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Productivity of Chilli and Soil Bacterial Population as Affected by Different Levels of Fungicides

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Abstract: Chilli (*Capsicum annum* L.) is of great nutritive and medicinal significance. It is also an important cash crop for both domestic and export markets. Several biotic and abiotic factors are responsible for the loss in chilli yield. Improper use of fungicides without any recommendation pollutes the edible portion of vegetables along with deterioration in soil fertility by affecting biological processes. Pot experiment was conducted to evaluate the effect of fungicides on plant growth and its residual effect on soil bacterial population. Four levels (control, half of recommended, recommended, and double of recommended dose) of two potential fungicides i.e. Foestyl-aluminum and Propineb were applied with 15 days interval after transplanting. Results showed that fungicides at recommended dose significantly improved all growth parameters as compared to control and other levels. It also improved the fruit shininess and fruit yield. The double of recommended dose not only deteriorates plant general appearance, but also produced negative impact on plant height, root length, and fruit yield. There was no significant hazardous effect on soil microbial population up to a recommended dose of both fungicides. However, the double of the recommended dose decreased soil microbial population which ultimately decreased root growth, root activity and soil health. It is concluded that fungicides should be applied carefully for plant protection measures as carelessness in their use lead to crop losses and deterioration of soil bacterial population..

Keywords: pesticide pollution; vegetables; soil health; beneficial bacteria; management.

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1 Introduction

Chilies or red pepper (*Capsicum annum* L.) is an important condiment consumed globally. It is grown for home consumption and as important cash crop by farmers with small land holding (Schreinemachers et al., 2015).It is a rich source of vitamins, antioxidants and phenolic compounds (Alam et al., 2018; Zachariah and Leela. 2018). The addition of these in dietary nutrition help in protecting several diseases and also act as natural food color (Chopan and Littenberg 2017; Marin et al., 2004; Materska et al., 2015; Przygodzka et al., 2014; Seth et al., 2018).Due to presence of capsaicin and dihydrocapsaic in red peppers are important food flavoring ingredient for many vegetarian and non-vegetarian food products and medicinal uses. . They are added for pungency and preservative properties (Momin et al., 2018; Ranjita et al., 2018). Red peppers have unique medicinal properties including analgesic, antibacterial, antifungal, anti-inflammatory, anti-microbial, antioxidant, hypercholesterolemia and hypoglycemic, etc. (Marrelli et al., 2016; Ranjita et al., 2018). Pepper cultivation is an important source of income and employment for millions of small landholding farmers (Kuivanen et al., 2016; Parvathi and Waibel, 2016). Pakistan is among the top five producers of red pepper / chilies in the world (Government of Pakistan, 2018).With the increase in area under peppers, incidence of diseases also reported to increase (Divakaran et al., 2018; Schreinemachers et al., 2015). A number of fungal, bacterial and viral diseases cause huge yield losses of red chillies (Ajithkumar et al., 2014). Powdery mildew (caused by *Leveillula taurica*) is among the major fungal diseases infecting field and greenhouse-grown chillies (Jo et al., 2017). Powdery mildew causes premature defoliation leading reduced photosynthetic area, photosynthates production, leading to not only yield reduction, but also damaging quality of harvest and making it unfavorable for marketing. Due to endophytic nature of powdery mildew, chemical control is difficult (Elad et al., 2007; Schor et al., 2017; Sudha and Lakshmanan, 2009).

In past few decades powdery mildew became a recurring problem on the peppers. Pesticides are used to control insect pests, diseases and weeds in vegetables especially pepper. A number of pesticides are highly toxic and even in very small quantities these pesticides can result in unintended consequences including chronic toxicity while exposure to a sufficient amount of almost any pesticide can cause death of humans and animals (Hallmann et al., 2014; Rohr et al., 2017). Pesticide application, in certain cases, can potentially increase pest outbreaks by adversely affecting their natural biological control (Break et al., 2018; Douglas et al., 2017; Main et al., 2018; Rohr et al., 2017; Rundlof et al., 2015) and can also cause non-target effects leading to disruption of functions and services of ecosystems (Tsvetkov et al., 2017; Woodcock et al., 2017). Pesticides became a novel agricultural practice, post 1945, increased in number and variety during the 1960s and became the standard practice in the 1970s. Annually, about 35% of potential crop production is lost due to pests, pathogens and weeds worldwide (Oerke and Dehne, 2004). The use of high dose of fungicides to control fungal diseases leaves residues in the vegetables and deteriorates soil fertility by affecting biological processes. However, the improvement in systemic resistance to abiotic stresses due to the application of fungicides has also been reported (Han et al., 2012). If pesticides are sprayed just-before harvest, pepper fruits may contain residues above the threshold of maximum residue limit by FAO/WHO. It may be hazardous to consumers and pose export problems. The use of pesticides is increasing day by day in Pakistan. These pesticides upon ingestion exert adverse effects on the soil environment and health in addition to disturbing ecosystem (Ahmed et al., 2011).

Excessive application of pesticide application decreases the crop growth, yield, and quality of produce. These inhibitory effects on growth parameters and soil microbial population have been observed in previous studies (Fox et al., 2007; Walia et al., 2014). A decrease in crop growth and yield has been reported due to the negative effects of pesticides on indigenous microbial population (Singh et al., 2015) and enzyme inhibition (Zablotowicz and Reddy, 2004; Gupta et al., 2013). It has been observed that fungicides application at lower rates are generally not toxic possibly because of the buffering nature of soils these chemicals become diluted (Ayansina, 2009).

Pesticides are toxic to plants and human, as many food crops including fruits and vegetables contain pesticides residues after being washed or peeled. The pesticides also affect the microbial population of the soil (Guene et al., 2003).A major factor of pesticide contamination or poisoning in developing countries is the unsafe use or misuse of pesticides. Although, world food supply got better due to pesticide use but their irresponsible and indiscriminate use lead to the serious consequences for the environmental health.

Keeping in view the above facts, the present study has been conducted to evaluate the effect of fungicides on productivity of chillies and to study the effect of these fungicides on soil bacterial population.

2. Materials and Methods

2.1. *In vitro* the effect of fungicides on plant growth and soil bacterial population

The experiment was conducted with different sets of treatments. Three levels of two potential fungicides i.e. Foestyl Aluminium (@1.25, 2.5, 5 gL^{-1}) and Propineb (@2.5, 5, 10 g L^{-1}) along with control (without fungicides) were applied one week after transplantation. Each treatment replicated thrice to remove the experimental error. Nursery of pepper plants was grown in pots using well prepared soil. The soil used in pots was collected, and analysed for physico-chemical characteristics. The pots were lined with polythene sheets before adding soil and there was no leaching provision in the pots as the only hole at the bottom was plugged with cork. Pots were arranged in wire house at ambient light and temperature in completely randomized design (CRD). Three plants of 30 days old nursery were transplanted and thinned to one plant after establishment of seedlings.

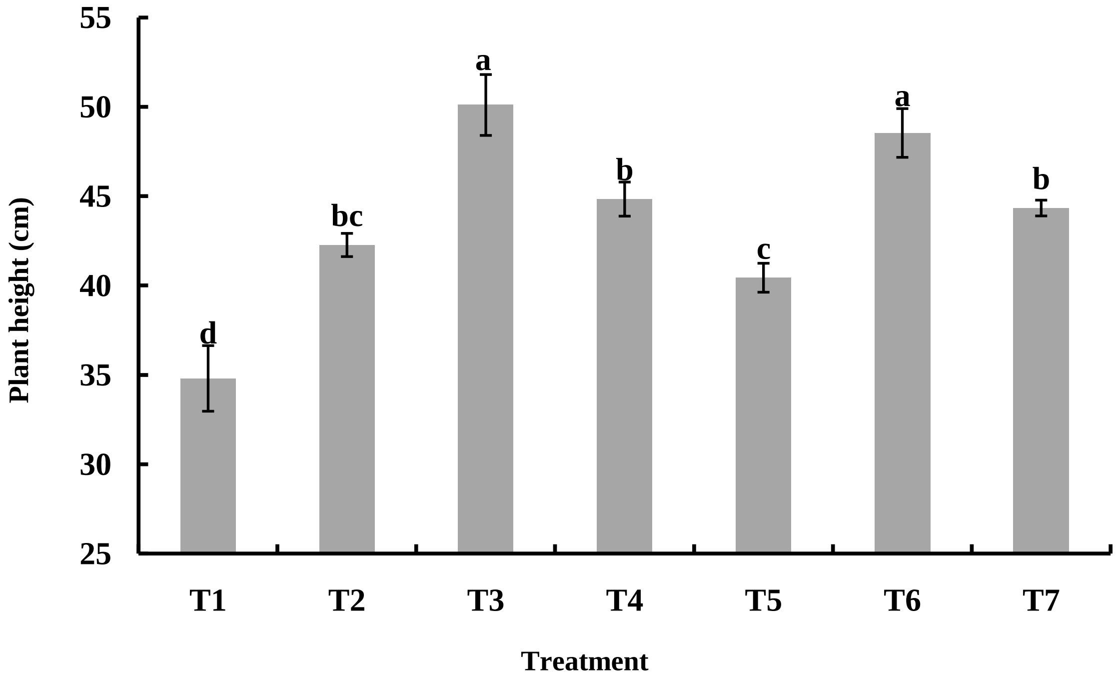

Fig. 1.Effect of fungicides on plant height of chillies, (n = 3); LSD ($p \leq 0.05$) = 3.677, T1: Control; T2-T4: Propineb @ 2.5, 5, 10 g/L; T5-T7: Foestyl-Al @ 1.25, 2.5, 5 g L^{-1}.

The recommended doses of N, P and K (@ 100: 40: 40 kg ha^{-1}) were applied as urea, diammonium phosphate (DAP) and sulphate of potash (SOP), respectively, were applied in each pot. All P and K fertilizers were applied as basal dose at the time of transplanting, while nitrogen was applied in two equal splits i.e. at transplanting and 20 days after transplanting. The plants were irrigated with good quality irrigation water meeting the irrigation quality criteria for crop (Ayers and Westcot, 1985). All recommended agronomic practices were followed. The fungicides were applied according to the treatment plan. The application of fungicides was started ten days after transplanting and repeated for six times at one week interval. The data was collected for different growth and yield parameters of red pepper at maturity. Relative water contents (RWC) were measured by the method as described by Barrs and Weatherley (1962). Rhizosphere soil samples were collected by uprooting plants. The soil adhering to plant roots was collected by scrapping for use as rhizospheric soil. Soil samples were placed individually into plastic bags, transported to the laboratory, and freeze-dried until further analysis. Composite sample was prepared by thorough mixing of soil collected from selected plants. Bacterial population (viable bacteria) was studied in the laboratory. Colony forming units (CFU) of bacteria were enumerated by standard serial dilution method as described by Alexander (1982). The data were analyzed statistically using analysis of variance techniques (ANOVA) and treatment means were compared using LSD at 5% level of probability (Steel et al., 1997).

3. Results

The results showed that application of fungicides significantly improved plant height (Fig. 1). Both the fungicides were equally effective in increasing height of pepper plants. The plant height was increased with increase in fungicide level up to recommended level and the maximum increase was reported in T3 and T6 where Propineb and Foestyl-Al were applied at 5 and 2.5 g L^{-1}, respectively, (recommended dose). With increase in level from recommended up to double of the recommended of both fungicides significantly decreased plant height.

Data showed that shoot fresh weight of plants was significantly increased with theapplication of both fungicides (Fig. 2). Maximum improvement in shoot fresh weight was observed in T3 and T6 where Propineb and Foestyl-Al were applied at their recommended rates.

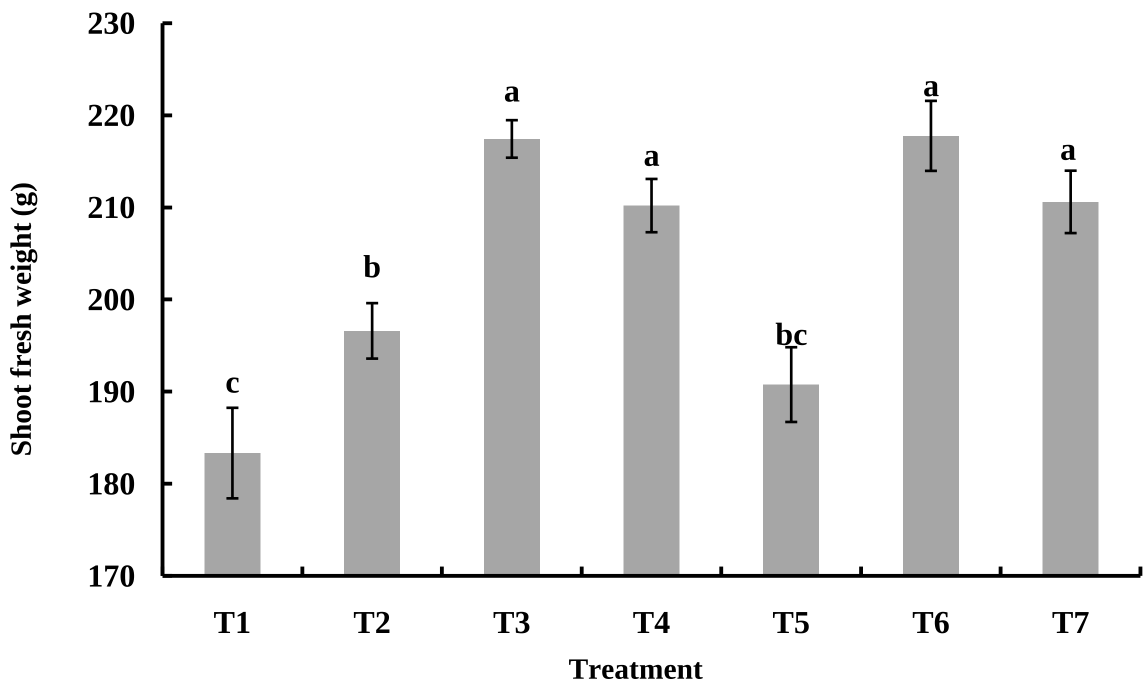

Fig. 2. Effect of fungicides on shoot fresh weight of chillies. (n = 3); LSD ($p \leq 0.05$) = 10.748; T1, Control; T2-T4: Propineb @ 2.5, 5, 10 g/L; T5-T7: Foestyl-Al @ 1.25, 2.5, 5 g L^{-1}.

The increase in fungicides dose from recommended to double of the recommended dose of both (T4 and T7) caused less increase in shoot fresh weight as compared to the recommended dose of both fungicides but it was statistically at par with each others. Similarly, application of both Propineb and Foestyl-Al lead to the significant improvement in the shoot dry weight of red pepper plants (Fig. 3).

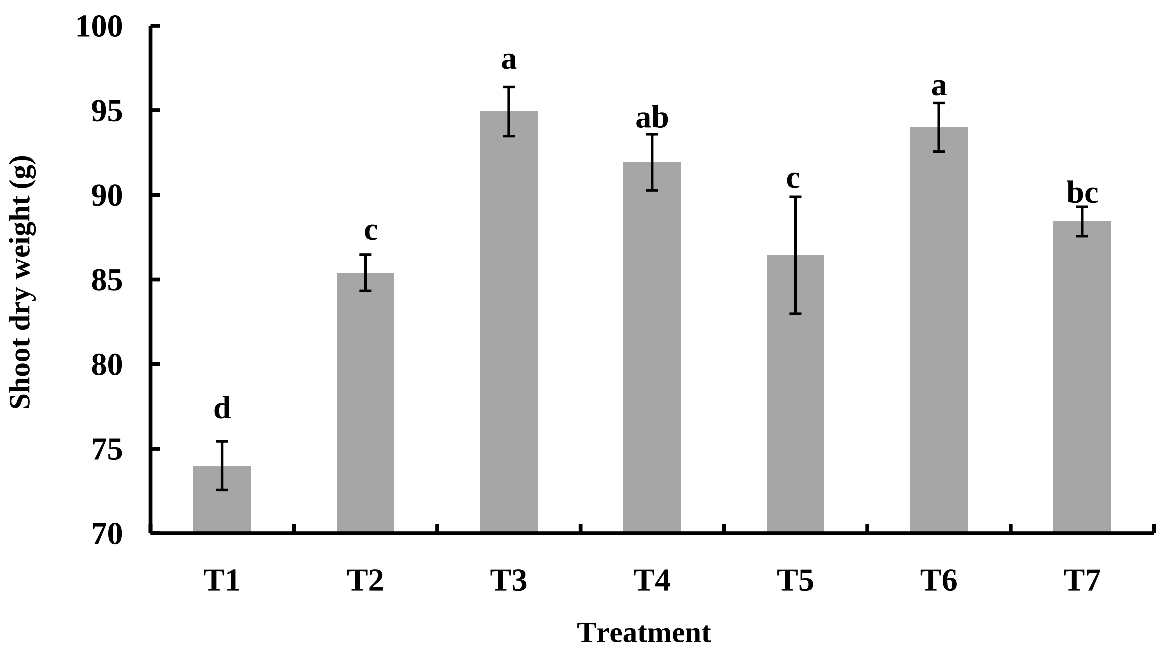

Fig. 3. Effect of fungicides on shoot dry weight of chillies. (n = 3); LSD ($p \leq 0.05$) = 5.473, T1: Control; T2-T4: Propineb @ 2.5, 5, 10 g/L; T5-T7: Foestyl-Al @ 1.25, 2.5, 5 g L^{-1}.

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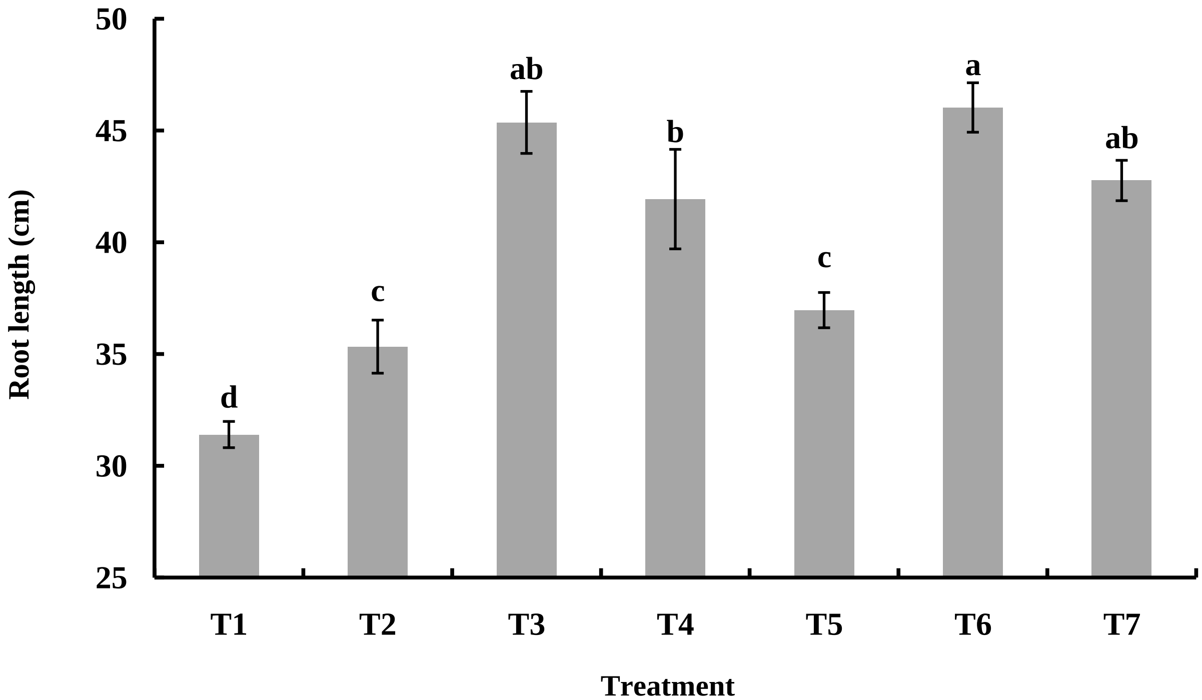

Fig. 4. Effect of fungicides on root length of chillies (n = 3). LSD ($p \leq 0.05$) = 3.85; T1: Control; T2-T4: Propineb @ 2.5, 5, 10 g/L; T5-T7: Foestyl-Al @ 1.25, 2.5, 5 g L^{-1}.

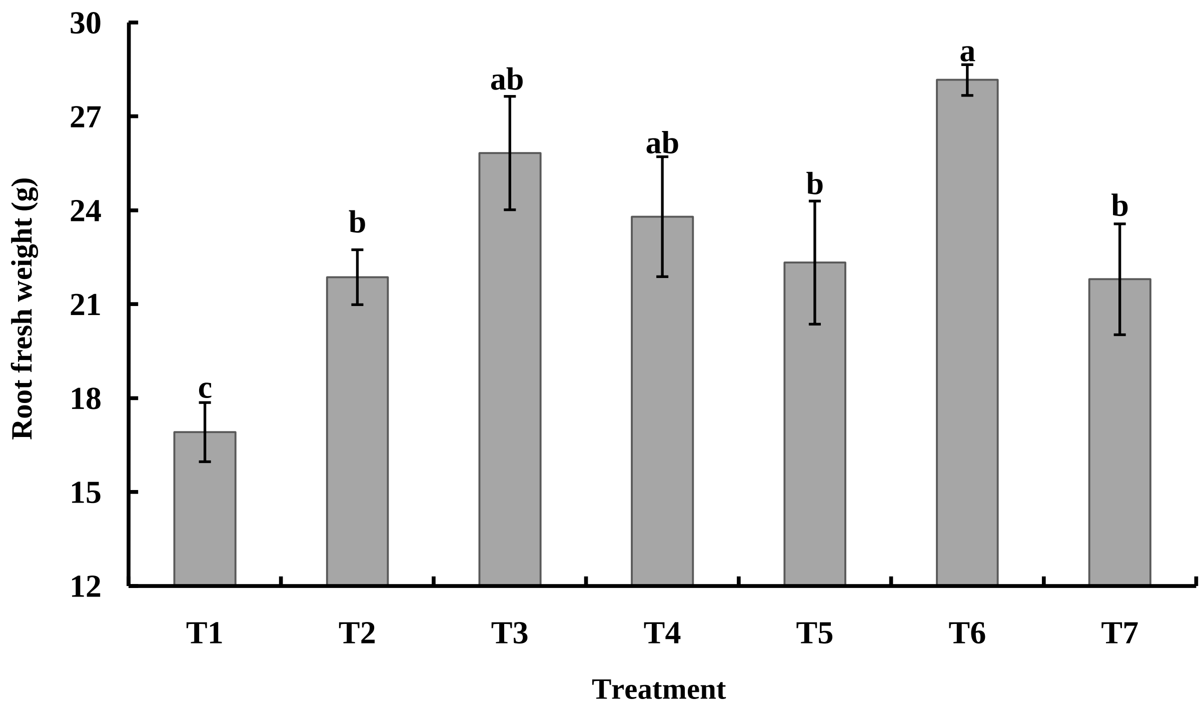

Fig. 5. Effect of fungicides on root fresh weight of chillies. (n = 3); LSD ($p \leq 0.05$) = 4.561; T1: Control; T2-T4: Propineb @ 2.5, 5, 10 g/L; T5-T7: Foestyl-Al @ 1.25, 2.5, 5 g L^{-1}.

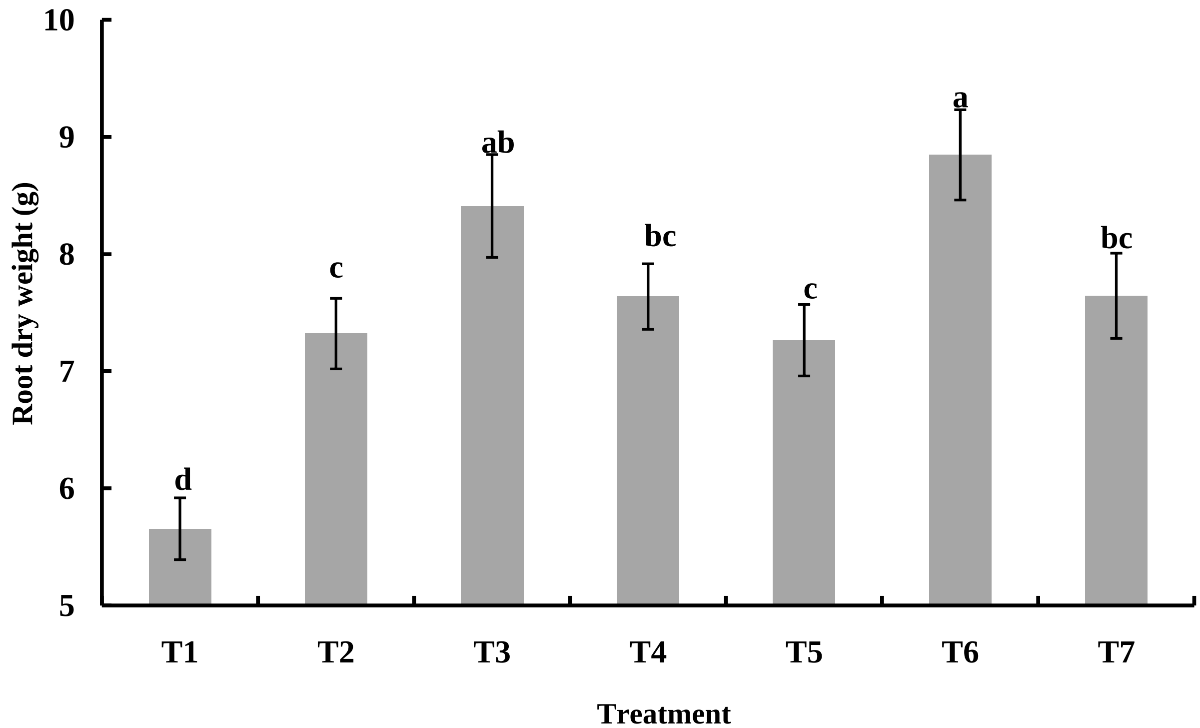

Fig. 6. Effect of fungicides on root dry weight of chillies. (n = 3); LSD ($p \leq 0.05$) = 1.028; T1: Control; T2-T4: Propineb @ 2.5, 5, 10 g/L; T5-T7: Foestyl-Al @ 1.25, 2.5, 5 g L^{-1}.

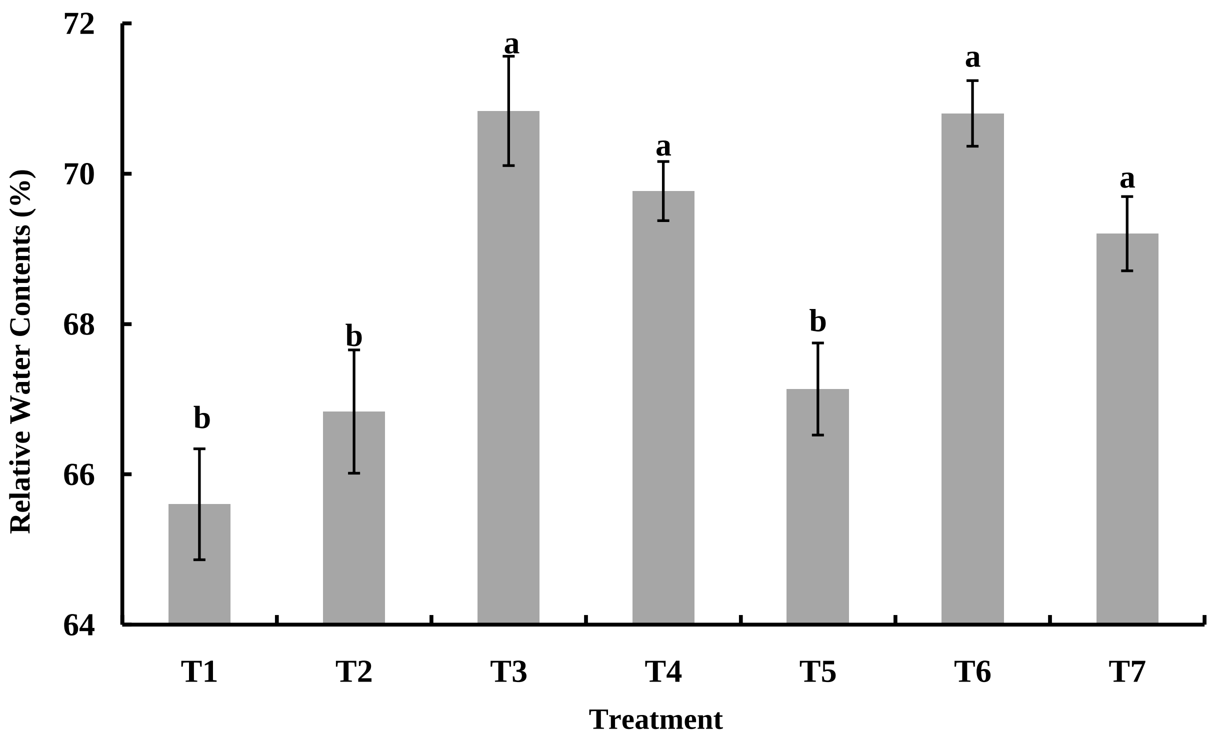

Fig. 7.Effect of fungicides on relative water contents in leaves of chillies. (n = 3); LSD ($p \leq 0.05$) = 1.885; T1: Control; T2-T4: Propineb @ 2.5, 5, 10 g/L; T5-T7: Foestyl-Al @ 1.25, 2.5, 5 g L^{-1}.

Likewise shoot fresh weight, maximum improvement in shoot dry weight was observed in T3 and T6 i.e. the recommended dose of both fungicides. The double dose of both fungicides was less effective and the improvement in shoot dry weight due to double dose of both fungicides was less as compared to recommended dose. The improvement in shoot dry weight due to double dose of Propineb was non-significant with its recommended dose; the same was significantly less than recommended dose in case of Foestyl-Al (T7).

There was no significant difference among the recommended dose of Propineb and Foestyl-Al but their under dose was less effective in improving the root length of pepper plants (Fig 4). The double dose also decreased the efficacy of fungicides and was less effective than the recommended dose of both Propineb and Foestyl-Al. The root fresh and dry weights of red pepper plants were improved with the application of Propineb and Foestyl-Al (Fig. 5 and Fig. 6). Likewise, the shoot fresh and dry weight, maximum improvement in root fresh and dry weight was observed in T3 and T6 i.e. the recommended dose of both fungicides. The improvement in root fresh and dry weight due to double dose was less as compared to recommended dose.

Results showed that fungicide application significantly increased the relative water contents (RWC) in leaves of **chilli** plants (Fig. 7). Maximum RWC were observed in the treatments where Propineb and Foestyl-Al were applied at their recommended rates. The increase in dose from recommended to double of recommended decreased the efficacy of fungicides. The increase, in this case, was less than recommended but it was non-significant with recommended rates. There was no significant difference between the results obtained by the application of two fungicides. The improvement in fruit yield of red pepper plants was observed with the application of Propineb and Foestyl-Al (Fig. 8 and Fig. 9). As in case of growth parameters, maximum improvement in number of fruits plant^{-1} and fruit fresh weight was observed in T3 and T6 where recommended dose of both fungicides was applied.

Data (Fig. 10) showed that there was non-significant effect of fungicides application on bacterial population in soil when Propineb and Foestyl-Al were applied at half of their recommended rates. However, these fungicides significantly increased total viable bacterial count when these were applied at their recommended rates.

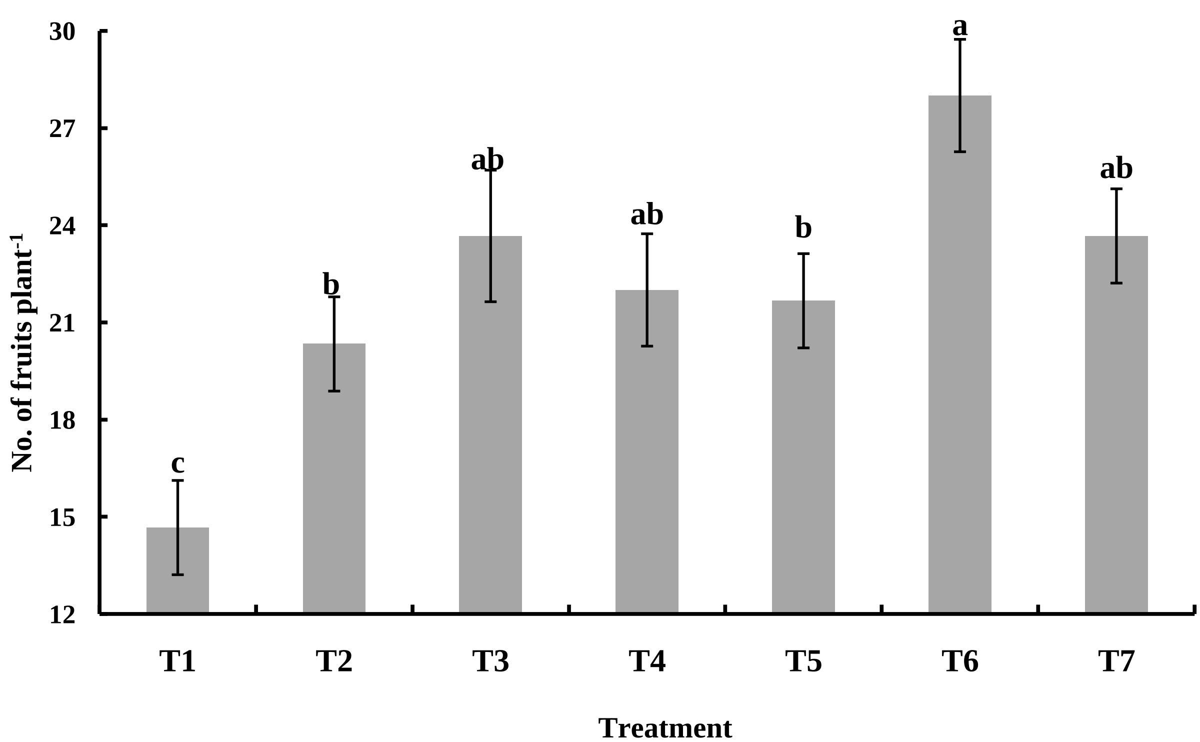

Fig. 8. Effect of fungicides on number of fruits plant^{-1} of chillies. (n = 3); LSD ($p \leq 0.05$) = 4.938; T1: Control; T2-T4: Propineb @ 2.5, 5, 10 g/L; T5-T7: Foestyl-Al @ 1.25, 2.5, 5 g L^{-1}.

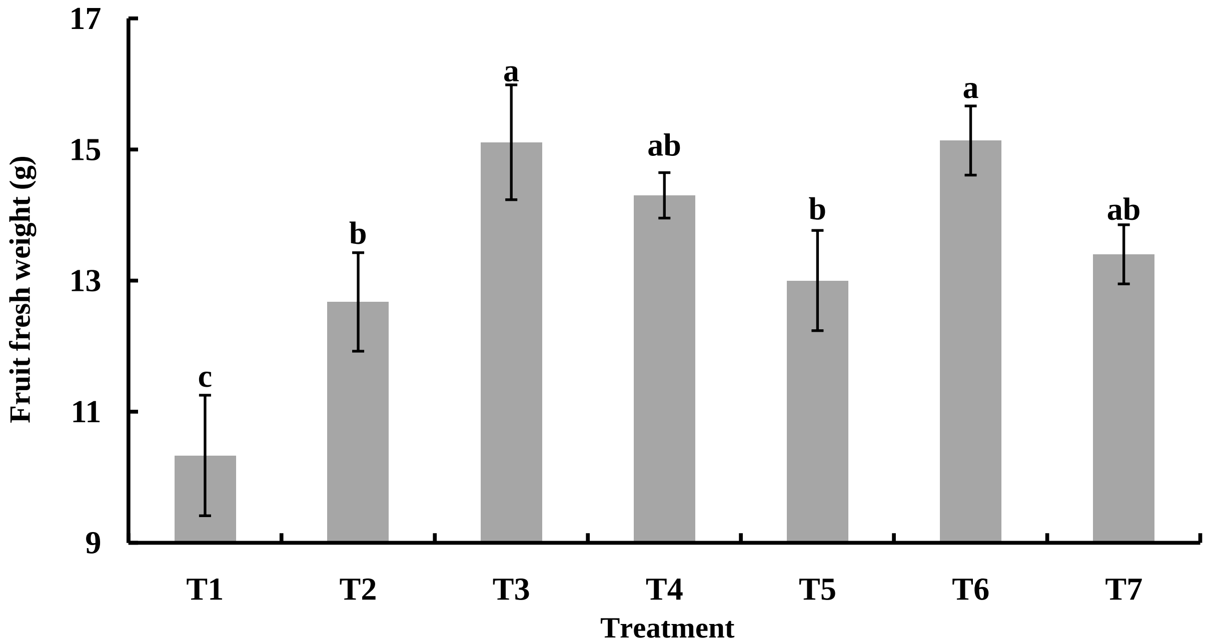

Fig. 9. Effect of fungicides on fruit fresh weight of chillies. (n = 3); LSD ($p \leq 0.05$) = 2.101; T1: Control; T2-T4: Propineb @ 2.5, 5, 10 g/L; T5-T7: Foestyl-Al @ 1.25, 2.5, 5 g L^{-1}.

The double of recommended dose of both fungicides caused a significant decrease in the total viable bacterial count in the rhizosphere. Foestyl-Al. was more destructive in this case as compared to Propineb and a significant decrease in total viable bacterial count was observed when Foestyl-Al. was applied at double of the recommended rate.

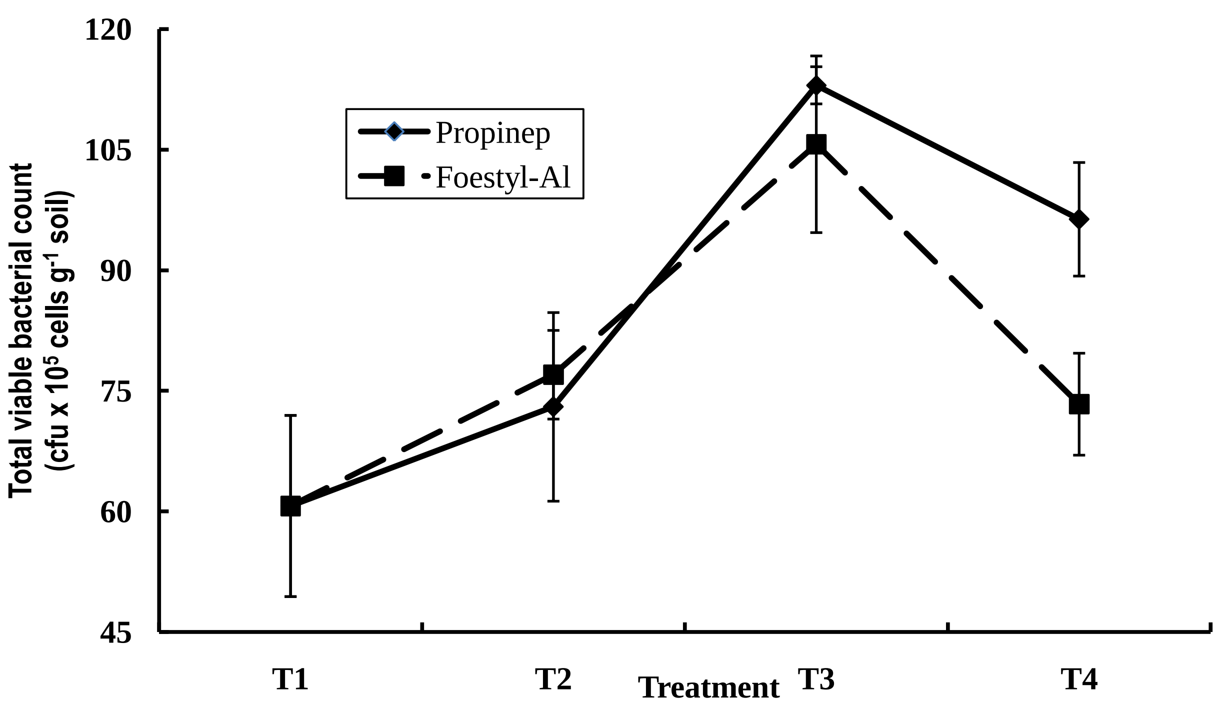

Fig. 10. Comparative residual effect of different doses of Propineb and Foestyl-Al on bacterial population in rhizosphere of chillies. T1: Control; T2: Half of the recommended dose of Propineb and Foestyl-Al; T3: Recommended dose of Propineb and Foestyl-Al; T4: Double of recommended dose of Propineb and Foestyl-Al..

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4. Discussion

The fungicides are being used on vegetable crops at alarming rates just to increase the yield and for improving the general appearance of vegetables (Esau et al., 2014; Goutam et al., 2018; Marine et al., 2016). The higher dose of these fungicides may enter in the food chain and cause toxic impacts in soil, plants and consumers of vegetables including humans (Pan et al., 2018; Singh et al., 2016; Zhang et al., 2015). In current studies, Propineb and Foestyl-Al. were applied on red pepper at different rates. It was observed that application of these fungicides up to their recommended rate showed significant effect on growth and yield parameters while a significant decrease in growth and yield parameters was observed when these fungicides were applied at double of the recommended rates. This may be due to toxic effects of pesticide on microbial activity which ultimately leads to decrease in growth and yield of pepper plants. Previous studies have reported excessive pesticides application can lead to decreased plant growth and yield (Fox et al., 2007), reduced indigenous microbial population (Guene et al., 2003; Singh et al., 2015) and enzyme inhibition (Zablotowicz and Reddy, 2004; Singh et al., 2015). Lower doses of fungicides are generally not toxic potentially due to buffering nature of soils (Ayansina, 2009). The positive effect of fungicides on growth and yield parameters at lower rates may be due to suppression of soil borne diseases which ultimately increased the root growth thus improving the growth and yield of pepper plants.

Soil fertility is directly related to microbial processes which are responsible to supply nutrients for plant growth (Rengalakshmi et al., 2018; Sun et al., 2018). Nutrient solubilization by rhizosphere bacteria is an important function of beneficial soil bacteria increasing the availability of nutrients to plants (Jacoby et al., 2017; Zhou et al., 2017). So, soil microbial population is the important parameter to describe the effect of fungicides on soil fertility. In our studies, the application of Propineb and Foestyl-Al. at their recommended rates have no significant negative effect on soil bacterial population but their double dose significantly decreased the soil bacterial population. This inhibitory effect may be attributed to the toxicity of fungicides for indigenous bacteria and rhizosphere microflora (Yang and Lee, 2008; Zaidi et al., 2009). This inhibitory effect of fungicides on soil microbial population leads to reduction in root activity, root and shoot growth and ultimately lower crop yields.

5. Conclusion

Recommended dose of fungicides significantly improved shoot growth, root growth, and fruit setting as compared to control and half of the recommended dose of both fungicides. The overdose of fungicides decreased plant height, root length and fruit yield, and deteriorated soil health that is evident from decrease in soil bacterial population. Recommended dose of both fungicides did not pose any significant hazardous effects on soil bacterial population. It is concluded that fungicides should be applied more carefully for plant protection measures as carelessness in their use not only decreases crop yield but also deteriorates soil fertility.

List of Abbreviations: CFU; Colony forming units; RWC; Relative water contents.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Authors Contribution: FN, MN and MA developed the idea, prepared initial research plan, coordinated with co-authors throughout the experimentation and manuscript development, and finalized the submission accordingly; MRK helped in the preparation of manuscript and finalized the bibliography; RAS guided throughout the preparation of the manuscript; WH and NM helped during the experimentation and data analysis.

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