IJARR, 7(7), 2022; 42-52

International Journal of Advanced Research and Review

www.ijarr.in

EVALUATION OF THE EFFICACY OF NATURAL COMPOUND AND CUPPER NANO PARTICLE COMPOUNDS IN CONTROLLING CERCOSPORA LEAF SPOT DISEASE OF SUGAR BEET

Asmaa M Alkolaly[1]*, Naser A. Ghazy[2] and Rodina A Hassan[3]

[1]Integrated Control Research Department, Plant pathology Research Institute, Agricultural Research Centre, 9 Gamaa street Giza 12619, Egypt. asmaa_alkollaly@yahoo.com
[2]Maiz and sugar crops diseases department, Plant pathology Research Institute, Agricultural Research Centre, 9 Gamaa street Giza 12619, Egypt
[3]Economic Entomology and Pesticides Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

ABSTRACT
In an attempt to search for alternative to fungicides, certain new natural formulation (Plco-free) and Copper nanoparticles (CuNps) beside Montoro 30% (difencoconazole + propiconazol) fungicide were evaluated against CercosporabeticolaSacc, the causative fungus of leaf spot disease of sugar-beet (*Beta vulgaris var. saccharifera*, L.) under field conditions. Also, effect of the tested materials on some parameters of sugar-beet *e.g.* root fresh weight, soluble solid content(T.S.S), Chl.a, Chl.b, leaf temperature ,flow transpiration, Carbon dioxide rates, diffusive resistance, conductive coefficient and rate of pushing juice were estimated. The results showed that the biocontrol formulation was the most effective treatments against the leaf spot of sugar-beet followed by Montoro and CuNps respectively in both growing seasons. Assayed materials represent potential effective alternatives for fungicides in controlling the leaf spot of sugar-beet.

Key words: Sugar beet**,** CuNps compounds, Leaf spot, Sugar-beet, Essential oils

INTRODUCTION
Lately, Sugar beet (*Beta vulgaris var.* saccharifera, L.) became the first crop for sugar production in Egypt in 2012, preceding sugar cane. In 2019, it contributed to the production of 62.2% of the total sugar yield, which reached to 2.458 million tons. Cercospora leaf spot disease, incited by *Cercospora baticola* fungus, where the most world widespread foliar disease of sugar beet (Holtschulte, 2000, Weil and Koch, 2004), this disease cause a serious reduction of 42% in sugar yield. The fungus spreads quickly from one region to another in the same country. Accordingly, it causes high losses in root and extractable sucrose yields, and increases impurity concentrations, resulting in higher processing losses (Lamey *et al*., 1987). Sugar beet cultivation in Egypt is vital for the sugar industry’s main goal since its natural properties of salinity tolerance and ability to thrive in a desert climate make it the main choice for sugar production Hager El-Zayat 2021. In the absence of appropriate control measures in the areas with a high disease incidence, severe epidemics of CLS can result in a significant reduction of root yield, recoverable sugar, sucrose concentration and an increase in impurities leading to higher processing costs. During the last few decades, the nanotechnology has evolved very rapidly, and nanoparticles are involved in most of our life branches from electronic industries, medicine, to

kitchenware (Filipina and Sutherland, 2013).Ingle, *et al*., (2013).Nanotechnology is a vital branch of science branches, as it is involved in solving many problems in many fields; many types of nanoparticles were manufactured and used in different fields for different applications. Due to the large surface area to volume ratio, copper nanoparticles have been used as an antimicrobial in many biomedical applications. And in agricultural fields, copper nanoparticles have been used as an antifungal that causes plant diseases. Natesan, *et al*., (2021). Reported that growth of *Poriahypolateritia* the causal pathogen of red root-rothypolateritia and *Phomopsistheae* which cause Phomopsis canker diseases of tea was inhibited by Copper nanoparticles. Also Copper nanoparticles reduced the spore germination and mycelial growth of *Cercospora coffeicola* (Natesan, *et al*., (2022). Highly significantly effect in suppressing Bird's eye spot disease of teawith higher stability in releasing the antagonistic activity during sporadic disease incidence comparing with control (Gnanamangai, *et al*., 2017). Antifungal activity of copper (Cu) nanoparticles also well reported against *Fusarium sp.* (Viet, *et al.*, 2016). Copper nanoparticles have the antifungal activity which differs for each fungal species; where induce strong morphological changes in the mycelium of *Fusarium solani*, *Neofusicoccum* sp., and *Fusarium oxysporum*, the damage of the cell membranes of the pathogens was revealed by microscopic observations with the three evaluated fungi (Pariona, 2019).Copper compound has the ability to suppress spores germination and decay the spores and conidia of fungus (Agrios, 2005). Copper play important and essential roles in plants, which play a role in functioning as cofactors or activators of enzyme systems. Many enzymes play pivotal roles in disease resistance in the production of defense barriers (Datnoff et al., 2007). Foliar spraying of copper was reduced i.e. brown spot (*Drechsleraoryzae*) and sheath blight (*Rhizoctonia solani*) diseases of rice. This effect is believed due to the biocidal effect of copper and the increase of the physiological properties of the plants itself, such as better lignifications and fortified cell membrane which is the primary defense mechanism to build up plants resistance (Liew et al., 2012).Copper sulfate and Montoro 30% fungicide were evaluated against *Cercospora* leaf spot disease on sugar beet under field condition. Foliar applications were significantly reduced the disease severity % of *Cercosporabeticola* fungi compared to control treatment (Ghazy, *et al*., 2020).Copper fungicides were used on large scale. 1882 noticed that leaves of diseased grape vines were retained through the season when sprayed with mixture of copper sulfate and lime. (Johnson, 1935), (Morton and Staub, 2008). Sulfur and copper are protective fungicides, the only non-organic fungicides allowed under organic agriculture guidelines (Letourneau and Van Bruggen, 2006).In 2012, different copper fungicides were recommended in Egypt to control different plant diseases and their classified according to active ingredient copper hydroxide, copper oxychloride, copper oxide and copper sulfate. Also, there were many other mixed fungicides in which copper was mixed with one or more other systemic active ingredient. (Kasana, *et al*., 2016). Due to the short shelf-life and low toxicity to the environment, the Essential oils (EOs) were representing a new class of crop protectants (Djordjevic, *et al*., 2013).In addition, the probabilities of creating new resistant strains by using essential oils as fungicidal agents are low since their constituents can act as synergists (Jobling, 2000). Usually, phenols, alcohols, ethers, carbohydrates, aldehydes and ketones are the main constituents of essential oils, which are responsible for the biological activity as well as for their fragrance (Bahraminejad *et al*., 2016). In fact in recent years, researchers have reported many mono- and sesquiterpene hydrocarbons as inhibitors of microbial pathogens (Cakir, *et al*., 2004 **)**Compounds such as carvacrol, thymol, linalool, cymene, pinene are known to exhibit antimicrobial activity (Knobloch, *et al*., 1986, Juven, *et al*., 1994, Harborne and Williams 1995, Cimanga, *et al*., 2002.)

These are the main components of essential oils with hopeful antifungal applications. There are some reviews on antifungal activity of plant extracts, generally structured according to the botanical family of plant species source of the active Eos (Tabassum and Vidyasagar 2013), or to the active compounds of plant extracts (Martínez, 2012).

The objective of the present study was to evaluate the efficacy of new natural fungicide and copper nanoparticles compounds against *Cercospora beticola*, Also, to investigate the efficacy of those compounds on sugar beet yield characters with respect to root fresh weight, soluble solid content, Chl.a and Chl.b, Leaf temperature, Flow Transpiration, Carbon dioxide rates, Diffusive resistance, conductive coefficient and rate of pushing juice.

MATERIAL AND METHODS

This work was carried out at Sakha Agricultural Research Station (latitude of 31.100 N and longitude 30.930 E, at an elevation of 14 m above sea level) Kafr El-Sheikh Governorate, Egypt in two growing seasons 2020/2021 and 2021/2022. A randomized complete block design with three replicates was done. The Plot area was about 18 m^2, including 5 rows of 6.0 m long and 60 cm in width, with 20cm apart between hills. Some materials were sprayed on beet tops to evaluate their influence in controlling cercospora leaf spot disease. Plco free (natural fungicide), copper nanoparticles compound CuNPs and fungicide Montoro 30%) as shown in Table 1.Were applied at 80, 95 and 110 days from sowing, when disease symptoms were detected. Untreated plots were left as control. Phosphorous was added in the form of superphosphate (15%) at the rate of 30 kg P2O5/fed during seedbed preparation. Nitrogen fertilizer was applied at 90 unit N/fed as ammonium nitrate (33.5% N) in two equal doses; the 1st was applied after thinning (4 true leaf stage) and one month later as recommended by the ministry of agriculture and land reclamation, Egypt. Multi-germ sugar beet variety viz" Oscarpoly" was sown in the 2nd week of September in the growing season, it is susceptible to Cercospora leaf spot disease. While harvesting took place at age of 210 days after sowing in both seasons. All recommended cultural practices were performed in both treated and untreated (control) plots.

Table 1: Fungicide, natural fungicide and nanoparticles compound

FUNGICIDES	RATE	ACTIVE INGREDIENT	FORMULATION
Montoro	50 cm/100 L water	Difenoconazole + propiconazole	30% EC
Plco-free	1L/300 L water	copper + natural oils	8% EC
Copper Nano Particle (CuNPs)	10 gm/100 L	copper sulphate pentahydrate	80% W.P

Copper nanoparticles (CuNPs) sample tested in this work was kindly donated by Prof. Dr., Mahmoud AbdElhalim AbdElgawad; Prof. of Nanotechnology and Head of Chemical Engineering Department, Faculty of Engineering-Elmenia Univ. Examination of physicochemical properties of the tested copper nanoparticles was performedin the Nanotechnology & Advanced Material Central Lab. (NAMCL), Agriculture Research Center

(ARC). Plco-free is a natural fungicide was obtained from Pure Land Fertilizers Company, Egypt.

1. **Disease severity%** ranged from 0 to 9 (death of older leaves and leaf spot progression to inner leaves) as a modified scale by Shane and Teng (1992). After15 days of the last treatment disease severity were recorded.

2. **Efficacy %** was determined according to the following equation

$$\text{Efficacy \%} = \frac{C-T}{C} x100$$

Where C = Control, T = Treatment

Physiological parameters

1-Total soluble solids (T.S.S. %) were estimated in fresh roots of sugar beet after 135 days from planting. Using a hand refract meter according to McGinnis (1982).

2-Root Weight Kg (10 roots)

3-Chl.a and Chl.b, Leaf temperature [leaf temp. = (˚C)], Flow = (Cm s), Transpiration [Trans. = (µg cm-2 S-1)], Carbon dioxide rates, Diffusive resistance [Diff. Res. = (S cm-1)], the rate of pushing juice, conductive coefficient were measured using a portable steady-state promoter (Publication No. 8210-0030, Serial No. SSP 275 and above, Copyright 1989. LI.COR, Inc.) on fourth leaves from two randomly selected plants in each plot during the mid-day period, and in the absence of cloud cover. Air temperature ranged from 18.0 to 22.0 °C at the time of measuring

Statistical analysis

Disease severity and physical characteristics were statistically analyzed as a complete randomized block design and multiple F test (Duncan, 1955), using Web Agri. Stat Package Computer Program (WASP).

RESULTS

Geochemical Instrumentation and Analysis

Examination of physicochemical properties of the tested copper nanoparticles using a Transmission Electron Microscope (TEM) and X-Ray Diffraction (XRD) revealed: The sample is copper sulfate pentahydrate (Cu SO4 (H2 O)5) with the empirical formula: CuH10O9S with the following crystal1 graphic parameters:

Crystall system: anorthic, space group: P –l, space group number: 2, a (A): 6.113 4, b (A): 10.711 6, c(A): 5.958 4, Alpha (°): 82.376 0, Beta (°): 107.323 0 and Gamma (°): 102.5940,volume of cell (10^6 prn-^3): 3 62.5 6, Z: 2 . 0 0, RIR: 0.9 9, and nanoparticles size ranged from 9.61 to 22.6 nm with very high purity (fig.1 and 2)

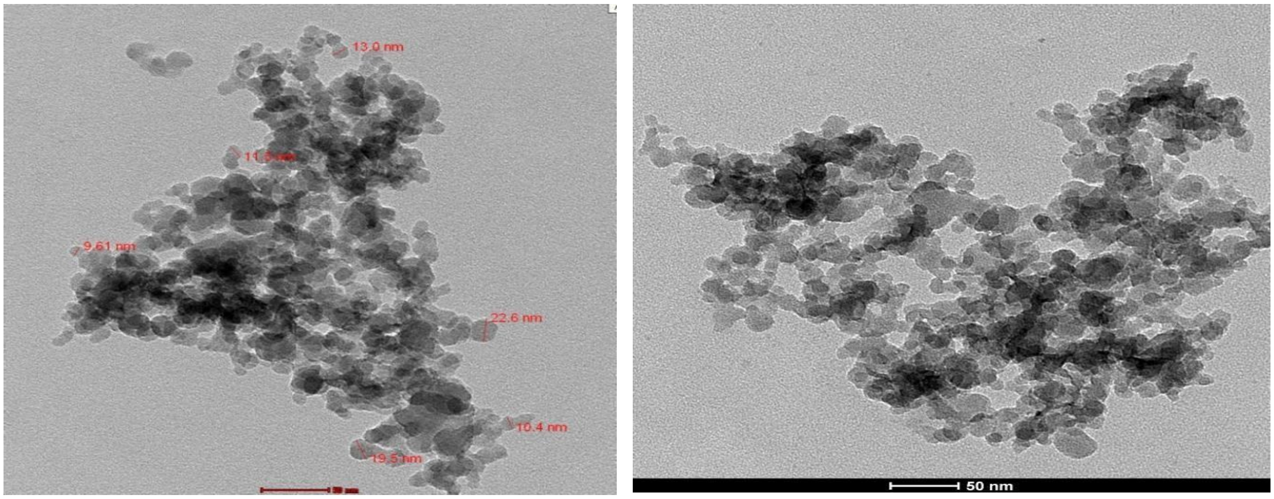

Fig. 1: TEM pictures show the geochemical properties of Cu nanoparticles, their Shape and size which ranged from 9.61 to 22.6 nm.

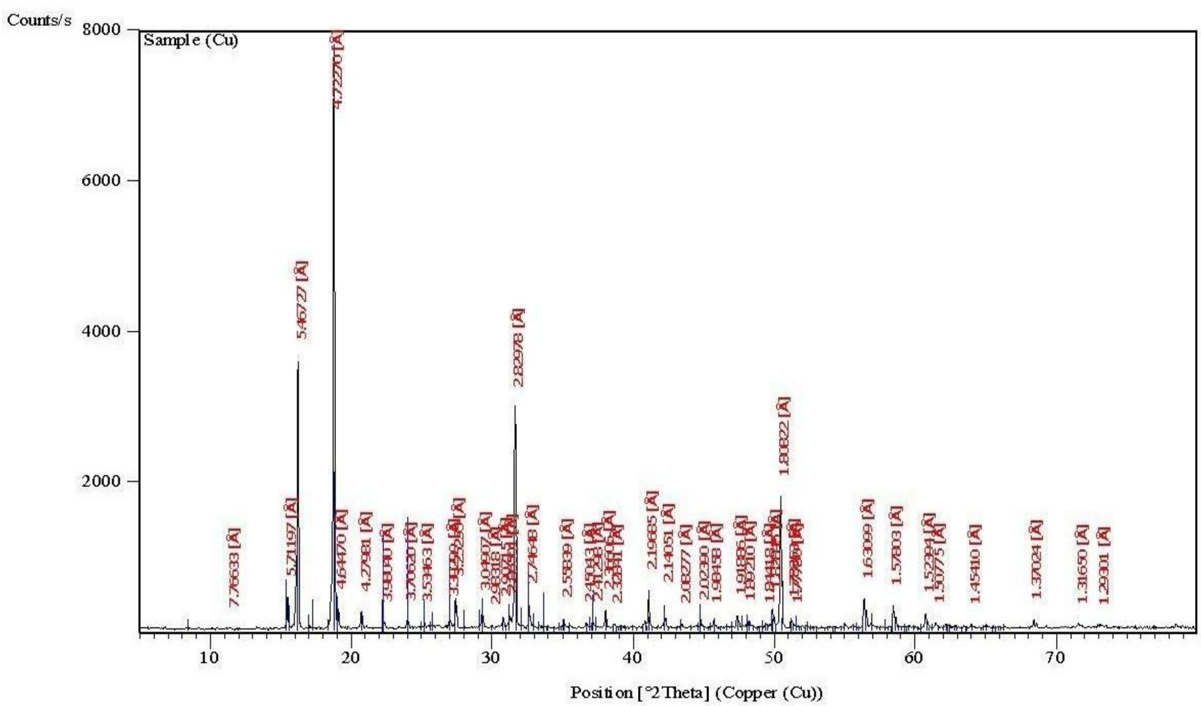

Fig. 2: XRD diagram shows Xr diffraction through Cu $SO_4(H_2O)_5$ nanoparticles crystals at the positions, 16, 19, 31.8 and 50.5 o2theta, absent of any other sharp pick confirms the high purity of the sample.

Field Experiment

A field trial was conducted under natural infestation with Cercospora leaf spot (CLS) in the Experimental field of Sakha agriculture research station, Kafr El-Sheikh Governorate, Egypt to

Evaluation the Efficacy of plco-free (natural component) and Cupper Nano Particles in Controlling Cercospora Leaf Spot Disease of Sugar Beet. Also, the efficacy of these treatments on yield characters with respect to root fresh weight, soluble solid content, the rate of pushing juice and conductive coefficient ,Chl.a and Chl.b, leaf temperature, flow transpiration, carbon dioxide rates and diffusive resistance were measured.

Data presented in Table (2).Show that fungicidal treatment caused a significant effect on the disease severity compared to control. Generally, this is correct in both seasons of experimentation. Significant differences in reducing the disease severity were found between treatments. Montoro gave the higher effect on CLS compared with the other treatments (Table 3). Plco free and CuNPs came after that fungicide. Whereas, it was observed that disease severity % was higher in the first season than in the second one.

Table (2): Effect of fungicides, natural fungicide and copper nano particle compound on Cercospora leaf spot under natural infestation during two growing seasons.

Treatments	%Disease severity		Efficacy%		Average of two seasons	
	2020 /2021	2021 /2022	2020 /2021	2021 /2022	% Disease severity	Efficacy%
MONTOR	2.16	1.66	87	89	1.88	88
Plco free	6.25	6	62.64	59	6.125	60.8
(CuNPs)	7.57	6.7	54.75	54.7	7.135	54.7
Control	16.73	14.8	/////////	//////////	15.8	//////
L.S.D 0.05	1.645	2.072				

Effect of fungicide, natural fungicide and (CuNPs) treatments on weight root, Total soluble solids (T.S.S. %)**,**chlorophyll a and b, Leaf temperature, Flow Transpiration, Carbon dioxide rates and Diffusive resistance of sugar beet plants:

Data presented in Tables (3&4) indicated that all treatments caused significant increase in root weight, %T.S.S and physiological parameters compared with the control. Except for chl.b, flow transpiration (F.T) and carbon dioxide rates were none significant with control measurements. Roots weight was higher with natural fungicide Plco free than fungicide Montoro and CuNPs in two seasons. Also other parameters were significant increase with Plco free in comparison with control and was close to fungicide Montoro and CuNPs despite the % efficacy of the fungicide Montoro was the best treatment in (table 2) but the physiological parameters, roots weight and % T.S.S were high with natural fungicide Plco free.

Table (3): Effect of fungicides, natural fungicide and copper nano particle compound on weight root, T.S.S. %,chl. a and b, leaf temperature (L.T.), flow transpiration (F.T), carbon dioxide rates and Diffusive resistance (D.R)the rate of pushing juice (P.J) and conductive coefficient (D.Co.) measured in sugar beet plants in 2020 /2021.

Treatments	Chl. a	Chl. b	T.S. S	L.T.	F.T	Co_2	D.R	P.J	D.Co.	R. W. (10 roots)
Montoro	3.115	1.6	19	18	32	1.3	14.3	81	0.0085	15.8
Plco free	2.178	1.5	21	20.3	33.3	1.53	4	80.6	0.0084	17
CuNPs	3	1.5	19.5	20.6	32.5	1.16	1.6	63	0.0066	15.6
Control	2.210	1.160	17.5	16.3	31	1.35	3.5	33.6	0.0075	10.7
L.S.D 0.05	0.733	non	0.751	1.174	non	non	3.696	1.012	0.005	1.010

Table (4): Effect of fungicides, natural fungicide and copper nano particle compound on Sugar beet root weight, T.S.S %, Chl.a and b, Leaf temperature (L.T.), Flow Transpiration (F.T), Carbon dioxide rates and Diffusive resistance (D.R) the rate of pushing juice (P.J) and conductive coefficient (D.Co.) as affected by treatment for cercospora leaf spot, during 2021/2022 growing season.

Traetments	Ch.a	Ch.b	T.S.S	L.T.	F.T	Co_2	D.R	P.J	D.Co.	R.W. (10 roots)
Montoro	3.3	1.8	20	19	31.6	1.2	11.6	85	0.0077	16.5
Plco Free	2.9	1.7	22	20.4	35	1.5	4	85	0.0087	17.3
CuNPs	3.2	1.8	20.5	20	34	1.1	2.1	65	0.0060	16.3
Control	2.7	1.4	18.5	18.4	32	1.3	3.7	37	0.0078	12.4
L.S.D 0.05	0.254	non	0.150	non	non	non	0.810	5.259	non	0.835

DISCUSION

In the area of Sakha agriculture research station, KafrEl-Sheikh Governorate, Egypt, where the experimental fields were established, Cercospora leaf spot is the most important foliar disease of sugar beet. Disease epidemics usually initiate during December and terminate in the middle of February, when cooler temperatures arrest disease development. Disease severity in the experimental fields generally was high during all 2 seasons of the study. All three treatments Montoro, Plco free and CuNPs suppressed Cercospora leaf spot significantly compared with untreated plots. However, there were significant differences in efficacy among them. The most effective was Montoro in case of %disease severity but when physical parameters were measured Plco free (Natural fungicide) gave the best results in yield and T.S.S% and other parameters. The lipophilic or hydrophobic nature of many EOs components allows them to interact directly with the fungal membrane, resulting in the alteration of membrane properties including the fluidity. An active transport via trans-membrane pumps has not been yet demonstrated (John and Sons; 2011). A recent study based on RNA-Seq-transcriptomic analysis of the fungus *Fusarium oxysporum*, responding to thymol, shows that most of glycosphingolipid and sphingolipid metabolism-related fungal genes were down regulated upon this treatment, while genes involved in antioxidant activity, chitin biosynthesis, and cell wall modification were up-regulated. The authors propose that the thymol acts by disrupting fungal cell wall and cell membranes by increasing the production of ROS on the fungal cell surface as well as by blocking the fungal molecular genes necessary for cell wall immunization and cell membrane synthesis (Zhang *et al*.,2018)Those molecular data are in line with the results obtained by (Gao *et al*., 2016),showing that thymol strongly inhibited conidial production and hyphal growth on *Fusarium graminacearum* via inducing lipid peroxidation and disrupting ergosterol biosynthesis, which150 Potential of Essential Oils are essential for plasma membrane structure. A similar mechanism of action was observed on carvacrol and thymol acting against vineyard and wine spoilage yeast (Chavan and Santosh 2014).The antifungal properties of EOs and their constituents have been reported in different studies, most of which are due to suppression of fungal mycelial growth in vitro. The mycelium supports all fungal activity, from the spore germination to the formation of the fruiting body, and thus represents a good indicator of fungus survival. Studies with plants of the Lamiaceae family have positive results in the control of various phytopathogenic fungi. The essential oils of oregano (*Oreganumvulgare*) and thyme (*Thymus vulgaris*) were effective against *Aspergillus niger, A.flavus, A. ochraceus*, *F.oxysporum*, *F. solani, Penicillium sp.*, *Phytophthora infestans, Sclerotinia sclerotiorum, Rhizoctonia solani, B. cinerea, Monilinia fructicola, Rhizopus stolonifer, Sclerotium rolfsii, Macrophomina phaseolina*, and *Pythium* sp.(El-Mohamedy*et al.*, 2013),Results were showed in field experiments. Citral, methyl anthranilate, and nerol tested at the concentration of 5.0 ml/L reduced 78.1 and 80% of Cercospora (*Cercospora beticola*) and (*Alternaria tenuis)* in sugar beet, respectively (Yaheia et al., 2011). Nanotechnology has recently played a vital role in all fields, especially in the field of antimicrobial (Ingle, *et al*., 2013).Copper nanoparticles were suppression the growth of fungi which reduced the spore germination and mycelial growth of different fungi (Natesan, *et al*., 2021). Also led to strong morphological changes in mycellium and causes damage of the cell membranes of different spaces of *Fusarium* Spp. (Pariona, 2019).Copper compound has the ability to suppress spores germination and decay the spores and conidia of fungus (Agrios, 2005).Copper play a role in functioning as cofactors or activators of enzyme systems. Many enzymes play pivotal roles in disease resistance in the production of defense barriers (Datnoff et al., 2007). Biocidal effect of copper and was increase the physiological properties of the plants itself, such as better lignification and fortified cell membrane which is the primary defense mechanism to build up plants resistance (Liew et al., 2012).

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