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PATHOGENICITY AND CHEMICAL CONTROL OF BASAL ROT OF ONION CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *CEPAE*

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ABSTRACT

Many *Fusarium* spp. have been reported to be associated with onion diseases and cause economically significant losses wherever onion is grown. *F. oxysporum* f. sp. *cepae* was isolated from diseased bulbs. Pathogenicity of isolated specie was confirmed with two methods of inoculation (seedling infestation method and soil infestation method). Both methods of inoculation showed substantial impact on disease development and plant growth. Seedling infestation method caused maximum reduction in plant germination, followed by soil infestation method as compared to control. Highest plant mortality as well as pathogen infection was recorded in soil infestation method, followed by seedling infestation method as compared to control. Four fungicides i.e. Antracol, Carbendazim, Copper oxychloride and Kingmil MZ with 10, 100, 1000 and 10000 ppm concentrations were evaluated against *F. oxysporum* under *in-vitro* as well as *in-vivo* conditions. A positive correlation was observed between concentrations of fungicides used and resulting inhibition of fungal growth. Among four fungicides, Carbendazim, followed by Antracol appeared as the most effective fungicides. Application of fungicides brought a remarkable increase in seedling emergence of treated plants inoculated with *F. oxysporum* as compared to the untreated plants. The most effective fungicide treatments, which produced highest seedling emergence (100%), were 10000 ppm of Antracol, Carbendazim and Copper oxychloride. Infection percentage and plant mortality decreased and minimum were recorded after the application of 10000 ppm of Antracol, Carbendazim, Copper oxychloride, Kingmil MZ and 1000 ppm of Carbendazim. Significant differences were also observed between plant growth (shoot length and weight, root length and weight) of treated and untreated (control) plants inoculated with *F. oxysporum*. Maximum plant growth was recorded in case of 10000 ppm of Carbendazim followed by Antracol 10000 ppm and Copper oxychloride. Whereas, minimum plant growth was observed in untreated plants inoculated with *F. oxysporum*, followed by Kingmil MZ 10 ppm and Copper oxychloride 10 ppm.

Keywords: Allium cepa, basal rot, fungicides, *Fusarium oxysporum,* inoculation methods.

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INTRODUCTION

Onion (*Allium cepa* L.) is an important vegetable crop worldwide and has great economic importance because of its nutritional and medicinal values (Nasri *et al*., 2012) including anti-inflammatory, anti-cholesterol, anticancer, antioxidant properties (Anonymous, 2013). China ranks first in total production of dry onion with 22600000 MT. India, United States and Iran follow it with 16308990, 3277460 and 2260000 MT, respectively. Pakistan is on number eight position, with total production of 1692300 MT (FAOSTAT, 2012).

Various foliar, bulb and root pathogens attack this crop and that reduces the production. Most important soil-borne fungal pathogens of this crop include *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., *Sclerotium* spp., *Sclerotinia sclerotiorum*, *Pyrenochaeta terrestris* and *Macrophomina phaseolina*. All over the world, these pathogens lead to severe production losses (Schwartz and Mohan, 1995). One of these pathogens, *F. oxysporum* f. sp. *cepae* causes basal rot (Cramer, 2000) and considered as one of the most important soil-borne diseases of onion, causes severe losses in the productivity both in the field and in storage condition (Coskuntuna and Ozer, 2008). Infected seeds and soil are the source of dispersal. The fungus causes infection at the basal stem plate of the onion bulb and degrades it; finally destroy the whole plant infections in dormant bulbs during storage allow secondary infections to occur (Cramer, 2000). Many methods have been developed to control plant pathogens, but only few have provided satisfactory control. Despite many attempts to control, the problem is still important throughout the world. The management practices generally employed for its control include resistant cultivars, chemical applications, cultural practices and biotechnological approaches. However, incorporation of integrated management provides a better opportunity to manage this disease (Chandel and Deepika, 2010). The chemical control based on the use of fungicides is most effective and reliable method. New fungicides with novel chemistry are being introduced and evaluated for plant disease control. Their application in the farmer fields can only be recommended against the causal pathogens after a successful laboratory evaluation. It, therefore, needs a constant watch and effort to evolve new fungicides along with some important nonchemical methods of controlling the diseases (Jamil and Kumar, 2010). The present study was carried out to confirm the pathogenicity and chemical control of basal rot of onion caused by *F. oxysporum*, so that better crop yield could be obtained to meet the domestic and export requirements.

MATERIALS AND METHODS

Sample collection and Isolation of the disease causing organism

Diseased samples of onion bulbs of commonly cultivated Nasarpuri variety was collected from different fields of district Hyderabad. In isolation, basal plate tissues of diseased samples were cut into small pieces and placed on the Petri dishes containing sterilized potato dextrose agar (PDA) medium after surface sterilization with a 5% solution of Sodium hypochlorite. These Petri dishes were incubated at 28±2^{o}C for five days. Predominant colonies were re-isolated, purified and identified on the basis of their morphological characteristics with the help of keys by Booth (1971); Ellis (1971); Barnett and Hunter (1972) and Singh (1987).

Pathogenicity test

In order to confirm the pathogenic nature of the isolated fungus (*F. oxysporum*), the pathogenicity test was done in earthen pots through seedling and soil infestation method. For this purpose, commonly growing onion variety "Nasarpuri" was used.

Preparation of conidial suspension

For inoculation, conidial suspension was prepared from 2 weeks old culture of *F. oxysporum*. Sterilized water was added in each culture plate in order to harvest spore of the fungus, the culture was gently rubbed with spatula. The spore suspension was collected in a sterilized glass beaker and strained through a muslin cloth. Spore concentration 2.5x10^{6} conidia/ml was maintained by adding water with the help of hymocytometer (Waller *et al*., 1998).

Seedling infestation method

Seedlings were dipped in 100 ml of spore suspension for five minutes. The un-infested seedlings were used as control. Five seedlings of both infested and un-infested (control) seedlings were sown in earthen pots (containing 2kg sterilized soil). These pots were kept in open air and watered as per requirement. After 45 days of sowing, the plants were uprooted carefully. The data on seedling emergence, plant mortality, plant growth and root infection were recorded. Re-isolation was done with the method described above. After that the growth of the fungus infection percent was calculated with the help of following formula:

$$\text{Infection (\%)} = \frac{\text{Number of pieces colonized by the fungus}}{\text{Total number of pieces studied}}$$

Soil infestation method

The already prepared fungal suspension was mixed with soil @ 2.5x10^{6} conidia/g of soil. Two kg soil was filled in each earthen pot. Five seedlings were sown in infested and un-infested (control) pots. The experiment was arranged as Randomized Complete Block Design. These pots were kept in open air and watered as per requirement. After 45 days of sowing, the observations were taken as described above.

Evaluation of fungicides on mycelial growth of *F. oxysporum*

Four fungicides namely, Antracol, Carbendazim, Copper oxychloride and Kingmil MZ were used to find out the effectiveness of different concentrations of fungicides on mycelia growth of targeted pathogen. The detail of fungicides including their trade name, chemical name, formulation, chemical group and mode of action are given in Table 1. The required concentrations of the fungicides were added to the PDA medium before pouring. Medium without fungicide served as control. After solidifying of the medium, 5mm diameter agar disk of test fungus was cut from 8-10 days old culture plate by using sterilized cork borer and placed in the center of the PDA plate. The inoculated plates were incubated at 25ºC. The radial colony growth of test fungus was recorded by drawing two perpendicular lines on the back of the Petri plates crossed each other in the center of the plate. The data on colony growth was recorded along with these lines in the millimeter after each 24 hours until the plates were filled in any treatment and calculated as inhibition (%) by using the following formula:

$$I=100(C-T)/C$$

Where, I = Inhibition percentage, C = Growth in control (check), T = Growth in treatment.

Evaluation of fungicides against basal rot of onion

Above given fungicides with the same concentration were used against basal rot disease in onion plant. The experiment was designed as Randomized Complete Block Design in earthen pots with four replications. The required concentration of each fungicide was incorporated in the sterilized soil before planting the seedlings. The onion seedlings were artificially infested with the pathogen inoculum as described in pathogenicity test. Plants inoculated with the pathogen (without fungicide treatment) and plant un-inoculated (without fungicide treatment) were kept as control. All the pots were kept in open air and watered as per requirement. After 45 days of sowing, the plants were uprooted carefully. The data were recorded on seed germination, infection percentage, mortality and plant growth.

RESULTS

Disease symptoms and associated pathogen

The diseased onion plants showed both above and below ground symptoms. The affected plants were characterized by general wilting, stunted growth with profound yellowing, followed by browning of leaves. Later on, these leaves became dry and plants showed die-back. The above ground symptoms comprise of rotting of roots and basal plate discoloration. Affected tissues appeared brown and watery. The bulbs become soft, exhibited semi watery decay initiated from the basal plate and move upwards. *F. oxysporum* f. sp. *cepae* predominantly isolated from diseased bulb and root pieces and identified with the help of keys developed by various workers as stated in materials and methods. Fungus produced the fluffy white colonies on the surface of the medium. The microconidia are abundant, mostly non-septate, ellipsoidal or cylindrical, straight or curved. The macroconidia are fusiform, slightly curved, pointed at the tip, mostly three septate.

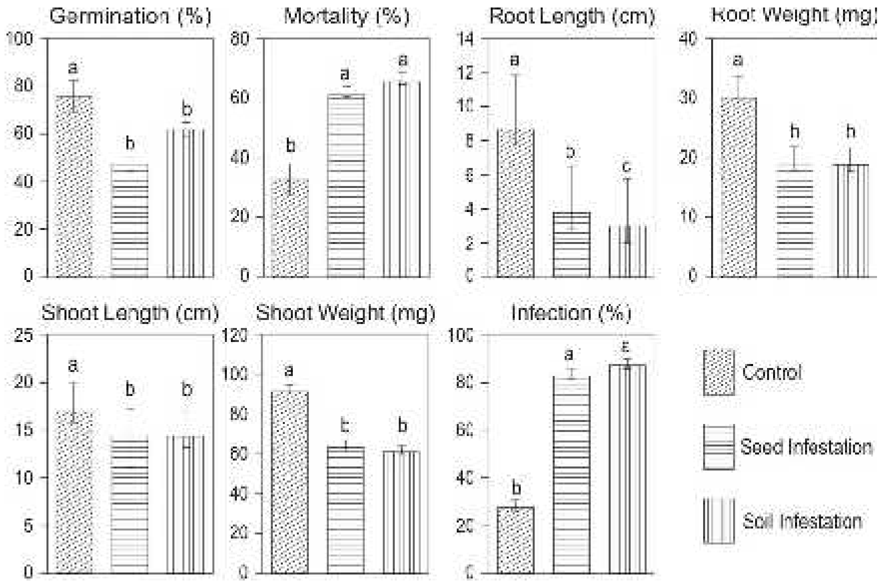

Figure 1. Effect of *F. oxysporum* on germination and plant mortality, root and shoot length, root and shoot weight and pathogen infection of onion plants. The data was statistically analyzed by using computer software “Statistix 8.1”. Bars with different letters indicating significant differences at $P<0.05$ between treatments. There were six replications of each treatment.

Pathogenicity test

Seedling infestation and soil infestation method were used to confirm the pathogenicity of the isolates. Both methods of inoculation showed a substantial impact on disease development and plant growth. Effect of *F. oxysporum* on germination and plant mortality, root and shoot length, root and shoot weight and root infection of onion plants are presented in Figure 1. Plant germination was significantly reduced in plants either inoculated by seedling infestation method or soil infestation method as compared to the un-inoculated plants (control). However, seedling infestation method caused maximum reduction in germination (53%), followed by soil infestation method (38%) as compared to control (24%). A similar trend was also observed in plant mortality, where highest plant mortality was recorded in soil infestation method (66.16%), followed by seedling infestation method (61.78%) as compared to control (32.8%). Significant differences were also observed between plant growth of inoculated and un-inoculated plants.

The plant growth parameters also significantly vary by method of inoculation used. Minimum root length and weight were recorded in soil infestation method followed by seedling infestation method as compared to control. Shoot length and weight significantly reduced in soil infestation method, followed by seedling infestation method as compared to control. Re-isolation of the inoculated fungus confirmed the pathogenic nature of the inoculated pathogen. *F. oxysporum* was re-isolated significantly in higher frequency in soil infestation method (87.78%) as compared to seedling infestation method (83.33%). However, the un-inoculated plants also showed some infection of *F. oxysporum* (9%), it could be either due to the seed-borne nature of the *F. oxysporum* or as a result of secondary infection from inoculated plants.

Table 1. Details of fungicides used in the experiment.

Trade name	Chemical name	Formulation	Chemical group	Mode of action
Antracol	Propineb	70% WP	Dithiocarbamate	Contact
Carbendazim	Carbendazim	50% WP	Benzimidazole	Systemic
Copper oxychloride	Copper oxychloride	50% WP	Copper compound	Contact
Kingmil MZ	Metalaxyl +Mancozeb	72% WP	Dithiocarbamates	Systemic

Table 2. Effect of different concentrations of fungicides on colony inhibition of *Fusarium oxysporum*. Values with different letters indicating significant differences at $P<0.05$ between treatments. There were six replication of each treatment.

Fungicides	Concentrations				
	1 ppm	10 ppm	100 ppm	1000 ppm	10000 ppm
Antracol	61.867g	67.022f	74.489e	81.822d	87.022b
Carbendazim	74.578e	80.440d	84.489c	93.6446b	98.667a
Copper oxychloride	34.622i	36.533k	46.11i	47.111i	56.756h
Kingmil MZ	19.556n	20.889n	24.000m	35.556kl	38.880j

Effect of different fungicides on inhibition of *F. oxysporum*

It is remarkably noticed that all the treatments tend to significantly inhibit the *F. oxysporum* growth *in-vitro.* Generally, higher concentrations of fungicides were more effective than the medium and lower concentrations. A positive correlation was observed between concentrations of fungicides used and resulting inhibition of fungal growth, means growth inhibition was gradually increased with an increasing concentration. The most effective treatment was 10000 ppm Carbendazim which provide maximum inhibition of targeted pathogen (98.67%), followed by Carbendazim 1000 ppm (93.64%) and Antracol 10000 ppm (87.02%) (Table 2). Among four fungicides, Carbendazim, followed by Antracol appeared as the most effective fungicides, which considerably reduced the fungal growth as compared to the other fungicides. On the other hand, Kingmil MZ and Copper oxycholoride were appearing as the slightly or completely ineffective fungicides, as targeted pathogen successfully grown even in the higher concentrations of these fungicides (Table 2).

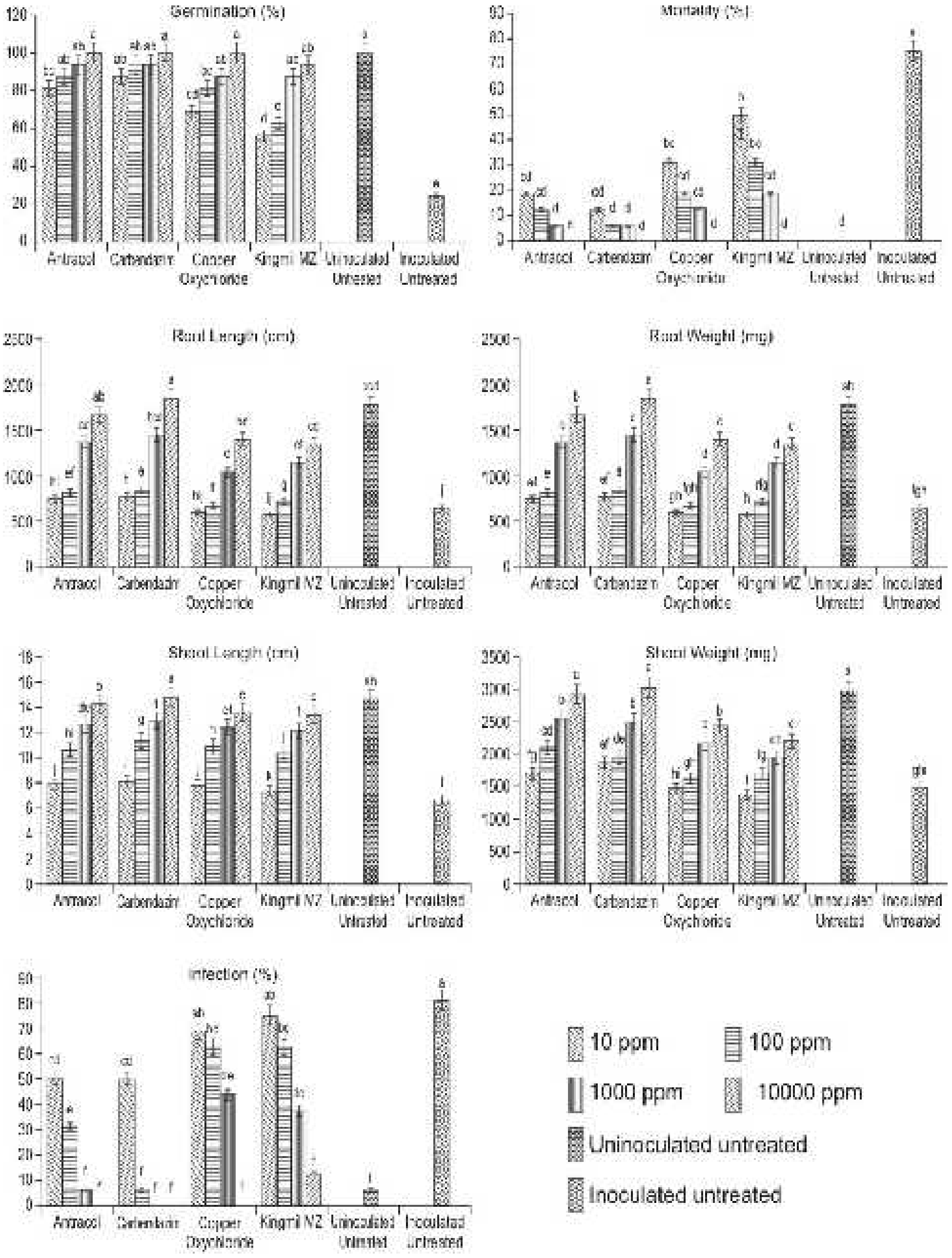

Figure 2. Effect of different concentration of fungicides on germination, mortality, root length and weight, shoot length and weight and infection on seedlings of onions inoculated with *Fusarium oxysporum.* Bars with different letters indicating significant differences at $P<0.05$ between treatments. There were six replications of each treatment.

Effect of fungicides on basal rot of onion

A pot experiment was carried out to evaluate the effect of different fungicides on plant growth and disease development on onion plants inoculated with *F. oxysporum.* The observations were recorded on germination (%), infection (%), plant growth (root length, shoot length, root weight and shoot weight) and plant mortality (%) of treated and un-treated onion plants presented in Figure 2. The most effective fungicide treatments which produced highest seedling emergence (100%) in the presence of inoculated pathogen were Antracol 10000 ppm, Carbendazim 10000 ppm and Copper oxychloride 10000 ppm, followed by Antacol 1000 ppm, Carbendazim 1000 ppm and Carbendazim 100 ppm, Kingmil MZ 1000 ppm (93.75%), Antracol 100 ppm (87.5%), Carbendazim 10 ppm (87.5%), Copper oxychloride 1000 ppm (87.5%) and (Figure 2). Among four fungicides, relatively all concentrations of the Carbendazim were more effective than corresponding concentrations of the other fungicides followed by Antracol.

Significantly maximum seedling mortality was recorded in case of untreated plants inoculated with *F. oxysporum* (75%), followed by Kingmil MZ 10 ppm (50%).The highest concentration (10000 ppm) of Antracol, Carbendazim, Copper oxycholoride and Kingmil MZ were appeared as highly effective as no plant mortality was occurred in treated plants.

Significant differences were observed between root length and weight of treated and untreated (control) plants inoculated with *F. oxysporum*. Moreover, significant differences were recorded between different concentrations of used fungicides. It appears that root length and weight were gradually increased with an increasing dose of each fungicide. Maximum root length and weight were recorded in case of 10000 ppm of Carbendazim (7.87 cm and 1850.0 mg)**.** Similar patterns of root growth was observed in shoot length and weight. Root infection was decreased in treated onion plants artificially infested with *F. oxysporum* f. sp. *cepea* by using the fungicide. No seedling infection was recorded in highest dose (10000 ppm) of used fungicides. Maximum seedling infection was recorded in untreated, but inoculated plants (81.25%) followed by the plants treated with Kingmil MZ 10 ppm (75.00%), Copper oxychloride 10 ppm (68.75%), Copper oxychloride 100 ppm and Kingmil MZ 100 ppm (62.5%).

DISCUSSION

Diseased plants show chlorotic, stunted and dying back symptoms as described by Dlahaut and Stevenson (2004). Stem plate discolouration and basal rot of the bulb were also apparent. In isolation attempt of disease causing organism, *F. oxysporum* f. sp. *cepae* was isolated predominately. The present study also confirms that it was the aggressive pathogen of onion caused characteristics symptoms of basal rot disease. Guillermo and Galván (2009), Bayraktar and Dolar (2010), Chandel and Deepika (2010) also reported the pathogenic nature of this pathogen.

Four different fungicides Antracol, Carbendazim, Copper oxychloride and Kingmil MZ were used to see their effect on the inhibition of *F. oxysporum* with four different concentrations by food poisoning method. Generally, higher concentrations of fungicides were more effective than the medium and lower concentrations. The most effective treatment was 10000 ppm Carbendazim, which provided maximum inhibition of targeted pathogen, followed by 1000 ppm of Carbendazim and Antracol. Ding *et al.* (2002) applied fungicides against *F. oxysporum* and *Rhizoctonia solani* and Carbendazim, had found the best inhibiting effects on both pathogens. Similarly, Song *et al.* (2004) and Rajput *et al.* (2006) tested Carbendazim and others for their inhibitory activities against the wilt pathogen *F. oxysporum.* Similarly, Kyada and Parakhia (2011) tested systemic and non-systemic fungicides *in-vitro* and found that Carbendazim and Thiophanate methyl were equally effective and completely inhibited the growth of the fungus. All the used fungicide at its used concentration significantly control the disease development on onion plants inoculated with *F. oxysporum.* It was also observed that seedlings emergence and plant growth of the treated plants were positively correlated with fungicide doses, in contrast to root infection and plant morality, which negatively correlated with fungicide doses. In general, the most effective fungicides are Carbendazim, followed by Antracol which significantly checked pathogen infection, thereby enhanced the plant growth in treating plants. Ozer and Koycu (2004) evaluated Benomyl, Thiram, Prochloraz and Tebuconazole for their effectiveness against *F. oxysporum* on seed and found that its application increased the emergence of seedlings and reduced the percentage of post-emergence damping-off when infested with *F. oxysporum*.

Infection percentage was decreased in treated onion plants artificially infested with *F. oxysporum* f. sp. *cepae* by using the fungicide. No seedling infection was recorded after the application of 10000 ppm of Antracol, Carbendazim, Copper oxychloride, and Kingmil MZ. Carbendazim 1000 ppm also inhibited the infection completely. Ashour *et al*. (1980) revealed that Benlate, Dithane A-40, Terraclor and Allisan reduced the relative activity of *F. oxysporum* f. sp. *cepae.* Similarly, Katiyar *et al.* (2001), Chandel and Deepika (2010) and Kagadi *et al.* (2002) observed that fungicides effectively controlled the disease compared with the control. Carbendazim increased the plant growth of cotton plants inoculated with *F. oxysporum* (Rajput *et al.,* 2006). The minimum disease intensity was recorded in a foliar application of Carbendazim closely followed by Carbendazim + Mancozeb. Carbendazim, Antracol and Copper oxychloride should be used for onion against basal rot disease caused by pathogenic fungus *F. oxysporum,* because these are found to be the best for better emergence of onion seedlings, plant growth (shoot, root and total plant length and weight) and to decrease disease infection and plant mortality.

CONCLUSION

Basal rot of onion caused by *Fusarium oxysporum* f.sp. cepae is one of the destructive disease of onion. Soil infestation method appears more effective in disease development as compared to the inoculation method. Basal rot of onion can be effectively controlled through fungicides such as carbendazim and antrocol. Fungicides are found to be better for plant growth and reduction in disease development.

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