol. Mixture of carboxin plus thiram and quintozene has been

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found most impressive in prohibiting sclerotial formation and mycelial growth (Yaqub and Shahzad, 2006). The present study was carried out to assess antifungal potential of some more fungicides against *in vitro* growth of *S. rolfsii* and *in vivo* management of collar rot disease of chickpea caused by this pathogen.

Table 1. Active ingredients and chemical groups of the fungicides.

Sr. No.	Trade mark	Active ingredient	Formulation	Chemical group
1	Tegula	Tebuconazole	12.5% EW	Triazoles
2	Thiophanate Methyl	Thiophanate Methyl	70% WP	Benzimidazole
3	Radomal Gold	Metalaxyl + Mancozeb	4%+64% WP	Acilalaninate
4	Mancozeb	Mancozeb	80% WP	Dithiocarbamate

MATERIALS AND METHODS

Laboratory bioassays: Four different fungicides namely Tegula, Thiophanate Methyl, Ridomil Gold and Mancozeb were used to evaluate their *in vitro* efficacy for the management of *S. rolfsii*. Active ingredients and chemical groups of the fungicides are given in Table 1.

Six concentrations of each of the four fungicides viz. 250, 200, 150, 100, and 50 ppm were prepared. Control treatment (0 ppm) was without the addition of any fungicide. Streptomycin capsules were added at the time of pouring of autoclaved medium in Petri plates to avoid bacterial contamination. Each treatment was replicated four times. After solidification, a disc of 5 mm diameter from actively growing margins of *S. rolfsii* colony was inoculated at the center of fungicide amended growth medium. The plates were incubated at 25±2 °C until full growth was observed in control. The colony diameter in each treatment was measured with a scale.

Pot trial: Two fungicides namely Mancozeb and Thiophanate methyl were selected for pot trial on the basis of their best activity in screening bioassays.

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] seeds were used for the preparation of fungal inoculum. Five hundred grams of seeds were soaked in water for 1 hour. After that, seeds were boiled mildly and excessive water was drained off. The boiled grains were put into 500-mL conical flasks, plugged with cotton and autoclaved for 50 min at 121 °C temperature and then cooled at room temperature prior to inoculation with an actively growing culture of *S. rolfsii*. Inoculated flasks were incubated at 25±2 °C for 10 days and stored in a refrigerator at 4 °C.

Plastic pots having diameter of 15 cm and 10 cm deep were filled with sandy loam soil at 1 kg pot^{-1}. The inoculum prepared on pearl millet seeds, was mixed in pot soil at 15 g per pot except in negative control. In negative control, 15 g of sterilized pearl millet seeds were mixed in each pot. Pots were watered and left for 10 days for establishment of the inoculum. Suspension of 150 ppm concentration of each of the two fungicides namely Mancozeb and Thiophanate Methyl were prepared and 500 mL of each was added to respective pots. Pots of positive control were prepared by only inoculating them with the fungus. Pots were irrigated with tap water whenever required. There were following 04 treatments in the pot trials:

T_1 Negative control

T_2 Positive Control (only *S. rolfsii* was added)

T_3 Thiophanate Methyl (500 mL of 150 ppm suspension)

T_4 Mancozeb (500 mL of 150 ppm suspension)

Each treatment was replicated 5 times.

Certified seeds of chickpea var. CMS-2118-2508 were obtained from National Agriculture Research Council (NARC) Islamabad, Pakistan. Seeds were surface sterilized in 1% (v/v) bleach solution for 3 minutes followed by repeated washings. Seeds were soaked in sterilized water for 6 hours. Imbibed chickpea seeds were sown in pot soil at the depth of 1-cm.

Plants were harvested 30 days after sowing. Data regarding plant mortality were recorded. Plants were washed carefully and dried on filter paper at room temperature and the roots and shoots were separated. Lengths of shoot and root were measured with a scale. For dry weight measurement, the shoots and roots were dried at 60 °C and weighed. Plant mortality was calculated as follows:

$$\text{Mortality (\%)} = \frac{\text{No. of plants died due to disease}}{\text{Total No. of plants}} \times 100$$

Statistical Analysis: In both laboratory bioassays and pots trial, standard errors of means of replicates were calculated on computer software Microsoft Excel. All the data were analyzed by analysis of variance (ANOVA)

followed by mean separation by Tukey HSD Test using computer software Statistix 8.1.

RESULTS AND DISCUSSION

The results about *in vitro* effects of fungicides on the radial growth of *S. rolfsii* were variable. Various fungicides used in the present study suppressed the fungal growth ranging from 39-100%. Similar variation in efficacy among the fungicides has also been reported against other fungal plant pathogens. Vipin *et al.* (2011) reported that Carbofuran, Thiophanate Methyl and Bavistin had variable efficacy against *Rhizoctonia solani*.

The effect of different concentrations of Mancozeb on *S. rolfsii* is presented in Figure 1A. All the concentrations of the fungicide significantly declined the fungal growth. Various concentrations reduced the fungal growth by 99-100% over control. Recently, Neha and Razia (2013) showed that the fungal species such as *Alternaria alternata, A. clamydophor, Aspergillus niger, A. flavus, Rhizopus oryzae, Rhizopus* spp., *Mucor* spp., *Fusarium* spp., *Drechslera australiensis, Penicillium* spp., *Curvularia lunata* and *Cladosporium,* isolated from stored grains of wheat, were effectively controlled by the use of Mancozeb. Similarly, Nandesha *et al.* (2013) reported that Mancozeb was highly effective against *Aspergillus niger*. Likewise, Nagarathnam *et al.* (2013) showed that the Mancozeb was highly useful for the control of *Marasmiellus* sp. causing banana stem rot disease. In the present study, application of Mencozeb significantly reduced plant mortality by 50% over positive control in pot trial. As a result, there was 233% and 33% increase in biomass of shoot and root over positive control, respectively (Figure 2&3). Similarly, Fravel *et al.* (2005) showed that the Mancozeb was effective for the control of *Fusarium oxysporum*.

The effect of different concentrations of Ridomil Gold on *S. rolfsii* is demonstrated in Figure 1B. All the concentrations of the fungicide significantly reduced the mycelial growth of *S. rolfsi.* The higher concentration viz. 250 and 200 ppm caused 100% reduction of mycelial growth whereas lower concentrations of the fungicide such as 50, 100 and 150 ppm inhibited the mycelial growth ranging from 39-93% over control. Presently, Ridomil Gold was found comparatively less effective than other fungicides in controlling the *in vitro* growth of *S. rolfsii.* Earlier, findings also demonstrated that Ridomil Gold was effective at its higher concentrations. In addition, Wheeler *et al.* (2005) showed that the Ridomil Gold was ineffective against *Pythium* species. Sukul and Spiteller (2000) explained that the Ridomil Gold performed poorly that might be due to either biodegradation of the fungicide or fungal resistance. The effect of different concentrations of Tegula (Tebuconazole) on *S. rolfsii* is presented in Figure 2C. The results revealed that all the concentrations of this fungicide significantly and completely arrested the mycelial growth of the fungus. Tebuconazole is among the most frequently used fungicides in plant disease management. It is very effective for protecting maize seedlings from head smut disease caused by *Sphacelotheca reiliana* (Yang *et al.,* 2014). This fungicide is also known to be very effective against many smut diseases of cereals including loose smut of wheat caused by *Ustilago segetum* var. *triticiy* (Srivastava *et al.,* 1997). It is also known to inhibit the growth of *Fusarium graminearum,* the cause of head blight disease of wheat (Spolti *et al.,* 2012).

The effect of different concentrations of Thiophanate Methyl on *S. rolfsii* is illusrated in Figure 1D. All the concentrations of this fungicide significantly arrested the mycelial growth of the targeted fungus. The inhibitory effect of the fungicide was ranging from 91-100% over control. The concentration 150 ppm and higher completely controlled the fungal growth. Previously, Javed *et al.* (2012) demonstrated that this fungicide provided the maximum fungal control for the storage of maize seed. In earlier studies, Thiophanate Methyl was found to be highly effective against *Rhizoctonia solani* (Sabet *et al.,* 2000; Khan and Mehnaz, 2003; Bharathi *et al.,* 2005). Similarly, Mokhtar *et al.* (2010) found that Thiophanate Methyl had highly inhibitory effects against *Macrophomina phaseolina* growth. In pot trial, the highest plant mortality (56%) was recorded in positive control treatment after 15 and 30 days growth stage. Thiophanate Methyl application reduced the plant mortality to 3% i.e. 95% decrease over positive control (Figure 2). Consequently, there was significant increase of 233% and 67% in shoot and root biomass over control, respectively (Figure 3 B & C). Earlier report showed that Thiophanate Methyl was highly effective for the control of *Fusarium* wilt of cotton caused by the *Fusarium oxysporum* (Qayoom *et al.,* 2006). The present study concludes that Mencozeb, Tegula and Thiophanate Methyl can control *in vitro* growth of *S. rolfsii.* Thiophanate Methyl followed by Mencozeb can be used effectively to control collar rot disease of chickpea *in vivo.*

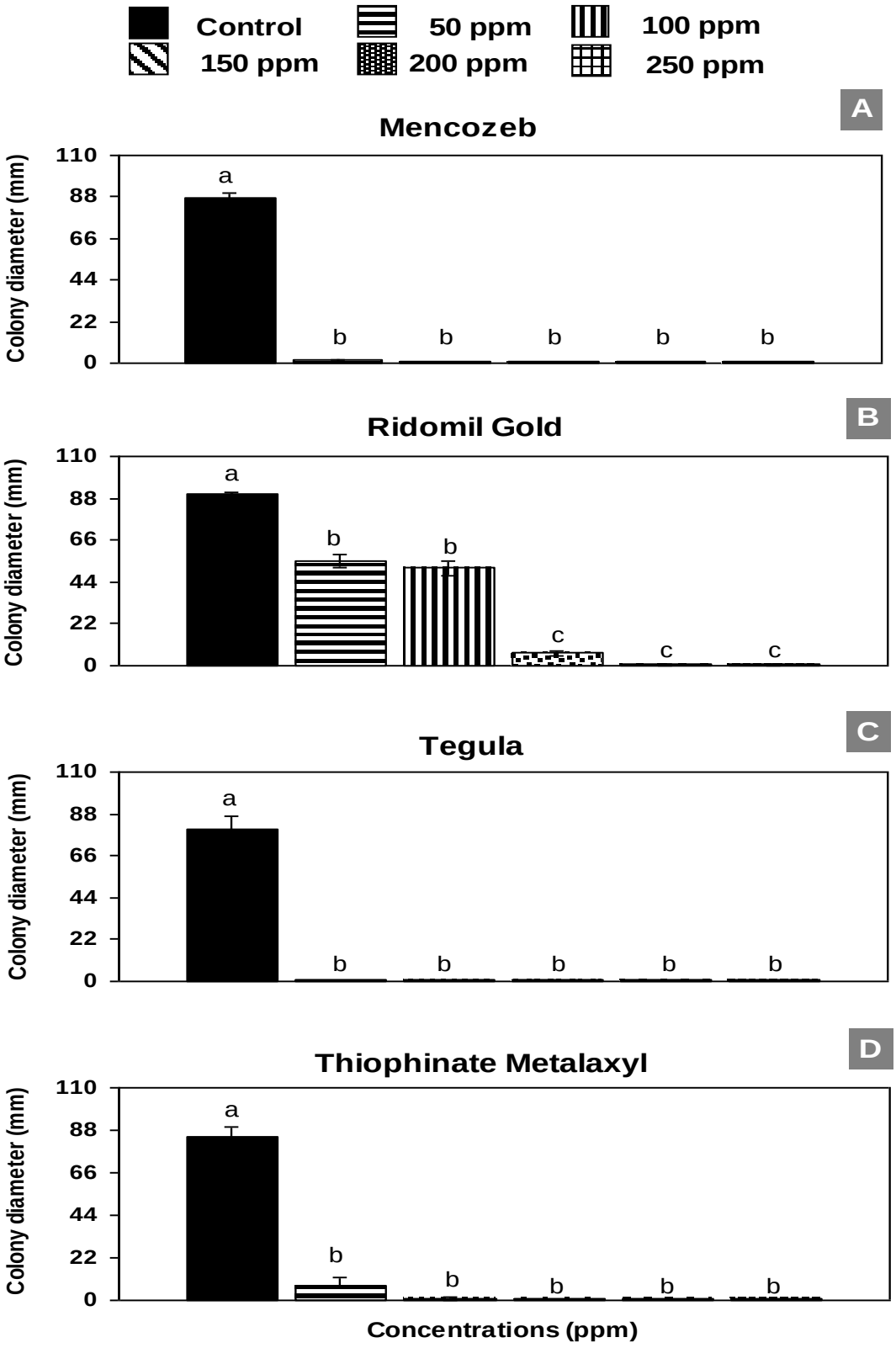

Figure 1. Effect of different concentrations of Mancozeb, Ridomil Gold, Tegula and Thiophinate Metalaxyl on radial growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey HSD Test.

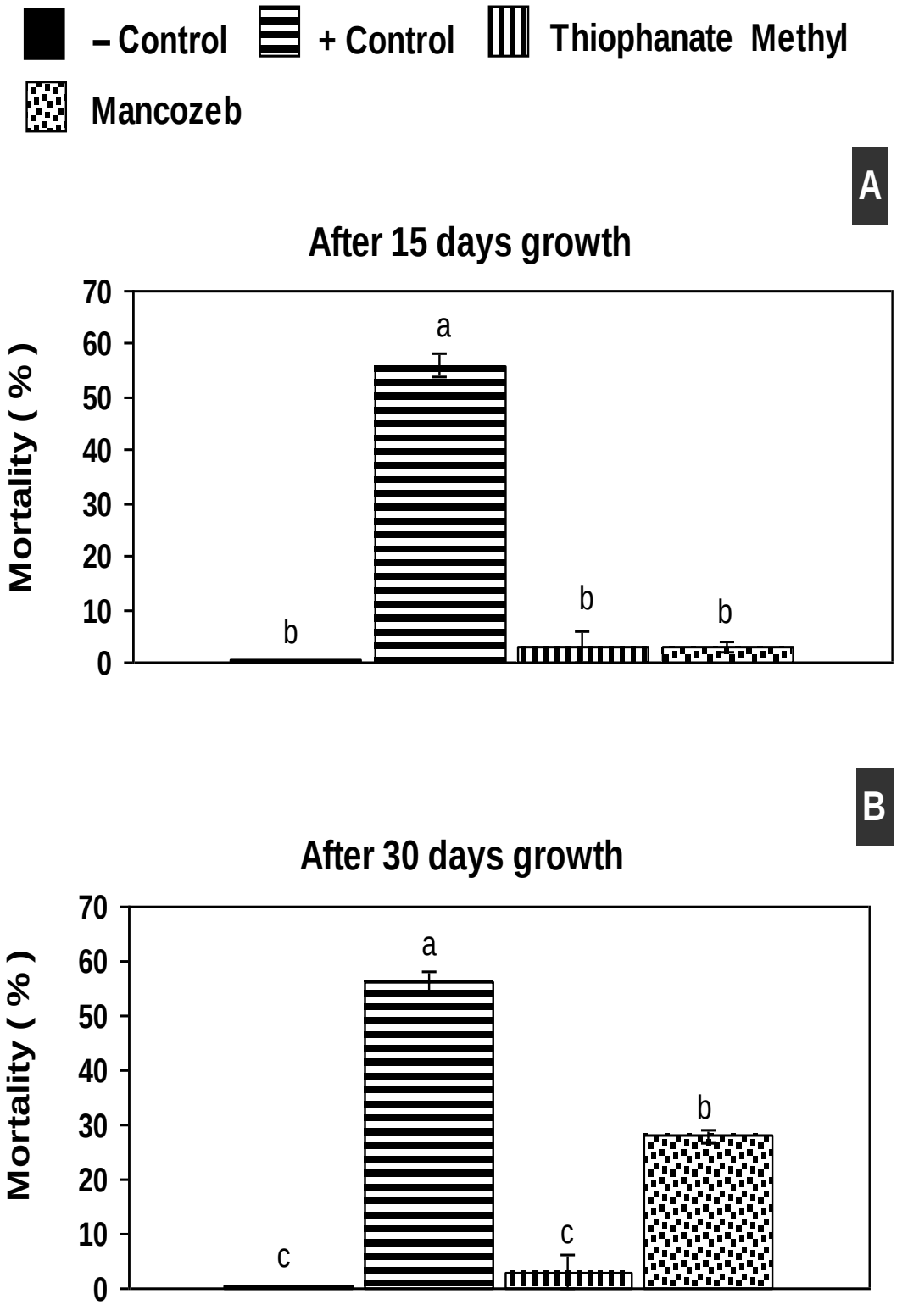

Figure 2. Effect of *Sclerotium rolfsii* inoculation and fungicides on disease incidence and mortality of chickpea. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey HSD Test.

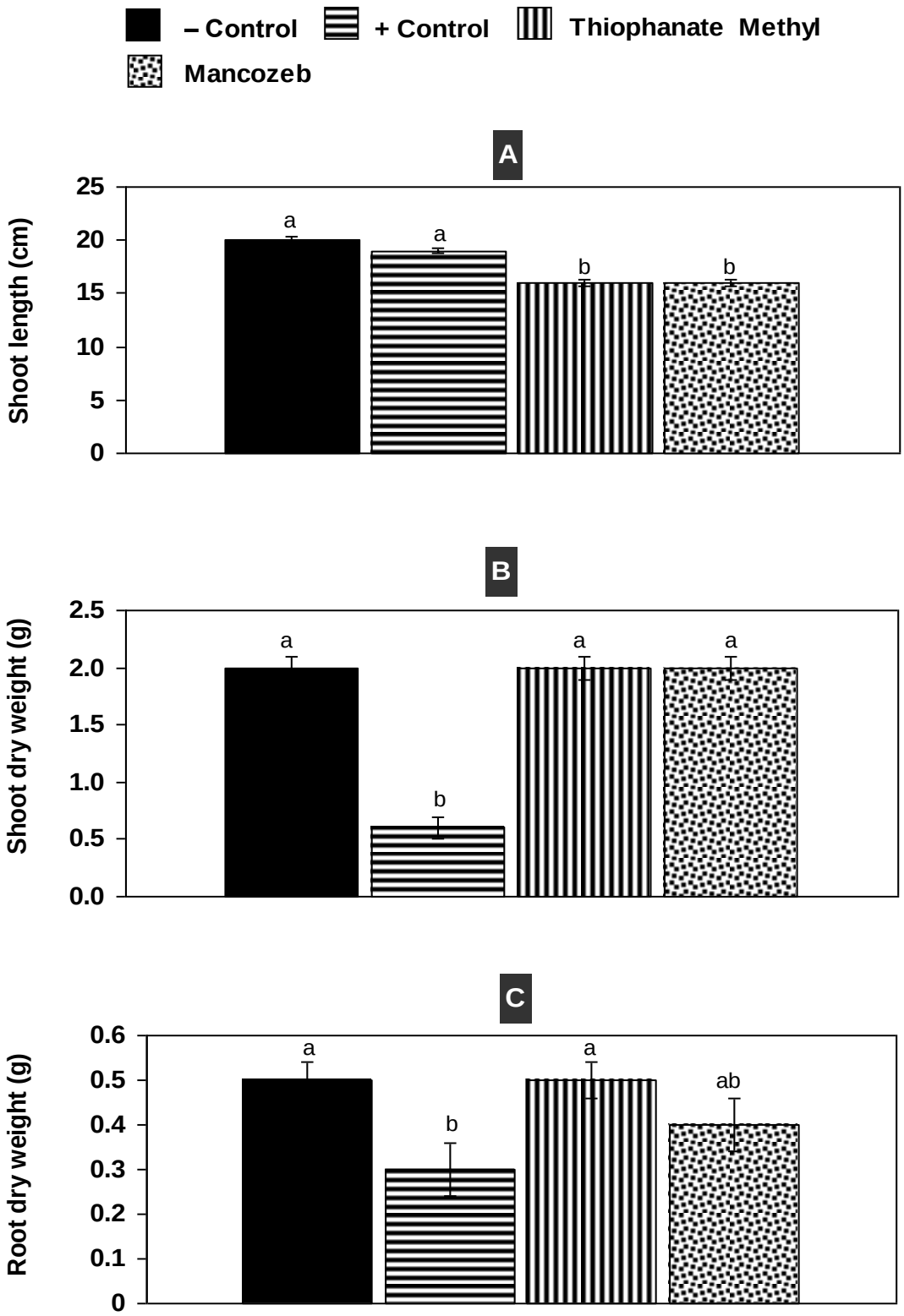

Figure 3. Effect of fungicides on shoot and root growth of chickpea under biotic stress of *Sclerotium rolfsii.* Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey HSD Test.

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