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Compatibility of *Bacillus subtilis* with selected antibiotics and fungicides commonly used in field crop

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Abstract
Nine antibiotics and fungicides *viz*. Streptomycin sulphate, Streptocycline, Chloramphenicol, Bactronol (Bromo - 2 – nitro propane -1,3 diol), Streptomycin sulphate + Copper oxychloride, Copper hydroxide, Validamycin, Copper oxychloride and Kasugamycin + Copper oxychloride were tested against *Bacillus subtilis in vitro*. The antibiotic sensitivity against thirty isolates was studied by paper disk technique. The Copper oxychloride + Streptomycin sulphate (each @ 100,250,500 ppm) was more effective in the form of zone of inhibition against phylloplane *Bacillus subtilis* isolate PBs6 and also the streptocycline (each @ 100,250,500 ppm) was more effective against rhizospheric *Bacillus subtilis* isolate RBs3.

Keywords: *Bacillus subtilis*, paper disk technique, antibiotic, fungicides

Introduction
Bacillus subtilis is very important bio-agent used for the management of plant diseases. *B subtilis* is a gram positive, motile, aerobic, rod shaped bacteria. It is a ubiquitous naturally occurring saprophytic bacterium that is commonly recovered from soil, water, air and decomposing plant material. Colony of *B. subtilis* is traditionally circular, with ragged edges, colored cream to white. It has ability to form a tough protective endospore, allowing the organism to tolerate extreme environmental conditions (Alexander, 1977) [1]. *Bacillus subtilis* is very effective against foliar diseases and it is becoming part of IDM. However, the research on management of diseases through use of *B. subtilis* limited and there is a need for development of information on bioefficacy of *B. subtilis* against foliar diseases. *Bacillus* is the most abundant genus in the rhizosphere of soil, are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion.
Biological management of plant pathogen by antagonistic microorganisms is a potential nonchemical means (Harman, 1991) [7] and is known to be a cheap, effective and eco-friendly method for management of crop disease (Cook and Baker, 1983) [6]. The use of bio-control agent as an alternative to chemical pesticides is increasing rapidly in the present day agriculture due to the deleterious effect of chemical pesticides. Though the bio-agents were effective but their compatibility need to be known with other pesticides like fungicides, insecticides, bactericides and nematicides which are used routinely in field crop cultivation for controlling many pests and diseases. The information on compatibility will help to (i) decide whether pesticide molecule can be mixed with or not, to reduce the cost incurred on individual spray (iii) to know deleterious effect of pesticide/disinfectant on survival and multiplication of bio-agents used. In this context current research has been focused on compatibility of bacterial bio-agents with commonly using agrochemicals in field crop cultivation.

Materials and Methods
Collection and isolation of *B. subtilis* isolates
Thirty isolates of *B. subtilis* were collected from different phylloplane and rhizosphere soil samples of cotton, soybean, pegionpea, paddy, green gram, sorghum, bean and chickpea crops of Vidarbha region of Maharashtra agro ecosystem by serial dilution technique on nutrient agar medium and designated as (PBs-1 to 15 and RBs-1-15) simultaneously.

Identification of the pathogen

The identification of the *Bacillus subtilis* was done as per available internals and by biochemical, morphological, cultural and physiological features of the pathogen as per standard microbiological procedures.

Preparation of bacterial culture

Thirty isolates of *B. subtilis* to be tested were inoculated on NA medium. The cultures were incubated cultures at 27 ± 2 ^{0}C for 3 to 5 days prior to inoculation. The 48 hrs old culture was used for the inoculation on NA medium.

***In vitro* efficacy of different antibiotics and fungicides against *Bacillus subtilis* by paper disc technique**

Sensitivity of the *Bacillus subtilis* isolates were tested by modified paper disc assay. The derived concentration of the antibiotics and fungicides *viz*. Streptomycin sulphate, Streptocycline, Chloramphenicol, Bactronol (bromo - 2 – nitro propane -1,3 diol), Streptomycin sulphate + Copper oxychloride, Copper hydroxide, Validamycin, Copper oxychloride and Kasugamycin + Copper oxychloride were freshly prepared in sterile distilled water. The bacterium *Bacillus subtilis* was multiplied by inoculating the loopful culture in 150 ml conical flask containing 50 ml of nutrient broth medium. The inoculated flasks were incubated at 27±2 ^{0}C for 72 h.

The 10ml of prepared bacterial suspension of each isolate was added to conical flask containing NA, when NA media get cooled and before to solidify the medium. The medium seeded with bacterial suspension was shaken well and immediately poured in sterilized Petri plates and allowed to solidify.

The concentrations of antibiotics and fungicides were prepared. The filter paper disc (Whatman No. 42) measuring 5 mm in diameter were prepared and sterilized before use. The sterilized filter paper discs were soaked in the respective concentrations of chemicals for five minutes and transferred onto the surface of the seeded medium in Petriplates. The plates were incubated at 27±2^{0}C for 72 hrs and observed for the production of inhibition zone around the filter paper discs. The paper discs soaked in sterile distilled water were served as control. The results thus obtained were analysed statistically.

Results and Discussion

Antibiotic sensitivity against *Bacillus subtilis* isolates by paper disc method

Antibiotic sensitivity against phylloplane *Bacillus subtilis* isolates

Antibiotic sensitivity is useful to assess the identification of the isolates, sensitive to particular antibiotics and fungicides.

The efficacy of antibiotics and fungicides was tested against fifteen isolates of phylloplane *Bacillus subtilis* (PBs1 to PBs15) by Paper disc method. Results indicated that the antibiotics and fungicides at various concentrations were significantly inhibited the growth of phylloplane *Bacillus subtilis* over untreated control.

Data presented in Table 1, revealed that, at lower concentration of 100 ppm, bacterial inhibition zone ranged from 0.00 to 12.66 mm. Zone of inhibition increased gradually with the increase of concentration of the antibiotics and fungicides. The highest zone of inhibition was observed in 500 ppm concentration. Among the antibiotics and fungicides tested COC+ Streptomycin sulphate (18.00 mm) in PBs6 followed by Streptocycline (17.33 mm) for PBs2 isolate. No inhibition zone was recorded in all the isolates of phylloplane *Bacillus subtilis* with Bactronol, Copper hydroxide, Conika (Copper oxychloride + Kasugamycin), Copper oxychloride and Validamycin (0.00 mm), respectively.

Antibiotic sensitivity against rhizospheric *Bacillus subtilis* isolates

The efficacy of antibiotics and fungicides was tested against fifteen isolates of rhizospheric *Bacillus subtilis* (RBs1 to RBs15) by Paper disc method. Results indicated that the antibiotics and fungicides at various concentrations were significantly inhibited the growth of rhizosphere *Bacillus subtilis* over untreated control.

Data presented in Table 2, revealed that, at 100 ppm, bacterial inhibition zone between from 0.00 to 12.67 mm. Zone of inhibition increased gradually with the increase of concentration of the antibiotics and fungicides. The highest zone of inhibition was observed in 500 ppm concentration. Among the antibiotics and fungicides tested, Streptocycline (16.66. mm) in RBs3 followed by COC+ Streptomycin sulphate (16.00 mm) for RBs2 isolate. Inhibition zone was not recorded in all the isolates of rhizospheric *Bacillus subtilis* with Bactronol, Copper hydroxide, Conika (Copper oxychloride + Kasugamycin), Copper oxychloride and Validamycin (0.00 mm), respectively.

Results of present study are in aggreement with the findings of Papavizas *et al*. (1981) [9] who reported that *Bacillus subtilis* was compatible with copper hydroxide (Kocide 3000) even at a high concentration of 300 ppm. Balakrishnan *et al*. (2003) [4] showed that all the *Bacillus* isolates were sensitive to tetracycline and chloramphenicol, two commonly used antibiotics in shrimp hatcheries and culture systems. Sarker *et al*. (2010) [10] studied the sensitivity of the *Bacillus thuringiensis* against ten different antibiotics. These strains are sensitive to streptomycin and chloramphenicol. Bautista *et al*. (2013) [5] studied the sensitivity of the Bt against ampicillin, amoxicillin, tetracycline, streptomycin and ofloxacin. The isolated strains of Bt were resistant to Blactams (amoxicillin and ampicillin). Valarmathi *et al*. (2013) [11] conducted the compatibility of copper hydroxide (Kocide 3000) with bacterial and fungal biocontrol agents under *in vitro* conditions. Bacterial biocontrol agents *viz*., *Pseudomonas fluorescens* and *Bacillus subtilis* were compatible with copper hydroxide (Kocide 3000) even at a high concentration of 300 ppm. Amruta Veena *et al*. (2014) [2] reported that out of 40 antagonistic bacteria isolated, 20 bacteria were obtained from rhizosphere soil and 20 from root as root endophytes in chickpea against dry root rot. In compatibility studies using spectrophotometric method, the isolate CREB-16 was more compatible with validamycin (84.13%) followed by copper oxychloride (78.27%). Avsar *et al*. (2017) [3] identified thirty-nine isolates as *Bacillus* spp. based on morphological and physiological properties. The isolates were 100% resistant to penicillin, rifampicine (66.6%), novobiocin, (23.7%), cefepime (48.7%), ceftazidime (87.1%), oxacillin (89.7%), streptomycin (2.5%), clindamycin (30.7%), tetracycline (7.6%), ampicillin/sulbactam (12.8%), gentamicin (56.4%), ceftriaxone (10.2%), polymyxin B (84.6%) and amikacin (12.8%). In addition, all the isolates were susceptible to imipenem, ciprofloxacin, meropenem and ofloxacin. Jayasudha *et al*. (2018) [8] did not find any growth of bacterial antagonistsin streptocyclin, K-cyclin and 2-bromo-2-nitropropane-1, 3-diol. Thus, present results of the investigations suggested the safe use of antibiotics *viz*.

Bactronol, Copper hydroxide, Conika (Copper oxychloride + Kasugamycin), Copper oxychloride and Validamycin with phylloplane and rhizosphere antagonistic *Bacillus subtilis* isolates obtained in this study for controlling foliar and soil borne fungal diseases.

Table 1: Sensitivity of different antibiotics and fungicides against phylloplane *Bacillus subtilis* isolates

Tre. No.	Treatments	Zone of inhibition (mm)								
		PBs1			PBs2			PBs3		
		100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm
T1	Streptomycin sulphate	10.00	13.00	16.66	10.33	12.00	15.67	11.66	14.00	16.00
T2	Streptocycline	8.33	10.66	13.33	12.33	15.00	18.00	11.00	14.33	17.33
T3	Copper oxychloride	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T4	COC + Streptomycin sulphate	5.66	10.66	14.66	10.00	13.66	16.00	11.00	13.33	18.00
T5	Bactronol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	Chloramphenicol	0.00	0.00	8.33	5.33	7.33	11.00	4.33	5.66	11.00
T7	Copper hydroxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	Kasugamycin + COC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T9	Validamycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T10	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F Test	-	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
SE (m) ±	-	0.20	0.20	0.18	0.15	0.25	0.24	0.25	0.22	0.28
CD (*P*=0.01)	-	0.94	0.94	0.84	0.73	1.20	1.12	1.20	1.02	1.34

Tre. No.	Treatments	Zone of inhibition (mm)											
		PBs4			PBs5			PBs6			PBs7		
		100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm
T1	Streptomycin sulphate	9.67	12.67	14.66	8.66	10.67	13.33	8.66	12.33	16.66	4.66	10.33	11.33
T2	Streptocycline	8.66	11.66	13.66	11.66	15.33	17.00	8.33	11.33	14.66	10.00	12.33	14.33
T3	Copper oxychloride	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T4	COC + Streptomycin sulphate	10.33	13.00	17.00	10.33	11.67	13.66	12.66	14.33	16.66	8.66	9.66	14.00
T5	Bactronol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	Chloramphenicol	4.66	7.00	12.33	5.66	7.00	8.66	5.33	6.67	9.33	6.00	6.66	9.33
T7	Copper hydroxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	Kasugamycin + COC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T9	Validamycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T10	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F Test	-	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
SE (m) ±	-	0.18	0.37	0.38	0.18	0.15	0.22	0.18	0.24	0.28	0.20	0.24	0.27
CD (*P*=0.01)	-	0.84	1.75	1.80	0.84	0.73	1.04	0.84	1.12	1.34	0.94	1.12	1.27

Tre. No.	Treatments	Zone of inhibition (mm)											
		PBs8			PBs9			PBs10			PBs11		
		100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm
T1	Streptomycin sulphate	7.33	12.66	16.00	0.00	8.00	13.66	8.66	12.00	17.33	4.33	5.00	5.67
T2	Streptocycline	10.33	13.66	16.33	8.66	13.33	15.00	10.00	13.33	15.33	7.33	8.67	9.66
T3	Copper oxychloride	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T4	COC+ Streptomycin sulphate	9.66	14.33	17.66	10.33	13.00	17.00	8.67	10.00	11.66	5.67	7.33	13.00
T5	Bactronol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	Chloramphenicol	0.00	6.00	9.66	5.00	6.00	8.33	5.00	6.33	9.66	0.00	5.00	8.66
T7	Copper hydroxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	Kasugamycin + COC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T9	Validamycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T10	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F Test	-	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
SE (m) ±	-	0.15	0.15	0.22	0.12	0.24	0.25	0.12	0.20	0.18	0.15	0.12	0.22
CD (*P*=0.01)	-	0.73	0.73	1.04	0.60	1.12	1.20	0.60	0.94	0.84	0.73	0.60	1.04

Tre. No.	Treatments	Zone of inhibition (mm)											
		PBs12			PBs13			PBs14			PBs15		
		100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm
T1	Streptomycin sulphate	8.33	11.33	13.33	4.67	7.33	13.66	6.00	7.67	11.00	10.00	12.33	14.67
T2	Streptocycline	5.67	7.333	9.67	4.66	13.00	15.66	5.67	9.33	11.00	5.33	8.67	11.00
T3	Copper oxychloride	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T4	COC + Streptomycin sulphate	9.67	11.33	15.67	8.33	9.66	14.00	8.67	9.67	16.00	10.33	14.33	15.00
T5	Bactronol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	Chloramphenicol	7.66	9.66	12.66	10.67	12.33	14.67	6.00	8.00	11.33	5.00	6.00	11.33

T7	Copper hydroxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	Kasugamycin + COC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T9	Validamycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T10	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F Test	-	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
SE (m) ±	-	0.18	0.18	0.18	0.24	0.22	0.22	0.20	0.15	0.28	0.20	0.15	0.30
CD (*P*=0.01)	-	0.84	0.84	0.84	1.12	1.04	1.04	0.94	0.73	1.34	0.94	0.73	1.40

Table 2: Sensitivity of different antibiotics and fungicides against rhizospheric *Bacillus subtilis* isolates

Tre. No.	Treatment	Zone of inhibition (mm)								
		RBs1			RBs2			RBs3		
		100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm
T1	Streptomycin sulphate	10.33	12.66	14.67	10.33	12.66	14.67	10.33	12.66	14.67
T2	Streptocycline	10.00	12.33	13.66	10.00	12.33	13.66	10.00	12.33	13.66
T3	Copper oxychloride	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T4	COC + Streptomycin sulphate	8.33	11.66	14.33	8.33	11.66	14.33	8.33	11.66	14.33
T5	Bactronol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	Chloramphenicol	0.00	5.00	9.00	0.00	5.00	9.00	0.00	5.00	9.00
T7	Copper hydroxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	Kasugamycin + COC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T9	Validamycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T10	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F Test	-	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
SE (m) ±	-	0.12	0.15	0.15	0.12	0.15	0.15	0.12	0.15	0.15
CD (*P*=0.01)	-	0.60	0.73	0.73	0.60	0.73	0.73	0.60	0.73	0.73

Tre. No.	Treatments	Zone of inhibition (mm)											
		RBs4			RBs5			RBs6			RBs7		
		100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm
T1	Streptomycin sulphate	5.00	6.00	8.67	9.33	11.33	13.67	8.67	12	14.67	8.33	11.33	13.66
T2	Streptocycline	11.33	13.00	14.67	8.33	10.33	12.33	9.33	11.33	12.66	10.33	12.66	14.33
T3	Copper oxychloride	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T4	COC + Streptomycin sulphate	9.33	12.67	14.67	9.00	11.33	13.33	10.33	12.67	15.33	8.67	11.67	12.67
T5	Bactronol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	Chloramphenicol	5.00	6.00	8.33	5.00	6.00	7.00	5.00	6.00	8.00	0.00	5.00	7.00
T7	Copper hydroxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	Kasugamycin + COC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T9	Validamycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T10	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F Test	-	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
SE (m) ±	-	0.12	0.24	0.18	0.12	0.22	0.15	0.15	0.25	0.15	0.15	0.22	0.15
CD (*P*=0.01)	-	0.60	1.12	0.84	0.60	1.04	0.73	0.73	1.20	0.73	0.73	1.04	0.73

Tre. No.	Treatment	Zone of inhibition (mm)											
		RBs8			RBs9			RBs10			RBs11		
		100	250	500	100	250	500	100	250	500	100	250	500
T1	Streptomycin sulphate	8.67	12.67	14.67	8.33	11.67	14.66	8.67	13.33	14.00	10.33	13.66	15.66
T2	Streptocycline	5.67	10.33	13.33	9.33	11.33	14.67	9.67	12.33	14.00	5.33	8.66	10.33
T3	Copper oxychloride	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T4	COC + Streptomycin sulphate	0.00	8.00	9.00	9.00	12.67	15.33	9.33	12.67	15.00	9.33	11.33	13.33
T5	Bactronol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	Chloramphenicol	5.00	7.00	9.33	5.00	6.00	8.00	5.00	6.33	10.00	0.00	5.00	6.00
T7	Copper hydroxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	Kasugamycin + COC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T9	Validamycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T10	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F Test	-	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
SE (m) ±	-	0.12	0.12	0.15	0.12	0.22	0.15	0.22	0.18	0.18	0.15	0.15	0.15
CD (*P*=0.01)	-	0.60	0.60	0.73	0.60	1.04	0.73	1.04	0.84	0.84	0.73	0.73	0.73

Tr. No.	Antibiotics	Zone of inhibition (mm)											
		RBs12			RBs13			RBs14			RBs15		
		100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm
T1	Streptomycin sulphate	10.66	13.33	15.33	10.67	13.00	15.33	5.33	7.33	9.67	5.00	6.00	7.67
T2	Streptocycline	7.33	8.00	10.33	11.33	12.66	14.33	10.67	13.00	14.00	5.33	7.00	8.00

T3	Copper oxychloride	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T4	COC + Streptomycin sulphate	10.33	12.00	14.67	9.33	9.33	13.67	6.00	8.67	9.67	9.00	11.33	12.67
T5	Bactronol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6	Chloramphenicol	0.00	5.00	6.00	0.00	0.00	6.00	0.00	5.00	6.00	5.00	6.00	8.00
T7	Copper hydroxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	Kasugamycin + COC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T9	Validamycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T10	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F Test	-	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
SE (m) ±	-	0.15	0.18	0.15	0.22	0.20	0.15	0.20	0.12	0.12	0.09	0.18	0.12
CD (*P*=0.01)	-	0.73	0.84	0.73	1.04	0.94	0.73	0.94	0.60	0.60	0.42	0.84	0.60

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