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EFFICIENCY OF SOME COPPER COMPOUNDS AND BACTERICIDES IN CONTROLLING ANGULAR LEAF SPOT DISEASE OF CUCUMBER

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ABSTRACT

Angular leaf spot disease caused by *Pseudomonas syringae* pv. *lachrymans* is worldwide bacterial disease of cucumber. The present study was undertaken to control the disease by using some copper compounds such as copper hydroxide, copper oxychloride, copper sulphate and cuprous oxide, as well as some bactericides such as Beltanol-L (Qchinosol), Starnar 20% (Oxolinic acid) and Streptorol 21.3% (Streptomycin sulphate). Different concentrations of chemicals and bactericides were applied *in vitro* and *in vivo* to study their effect on the growth of *P.lachrymans* and reducing the disease in greenhouse. The inhibitory effect was determined as a minimum inhibitory concentration (MIC) and by using the disc diffusion method. The results revealed that chemicals and bactericides were differed in their effect on the bacterium. However, the bactericides effect were more effective than the copper compounds in reducing the bacterial growth. Starner 20% showed the largest inhibition zone (18mm) followed by streptorol 21.3% (14mm), while beltanol-L showed the lowest effect (13mm), with respect of copper compounds, cuprous oxide, showed largest inhibitory effect, followed by copper hydroxide and copper oxychloride respectively, while copper sulphate showed no effect on the bacterial growth. In greenhouse experiments, all chemicals used significantly reduced the disease incidence comparing with the control treatment. Starner 20% was the most effective followed by beltanol-L, copper hydroxide, streptorol 21.3% or copper oxychloride and cuprous oxide. While copper sulphate was the least effective compound in controlling the disease.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is considered an important worldwide fresh vegetable. In Egypt cucumber is grown in winter in greenhouse for both local consumption and exportation.

Angular leaf spot disease of cucmber caused by the bacterium *Pseudomonas syringae* pv. *lachrymans* was recorded as one of the most widely distributed disease in many countries including USA, Germany, Brazil, England, Hungary, USSR, Canada, India, China and Japan (Saleh and Korobko, 1981). In Egypt, El-Sadek *et al*. (1992) reported the disease on cucumber plants grown in greenhouse at El-Minia governorate. Galal *et al*., 2003, reported that the disease is found in some fields at El-Minia governorate.

Severin *et al*., 1973 and Umekawa & Watanabe, 1978, used hot water and dry heat in controlling the bacterium in seeds. In the same trend (Cholakov and Kona, 2004) used laser and gamma irradiation. Biological control by using *Bacillus subtilis, Pseudomonas fluorescens* and *Pseudomonas putida* (Abd El-Ghafar, 2000) was also applied for reducing the disease .

It is important to find an additional methods to control the disease that which may be effective and inexpensive. Spraying with bactericides are widely used for minimizing the disease in greenhouse and fields.

The effectiveness of copper sulphate for the control of both bacteria *Pseudomonas corrugata* and *Clavibacter michiganensis* on tomato were studied by Prishchepa and Zherdetskaya, 2004. The effectiveness of copper oxychloride on a number of bacterial diseases of cucumber was studied by (Cheng *et al*., 2000 and Chromova, 2004). The role of copper hydroxide and cuprous oxide on the disease severity was also studied by Marinescu, 1982a and b; Severin *et al*., 1990 and Mahmoud, 2002.

Marinescu, 1982b reported that *Pseudomons syringae* pv. *lachrymans* on cucumber seeds could be controlled by Terramycin (oxytetracycline) at 0.05%; Khlaif & Abu-Blan, 1994 found that bacterium was inhibited by Agrimycin 17% (streptomycin sulphate). *Acidovorx aveneae* subsp. *citrulli* on melon fruits decreased significantly after seed treatment with streptomycin+tetracycline 10% WP kasugamycin + copper oxychloride 81.3% WP (Cheng *et al*., 2000). In Egypt, watermelon showing hypocotyl rot disease symptoms caused by *Bacillus polymixa* was controlled by erythromycin (Abd Alla *et al*., 2001).

The present work was carried out to test the efficiency of some copper compounds and bactericides to inhibit the growth of *P. syringae* pv. *lachrymans in vitro*, and their effectiveness in controlling angular leaf spot disease of cucumber under greenhouse condition.

MATERIALS AND METHODS

Bacterial isolate of the causal organism

Isolate of *P. syringae* pv. *lachrymans* (Psl 517) obtained from the Bacterial Disease Dept. Plant pathology Res. Institute, ARC, Giza, Egypt was used in this investigation. The isolate was grown in King's B broth at 28ºC for 48 hours and optically standardized to gives 10^8 cfu/ml. The inoculum was prepared for leaves inoculation of cucumber plants.

Seeds and sowing

Cucumber seeds (cv. Greendew F_1) were obtained from Horticulture Res. Institute, ARC, Giza, Egypt. Seeds were sown in pots 25cm diameter containing sandy loam soil for 4 weeks. They were watered regularly and kept under greenhouse condition.

Table(1):Chemical structure, common and trade names, formulations and rates of applications of the four tested copper compounds and the three bactericides used.

Common names	Trade names	Chemical structures	Concentration of formulations	Rates of application /100L
Cuprous oxide	Cupronox	Cu_2O	50% WP	150g
Copper oxychloride	Coppril	$CuCl_2$. 3 Cu $(OH)_2$	50% WP	250 g
Copper hydroxide	Kocide 2000	$Cu(OH)_2$	53.8%WP	180g
Copper sulphate	Del Cup 6%	$CuSO_4.5H_2O$	23.5% SL	250ml
Qchinosol	Beltanol-L	8-hydroxy qunoline sulphate	50% SL	200 ml
Streptomycin sulphate	Streptorol	Streptomycin sulphate	21.3% WP	50 g
Oxolinic acid	Starnar	Oxolinic acid	20% WP	150 g

Effect of copper compounds and bactericides on the growth of *Pseudomonas syringae* pv. *lachrymans, in vitro*

Different concentrations of copper compounds and bactericides Table (1) were tested for their effect on the growth of *Pseudomonas syrinage* pv. *lachrymans in vitro* the effectiveness of these compounds were screened by the following methods:

A- Determination of the minimum inhibitory concentration (MIC) of the copper compounds and bactericides.

The MIC of the copper compounds and bactericides was determined by using the agar dilution method (**Hammer *et al*., 1999)**. Serial dilutions of each copper compound or bactericide ranged from 50 to 1500 (ppm) and mixed in King's B medium. The medium was poured in sterilized Petri dishes of 9cm diameters..

The surface of the media was dried prior inoculation with *P.lachrymans*, spot inoculation technique was used while 10µl of bacterial suspension was dropped and replicated three times. Dishes without copper compounds or bactericides were sowed as control. Inoculated plates were incubated at 28ºC for 48 hrs.

The MICs were determined as the lowest concentration of copper compounds or bactericides inhibiting the growth of the bacterium on the agar plates.

B- Filter paper disc method

Filter paper discs were used to test the efficacy of the antimicrobial activity of copper compounds and bactericides against growth of *P. lachrymans.* Agar plates of King's medium B were seeded by 0.1 ml *P. lachrymans,* standardized to give 10^8 cfu/ml. filter paper discs (Whatman No.1, 5 mm diameter) impregnated with copper compounds at the concentrations of 500, 1000 and 1500ppm or bactericides at the concentrations of 100, 200 and 3000 ppm were applied to agar surface in three replicates. The plates were incubated at 28ºC for 24 hours. The diameters of inhibition zones were measured in millimeter.

Effect of bactericides and copper compounds on the occurrence of the disease on cucumber plants

Cucumber plants were grown in 25 cm, pots containing sandy loam soil. Each pot contains three plants, three pots were used as replicates for each treatment. After 4 weeks the plants sprayed by different concentrations of bactericides or copper compounds 48 hours before leaf inoculation with *P. lachrymans* under mist condition. Data were determined after 7 days from inoculation.

Disease assessment

Disease severity was estimated after 7 days from inoculation by using a modified scale of Horsfall and Barrett (1945). The modified scale was ranged from 0 to5,. Where 0=no symptoms, 1= 1-6%, 2= 7-25%, 3= 26-75%, 4= 76-94% and 5= 95-100% of infected tissue.

The bactericidal efficiency was calculated according to the following equation developed by Fröhlich (1979).

$$E = \frac{C - t}{C} \times 100$$

Where : E = bactericidal efficiency
C= disease severity in control
t= disease severity in treatment

Experiments were designed by a completely randomize of factorial arrangement in three replicates while the statistical analysis of variance was done as outlined by Steel and Torrie (1980).

RESULTS

Effect of copper compounds and bactericides on the growth of *P. lachrymans, in vitro*

A- Determination of the minimum inhibitory concentration (MIC) of the copper compounds and bactericides

Data presented in Table (2) revealed that the MIC of bactericides were shown at low concentrations when compared with copper compounds. The effective concentrations of the tested bactericides were 100, 150 and 200 ppm for Starner 20%, Beltanol-L and Streptorol 21.3%, respectively. The effective concentrations of copper compounds were 1000 ppm for Cuprous oxide and 1500 ppm for Copper hydroxide, Copper oxychloride and Copper sulphate.

B- Filter paper disc method

Filter paper discs impregnated with copper compounds or bacteicides reduced the growth of *P.lachrymans* compared with the control (Table 3). The results indicate that the growth of the bacterium was significantly decreased by increasing the concentration of both copper compounds and bactericides. Bactericides were more effective against growth of the pathogen, than the copper compounds. The bactericides Starner 20% at 300 ppm showed the greatest inhibitory effect (18mm) against *P. lachrymans* followed by Streptorol 21.3%, at 300 ppm give 14 mm zone while the Beltanol-L at 300 ppm showed the lowest effect (13mm). Cuprous oxide showed the greatest inhibitory (20 mm) effect at 1500 ppm followed by copper hydroxide (18mm) and copper oxychloride (17mm) when used at the same concentration. Copper sulphate showed minimal inhibition zones when used at the same concentrations of the other tested copper compounds.

Table (2): Minimum inhibitory concentrations of the bactericides and copper compounds on the growth of *P. lachrymans*.

Concentrations (ppm) / Copper compounds and bactericides	50	100	150	200	250	500	1000	1500
Starner 20%	+	-	-	-	-	-	-	-
Beltanol-L	+	+	-	-	-	-	-	-
Streptorol 21.3%	+	+	+	-	-	-	-	-
Cuprous oxide	+	+	+	+	+	+	-	-
Copper hydroxide	+	+	+	+	+	+	+	-
Copper oxychloride	+	+	+	+	+	+	+	-
Copper sulphate	+	+	+	+	+	+	+	-

+ Bacterial growth.
- No bacterial growth.

Table (3): Effect of different concentrations of the bactericides and copper compounds on growth of *P. lachrymans* by using filter paper disc.

Concentrations (ppm) / Copper compounds and bactericides	Inhibition zone (mm)					
	Bactericides			Copper compounds		
	100	200	300	500	1000	1500
Starner 20%	7	11	18	-	-	-
Streptorol 21.3%	7	9	14	-	-	-
Beltanol-L	5	9	13	-	-	-
Cuprous oxide	-	-	-	7	15	20
Copper hydroxide	-	-	-	8	10	18
Copper oxychloride	-	-	-	7	10	17
Copper sulphate	-	-	-	5	5	5

Effect of bactericides and copper compounds on disease severity of angular leaf spot caused by *P.lachrymans*, under artificial inoculation.

Data in Table (4) showed that the three tested bactericides and four copper compounds significantly reduced the disease incidence as compared with the control treatment. They varied in their effect on disease reduction, with significant variations between them. Starner 20% showed to be the most effective bactericide on controlling the disease followed by beltanol-L, copper hydroxide, streptorol 21.3% or copper oxychloride and cuprous oxide. While copper sulphate was the least effective copper compound in controlling the disease.

Table (4): Effect of bactericides and copper compounds on disease severity of cucumber angular leaf spot caused by *P.lachrymans*, under artificial inoculation.

Bactericides and copper compounds	Dose g/L	Disease infection %	Efficiency of bactericides and copper compounds
Starner 20%	1.2	0.67	86.60
	1.5	0.33	93.40
	1.8	0.00	100.0
Beltanol-L	1.7	1.67	66.60
	2.0	1.00	80.00
	2.3	0.67	86.60
Streptorol 21.3%	0.2	2.67	46.60
	0.5	2.00	60.00
	0.8	1.67	66.60
Copper oxychloride	2.2	2.67	46.60
	2.5	2.00	60.00
	2.8	1.67	66.60
Cuprous oxide	2.2	2.67	46.60
	2.5	2.33	53.40
	2.8	2.00	60.00
Copper hydroxide	2.1	2.33	53.40
	1.8	2.00	60.00
	1.5	1.67	66.60
Copper sulphate	2.2	4.33	13.40
	2.5	3.67	26.60
	2.8	3.33	33.40
Control	0.0	5.00	
L.S.D at 5%		**0.784**	

DISCUSSION

The use of copper compounds and bactericides in controlling bacterial diseases as a disinfectant compounds were studied by a number of investigators; Cheng *et al*., 2000; Abd Alla *et al*., 2001; Mahmoud, 2002; Chromova, 2004 and Prishchepa & Zherdetskaya, 2004.

The represent study was undertaken to determine the effect of copper compounds and bactericides against the growth of *P.lachrymans*.

The results of this study revealed that the three tested bactericides and the four copper compounds presented had an antimicrobial effect against *P. syringae* pv. *lachrymans*. They inhibited the growth of the bacterium at concentrations ranged from 100-150 ppm for the bactericides and the concentrations ranged from 1000-1500 ppm for the copper compounds. The disc diffusion method, however, showed considerable variation between the tested chemicals in inhibiting the bacterial growth. Copper sulphate did not antimicrobial effect on the bacterial growth even at high concentration which reached 1500 ppm. In contrary, cuprous oxide showed the highest effect followed by copper hydroxide and copper oxychloride, respectively. The bactericides Starner 20% showed the highest effect on the bacterial growth *in vitro* followed by Streptorol 21.3% and Beltanol-L respectively. These results are in agreement with the results obtained by several investigators (Menkissoglu and Lindow, 1991; Khalif and Abu-Blan, 1994; Scheck and Pscheidt, 1998 and Mahmoud, 2002) they indicated that several antibiotics and certain fixed copper compounds, significantly reduced severity of

bacterial disease.

According to the mode of action of bactericides, starner 20% has high antibacterial activity against G-ve bacteria, thus it acts to DNA, resulting the inhibition of reproduction of DNA and the bacteria treated with starner 20% lose the activity of multiplication. So it is of great importance to make preventive application is recommended for achievement of its effective control (Tawfik *et al*., 2001) .

Regarding the effect of the copper compounds and the tested bactericides on growth of *P.lachrymans* and disease severity on cucumber plants under artificial inoculation, all tested compounds inhibited the growth of *P. lachrymans* and reduced the severity of the disease under artificial inoculation, compared with the control. A parallel increase in disease suppression was obvious when increasing the concentrations of each compound used in this investigation to control the bacterium *P.lachrymans*. Menkissoglu and Lindow (1991) reported that the concentration of Cu^{2-} on leaves depends on the equilibrium established with the complexed and soluble forms of copper. Cu^{2-} was shown to be the only form of copper toxic to bacterial cells either *in vitro* or on leaves, and no strong relationship was found between the total amount and the concentration.

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فعاليـة بعـض المركبـات النحاسـية والمبيـدات البكتيريـة فـى مقاومـة مـرض التبقـع البكتيرى الزاوى فى الخيار

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يعتبـر مـرض التبقـع البكتيـرى الـزاوى علـى أوراق الخيـار المتسـبب عـن البكتريـا بسيدوموناس سيرنجى لكريمانس أحد الأمراض البكتيرية الهامة. وتهدف هذه الدراسة إلى مكافحة المرض باستخدام بعض مركبات النحاس مثل هيدروكسيد النحاس، اوكسى كلور النحاس، كبريتات النحـاس وأكسـيد النحاسـوز بالإضـافة إلـى بعـض المبيـدات البكتيريـة مثـل بلتـانول –إل (كينوسـول) وستارنر ٢٠% (أوكسولينيك أسد) وستربتورول ٢١,٣% (ستربتوميسين سلفات) حيث استخدمت بتركيـزات مختلفـة لدراسـة تأثيرهـا علـى نمـو المسـبب المرضـى فـى المعمـل ومكافحـة المـرض فـى الصوبة. وبدارسة تأثيرها المثبط على المسبب المرضى على أساس قياس قيمة أقل تركيز مثبط وكذلك طريقة أقراص ورق الترشيح المشبع كان هناك اختلافاً كبيراً فى التأثير المثبط من مركب لآخـر. ومـع ذلـك اتضـح أن المبيـدات البكتيريـة كانـت أعلـى تـأثيراً مـن مركبـات النحـاس، وكـان للاستارنر ٢٠% أعلى تأثير (١٨مم) يليه ستربتورول ٢١,٣% (١٤مم) بينما أعطى بلتانول –إل أقـل تـأثير (١٣ مـم)، أمـا بالنسـبة لمركبـات النحـاس، كـان لأوكسـيد النحاسـوز أعلـى تـأثير يليـه هيدروكسيد النحاس وأكسى كلور النحاس على الترتيب، فى حين أن كبريتات النحاس لم يكن لها أى تأثير باستخدام هذه الطريقة. وفى تجارب الصوبة، كان لجميع المركبات المختبرة تأثير معنوى فى مقاومة المرض مقارنة بمعاملة الكنترول. ولقد اختلفت فى فاعليتها فى مقاومة المرض، حيث كان هنـاك اختلافـات معنويـة فيمـا بينهـا، حيـث كـان سـتارنر ٢٠% الأعلـى تـأثيراً يليـه بلتـانول-إل، ثم هيدروكسيد النحاس فـ ستربتو رول (٢١,٣%)، أو أوكسى كلور النحاس، وأوكسيد النحاسوز، بينما كان لكبريتات النحاس أقل تأثير فى هذا الشأن.