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Research Article

In vitro evaluation of antibiotic performances on *Trichoderma harzianum* and some crop infecting fungi

Md. Moshiur Rahman Akonda*, Raihan Mujib Himel, Mohammad Ali and Md. Syeful Islam

Division of plant pathology, Bangladesh Tea Research Institute, Bangladesh

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Corresponding Author
Md. Moshiur Rahman Akonda
moshiur.ado@gmail.com

Abstract

An *in vitro* experiment was carried out to evaluate the effectiveness of antibiotics at different concentrations on growth and development of *Trichoderma harzianum*, *Phytophthora infestans*, *Colletotrichum gloeosporioides*, *Corticium theae* and *Fusarium oxysporum* found in tea plantation. Three samples viz. sample-1 (Validamycin 60% w/w) @ 45, 60 and 75 ppm, sample-2 (Hexaconazole 2.5% w/w+Validamycin 8.5% w/w) @ 55, 82.5 and 110 ppm and sample-3 (Streptomycin 9% w/w+Tetracyclin hydrochloride 1% w/w) @ 50, 75 and 100 ppm were tested. The result showed that Antibiotics have inhibitory effects on *T. harzianum*. Unsatisfactory performances in terms of per cent growth inhibition (<80) were recorded on crop infecting fungi. *C. theae* treated with sample-1 @ 75 ppm and *C. gloeosporioides* with sample-2 @ 110 ppm had shown maximum 25.50 and 54.19 per cent growth inhibition, respectively. The highest 70.53 per cent growth inhibition of *C. theae* was observed in sample-3 treated @ 100 ppm. Considering the findings it can be recommended not to use above antibiotics with their respective concentrations in plant agriculture for controlling diseases caused by the said fungi.

Keywords
Antibiotic; Performance; Growth inhibition; Fungi; Tea; Agriculture

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Introduction

In 1928, Alexander Fleming, a Scottish scientist, identified penicillin, the first chemical compound with antibiotic properties derived from *Penicillium* fungi (Wikipedia, 2016). In 1942, Selman Waksman and his collaborators in journal articles first used the term *antibiotic* to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution (Waksman, 1947). Since then the role of antibiotics has expanded from treating serious infections to preventing infections in surgical patients, protecting cancer patients and people with compromised immune systems, promoting growth and preventing disease in livestock and other food animals, including their uses in plant agriculture for controlling diseases (Gelband *et al.*, 2015).

In plant agriculture, several antibiotics have been commercialized for controlling bacterial diseases, especially fire blight of pear and apple caused by *Erwinia amylovora* and bacterial spot of peach. In USA, 90% of the streptomycin has been using in plant agriculture for control of fire blight (McManus *et al.*, 2002). Streptomycin is currently also registered for fire blight control in Israel, New Zealand, Canada and Mexico. It has been permitted on an emergency use basis, subject to annual review and under tightly restricted conditions in Germany, Austria and Switzerland. Oxytetracycline is used in Mexico and Central America to control *E. amylovora* on apple, and diseases caused by *Pectobacterium* spp., *Pseudomonas* spp. and *Xanthomonas* spp. on several vegetable crops. Gentamicin is also used in Mexico and Central America to control various bacterial diseases of vegetable crops caused by species of *Pectobacterium*, *Pseudomonas*, *Ralstonia*, and *Xanthomonas* (Vidaver, 2002). In the 1990s, an attempt to register gentamicin for plant agriculture in the USA was contested, owing to its clinical importance, and the application was withdrawn without prejudice (McManus *et al*., 2002). Oxolinic acid, a synthetic quinolone antibiotic, is used only in Israel to manage fire blight of pear and related plants, especially in areas where *E. amylovora* is resistant to streptomycin (Shtienberg *et al*., 2001). Oxolinic acid also is registered in Japan for management of bacterial panicle blight of rice, caused by *Burkholderia glumae* (Maeda *et al*., 2004; Nandakumar *et al*., 2009). In rice production, seeds and plants during panicle emergence or flowering are treated with oxolinic acid (Maeda *et al*., 2004).

Some multinational pesticide companies come up with antibiotics like validamycin, streptomycin and tetracycline hydrochloride for controlling fungal diseases of crops. Validamycin, a non-systemic antibiotic with fungistatic action, produced from fermentation of *streptomyces hygroscopicus* var. *limoneus* used effectively for controlling the diseases caused by *Rhizoctonia solani* in rice, potatoes, vegetables, strawberries, tobacco, ginger and other crops; damping-off diseases of cotton, rice and sugar beet, etc. (Thomson, 1982; NIOSH, 1993; Anon., 1994; Meister, 1994; Tomlin, 2000). Streptomycin is a human antibiotic drug which also is used to control bacteria, fungi, and algae in crops (EPA, 1988). It is obtained by fermentation of *Streptomyces griseus*, isolated as sesqui sulfate. It has systemic action and effectively used against the diseases especially those caused by gram-positive species of bacteria (Tomlin, 2000). Tetracyclines are a group of broad-spectrum antibiotics whose general usefulness has been reduced with the onset of antibiotic resistance. Tetracycline hydrochloride is used only to treat or prevent infections that are proven or strongly suspected to be caused by susceptible bacteria (Klajn, 2001).

Global consumption and demand for antibiotics to control diseases continue to rise. More dependency on antibiotics accelerates to develop antibiotic-resistant mechanism in the pathogen(s) that is recognized as a major threat to control bacterial diseases and infections worldwide (Stockwell, 2012; CDC, 2013; Gelband, 2015). When a pathogen became resistant to an antibiotic, a new antibiotic with potent active ingredient is introduced in the market that causes more access to antibiotics and expenditure.

However, indiscriminate and overuse of chemical pesticides for controlling pests without prescription, and their adverse effects on food chain, human health and environment are common phenomena. So, introduction of antibiotics in crop husbandry for controlling fungal diseases, their effectiveness against pathogen(s) and residual effects in treated crops have claimed special attention from the researchers. Beside these, no works have been done on antibiotics for controlling fungal diseases in plant agriculture in Bangladesh. In view of the points, the present research work has been undertaken to evaluate the effectiveness of antibiotics on *T. harzianum*, a beneficial fungus and some crop infecting fungi viz. *P. infestans* (late blight of potato, damping-off of seedlings), *C. gloeosporioides* (die-back, anthracnose, rot of fruits, vegetables and other crops), *C. theae* (black rot of tea) and *F. oxysporum* (wilt, damping-off, die-back, foot and collar rot of vegetables, etc.).

Materials and methods

The experiment was carried out in the laboratory of plant pathology, Bangladesh Tea Research Institute (BTRI) during October 2014-September 2015. Three samples containing antibiotic were tested on *T. harzianum*, a beneficial and some crop infecting fungi *viz*. *P. infestans*, *C. gloeosporioides*, *C. theae* and *F. oxysporum* found in tea plantation. Potato dextrose agar media (PDA), necessary glassware and equipment required for isolation and culturing fungi were sterilized in advance by autoclave at 121°C temperature for 22 minutes at 15 PSI.

Isolation of fungi

1. ***Dilution plate method***

T. harzianum, a beneficial fungus having antagonistic behavior against some harmful fungi and *P. infestans*, a late blight of potato pathogen that was found surprisingly in tea ecosystem were isolated from the subsoil (0-9” depth) following dilution plate method. In this method, 1g of sun-dried soil known as working sample was taken in a test tube containing 9ml of sterile water. The test tube was shaken thoroughly to obtain a uniform stock solution. Now, 1 ml of stock solution was transferred to a test tube containing 9 ml of sterile water and shaken thoroughly to make 1:10 dilution. Similarly, a serial dilution of 1:1,000 was prepared by transferring 1 ml of soil suspension to two consecutive test tubes. Finally, 1 ml of soil suspension from 1:1,000 dilutions was spread over the agar medium and then shaken smoothly for few seconds. The agar plates were kept in controlled condition for observing the fungal growth.

2. ***Tissue planting method***

Tea infecting three fungi species viz. *C. gloeosporioides* (die back of tea); *C. theae* (black rot of tea) and *F. oxysporum* (gall of tea) were isolated from infected plant materials using tissue planting method. In this method, infected sample was clean with sterile water and cut into 1.5-2 mm pieces containing both healthy and diseased part. The inocula were then surface-sterilized by using 30% ethanol for 2 minutes and washed three times with consecutive changes of sterile water. Excess water remained onto the inocula was removed by sterilized blotting papers. Four inocula were plated in each of five petri dishes containing PDA media. The whole operation was done in a laminar air flow cabinet. The petri plates were incubated at 29±1^{0}C and kept under observation for 2 weeks.

Preparation of antibiotic concentrations

Three samples containing antibiotic viz. sample-1 60 SP (Validamycin 60% w/w) @ 45, 60 and 75 ppm, sample-2 11 WP (Hexaconazole 2.5% w/w+Validamycin 8.5% w/w) @ 55, 82.5 and 110 ppm; and sample-3 10 SP (Streptomycin 9% w/w +Tetracyclin hydrochloride 1% w/w) @ 50, 75 and 100 ppm were tested on isolated fungi. Concentrations in ppm were calculated on the basis of their doses i.e. 75, 100 and 125 g/1,000 liter of water/ha for sample-1 and 500, 750 and 1,000 g/1,000 liter of water/ha for sample-2 and sample-3, respectively. The lowest doses of all antibiotics were regarded as expected/supplied commercial doses. In the laboratory, calculated volume of antibiotic was added with 20 ml of melted PDA medium maintaining the respective concentrations of each. The poisoned growth medium was then poured in the sterilized Petri dish.

Inoculation of fungi into poisoned growth media

Previously isolated and cultured fungi were inoculated into the poisoned growth media separately. Three replications were maintained for each concentration along with their control plates. Inoculated plates were then incubated for further observations.

Observation and data collection

Observations of fungal growth on poisoned media were made every 24 hours of incubation. Hyphal growth extension (diameter) was measured by millimeter scale. Growth inhibition was calculated when the whole control plate (90 mm) covered by fungus and then expressed in per cent by using the following formula:

$$\text{Per cent growth inhibition} = \frac{A\text{-}B}{A} \times 100$$

Where, 'A' is defined as growth in control plate and 'B' means growth in poisoned medium. Finally data were analyzed by statistical program using MSTAT C (version 2.10) with the help of computer.

Results

In vitro results revealed that all the antibiotics have inhibitory effects on *T. harzianum*, but their performance in terms of per cent growth inhibition on some crop infecting fungi viz. *P. infestans*, *C. gloeosporioides*, *C. theae* and *F. oxysporum* were quite unsatisfactory (Table 1, Fig. 1).

Sample-1 containing Validamycin tested @ 45 ppm had shown maximum 14.37% growth inhibition on *P. infestans* followed by 11.03, 5.09 and 4.03% on *C. theae*, *F. oxysporum* and *C. gloeosporioides*, respectively. But no inhibition was recorded on *T. harzianum* treated with 45 ppm. At 60 ppm, maximum 20.26% growth inhibition was observed on *P. infestans* followed by 19.09, 12.64, 10.18 and 2.30% on *C. theae*, *F. oxysporum*, *C. gloeosporioides* and *T. harzianum*, respectively. The highest 25.50% inhibition was recorded on *C. theae* treated @ 75 ppm followed by 21.96, 21.11, 18.25 and 4.05% on *P. infestans, F. oxysporum, C. gloeosporioides* and *T. harzianum*, respectively (Table 1).

Sample-2 containing validamycin+hexaconazole tested @ 55 ppm had shown maximum 24.45% growth inhibition on *P. infestans* followed by 20.44, 15.33, 14.06 and 13.60% on *C. gloeosporioides*, *T. harzianum, C. theae* and *F. oxysporum*, respectively. At 82.5 ppm, maximum 50.34% inhibition was observed on *P. infestans* followed by 44.07, 34.07, 21.07 and 17.40% against *C. gloeosporioides*, *T. harzianum, F. oxysporum* and *C. theae*, respectively. The highest 54.19% inhibition was recorded on *C. gloeosporioides* treated @ 110 ppm followed by 52.84, 45.77, 30.01 and 25.55% on *P. infestans, T. harzianum, F. oxysporum* and *C. theae*, respectively (Table 1).

Sample-3 containing streptomycin+tetracyclin hydrochloride tested @ 50 ppm had shown maximum 61.69% growth inhibition on *C. theae* followed by 28.73, 21.74, 15.32 and 14.47% on *T. harzianum, C. gloeosporioides*, *F. oxysporum* and *P. infestans*, respectively. At 75 ppm, maximum 65.29% inhibition was observed on *C. theae* followed by 54.69, 49.43, 47.59 and 21.18 % against *P. infestans, C. gloeosporioides*, *T. harzianum* and *F. oxysporum*, respectively. The highest 70.53% inhibition was recorded on *C. theae* treated @ 100 ppm followed by 66.66, 59.12, 58.17 and 30.36% on *C. gloeosporioides, T. harzianum, P. infestans* and *F. oxysporum*, respectively (Table 1).

Discussion

Three antibiotic containing formulations viz. validamycin, hexaconazole+validamycin, and streptomycin+tetracyclin hydrochloride were used in the experiment to evaluate their effectiveness on *T. harzianum*, a beneficial and some crop infecting fungi viz. *P. infestans*, *C. gloeosporioides*, *C.theae* and *F.oxysporum*. All the antibiotics showed unsatisfactory performance at lower (commercial/supplied) as well as increased

Table 1. Evaluation of antibiotic performances on *Trichoderma harzianum* and some crop infecting fungi

Treatment	Doses (ppm)	% growth inhibition				
		P. infestans	*T. harzianum*	*C. gloeosporioides*	*C. theae*	*F. oxysporum*
		4^{th} day	5^{th} day	14^{th} day	16^{th} day	17^{th} day
T_0	-	00	00	00	00	00
	-	00	00	00	00	00
	-	00	00	00	00	00
T_1 (Sample 1)	45	14.37	00	4.03	11.03	5.09
	60	20.26	2.30	10.18	19.09	12.64
	75	21.96	4.05	18.25	25.50	21.11
	lsd (.05)	0.815	0.30	0.432	0.253	0.294
T_2 (Sample 2)	55	24.45	15.33	20.44	14.06	13.60
	82.5	50.34	34.07	44.07	17.40	21.07
	110	52.84	45.77	54.19	25.55	30.01
	lsd (.05)	0.415	0.195	0.324	0.015	0.248
T_3 (Sample 3)	50	14.47	28.73	21.74	61.69	15.32
	75	54.69	47.59	49.43	65.29	21.18
	100	58.17	59.12	66.66	70.53	30.36
	lsd (.05)	0.346	0.588	0.271	0.588	0.100

To=Control, T1=Validamycin, T2=Validamycin+Hexaconazole, T3=Streptomycin+Tetracyclin hydrochloride

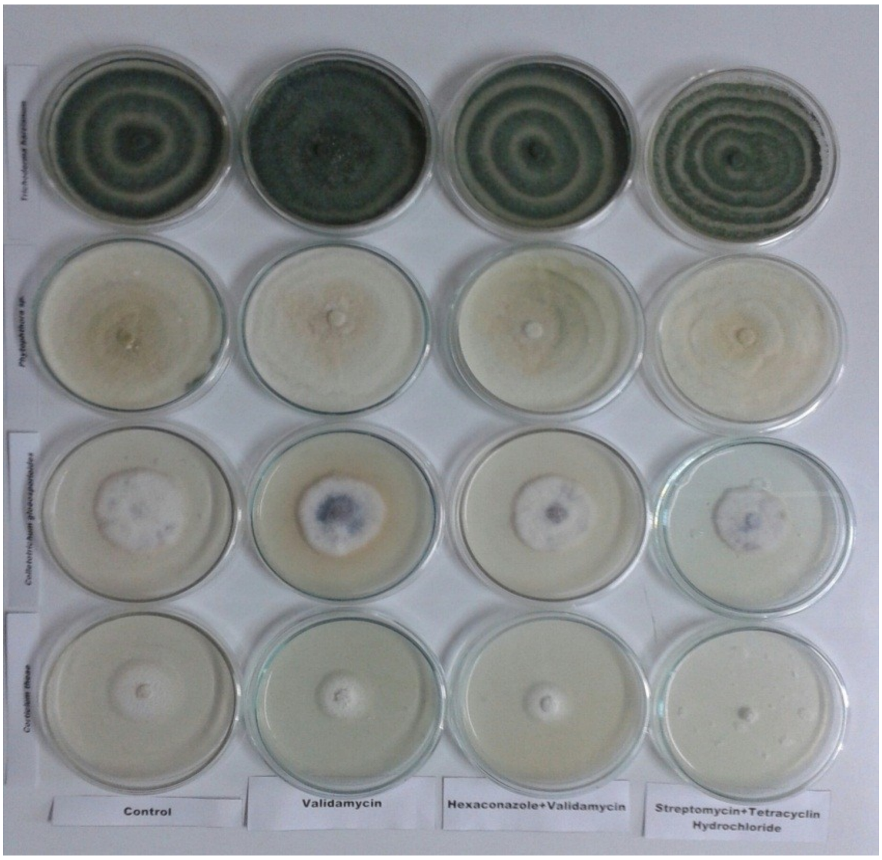

Fig. 1. Antibiotic performances against *T. harzianum* and some crop infecting fungi

concentrations on test fungi. Earlier study had shown that all fast-growing species (except *Pythium ultimum*) were efficiently inhibited but not completely suppressed by validamycin (Laar, 1982). In 2000, Tomlin reported that validamycin applied as a foliar spray, soil drench, seed dressing, or by soil incorporation, at 1.25 g/ha (liquid) for controlling *Rhizoctonia solani* in rice, potatoes, vegetables, strawberries, tobacco, ginger, and other crops; damping off diseases of cotton, rice, sugar beet, etc. Hexaconazole, a fungicide mixed with an antibiotic validamycin introduced in the market for controlling fungal diseases of crops. But this formulated product had shown unsatisfactory performance on fungi at different concentrations in the laboratory. Streptomycin and Tetracyclin hydrochloride are basically antibacterial compounds used as human drug commercially. Streptomycin also is used as a pesticide, to combat the growth of bacteria, fungi, and algae (EPA, 1988; Wikipedia, 2016). This compound is extensively used in controlling fire blight of apples and pears (50-100 ppm), crown gall of rose (50-200 ppm), bacterial spots of peppers and tomatoes (200 ppm), wildfire and blue mold of tobacco (100-200 ppm), soft rot and blackleg of potatoes (100 ppm) in North America (Anon., 2006). Combined formulation of streptomycin with tetracycline hydrochloride-a broad-spectrum human antibiotic, for controlling fungi was found to ineffective at different concentrations in the present investigation. The impractical performances of such antibiotics might be due to their inappropriate active ingredients mixed with the formulated products or unwise guidelines prescribed by the marketing companies to convince the growers though their antibiotics are not effective in controlling fungal diseases of crops.

Conclusion

The *in vitro* results showed that antibiotics have inhibitory effects on *T. harzianum*. Unsatisfactory performances in terms of per cent growth inhibition (<80) were observed on crop infecting fungi. So, considering the findings it can be recommended not to use tested antibiotics with their respective concentrations for controlling plant diseases caused by the said fungi. Further researches on effectiveness of antibiotics, impacts on human health and environments, residual effects on test crops, and growing antibiotic-resistant in pathogenic fungi should be carried out before introducing them in plant agriculture.

Competing Interest

The authors declare that they have no competing interests.

Author's Contribution

Md. Moshiur Rahman Akonda: Performed the experiment and writing the manuscript. Raihan Mujib Himel: Performed the experiment as a co-researcher. Mohammad Ali: Monitored the research work for authentication. Md. Syeful Islam: Analyzed the experimental data by MSTAT C.

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References

Anonymous. 1994. The Agrochemicals Handbook. Royal Society of Chemistry Information Systems. Third Edition. Unwin Brothers Ltd., Surrey, England.

Anonymous. 2006. Ag Streptomycin. Makhteshim Agan of North America. Inc. 4515 Falls of Neuse Road, Suite 300 Raleigh, NC 27609. EPA Reg. No. 66222-12.

CDC. 2013.Antibiotic Resistance Threats in the United States. Atlanta, GA; 2013.P. 7, 36-7.

EPA. 1988. US Environmental Protection Agency. Fact Sheet Number 186 Streptomycin. USEPA. Washington, DC.

Gelband, H., M. M. Petrie, S. Pant, S. Gandra, J. Levinson, D. Barter, A. White and R. Laxminarayan. 2015. The State of the World's Antibiotics 2015. Center for Disease Dynamics, Economics & Policy 2015. CDDEP: Washington, D.C.

Klajn, R. 2001. Tetracycline: Chemistry and Chemical Biology of Tetracyclines. Accessed from www.chm.bris.ac.uk/motm/tetracycline/tetracycline.htm

Laar, W. V. and W. Gams. 1982. The use of Solacol (validamycin) as a growth retardant in the isolation of soil fungi. Netherlands Journal of Plant Pathology, Volume 88, Issue 2, pp 39-45.

Maeda, Y., A. Kiba, K. Ohnishi and Y. Hikichi. 2004. Implications of amino acid substitutions in GyrA at position 83 in terms of oxolinic acid resistance in field isolates of *Burkholderia glumae*, a causal agent of bacterial seedling rot and grain rot of rice. Appl. environ. Microbiol., 70, 5613–5620. doi: 10.1128/AEM.70.9.5613-5620.2004

McManus, P. S., V. O. Stockwell, G. W. Sundin and A. L. Jones. 2002. Antibiotic use in plant agriculture. Annu. Rev. Phytopathol., 40, 443–465. doi: 10.1146/annurev.phyto.40.120301.093927

Meister, R. T. 1994. Farm Chemicals Handbook '94. Meister Publishing Company. Willoughby, OH.

Nandakumar, R., A. K. M. Shahjahan, X. L. Yuan, E. R. Dickstein, D. E. Groth, C. A. Clark, R. D. Cartwright and M. C. Rush. 2009. *Burkholderiaglumae* and *B. gladioli* cause bacterial panicle blight in rice in the southern United States. Plant Dis., 93, 896–905. doi: 10.1094/PDIS-93-9-0896

NIOSH. 1993. National Institute for Occupational Safety and Health. Registry of Toxic Effects of Chemical Substances (RTECS). NIOSH. Cincinnati, OH.

Shtienberg, D., M. Zilberstaine, D. Oppenheim, Z. Herzog, S. Manulis, H. Shwartz and G. Kritzman. 2001. Efficacy of oxolinic acid and other bactericides in suppression of *Erwinia amylovora* in pear orchards in Israel. Phytoparasitica, 29, 143–154. doi: 10.1007/BF02983958

Stockwell, V. O. and B. Duffy. 2012. Use of antibiotics in plant agriculture, Rev. sci. tech. Off. int. Epiz., 31 (1), 199-210.VII.

Thomson, W. T. 1982. Agricultural Chemicals Book IV Fungicides. Thomson Publications. Fresno, CA.

Tomlin, C. D. S. 2000. The Pesticide Manual. British Crop protection Council, Twelfth Edition. 49 Downing Street, Farnham, Surrey GU9 7PH, UK.

Vidaver, A. K. 2002. Uses of antibiotics in plant agriculture. Clin.infect. Dis., 34, S107–110. doi: 10.1086/340247

Waksman, S. A. 1947. What Is an Antibiotic or an Antibiotic Substance? Mycologia 39 (5): 565–569. doi: 10.2307/3755196. JSTOR 3755196. PMID 20264541.

Wikipedia. 2016. https://en.wikipedia.org/wiki/*Penicillin*

Wikipedia. 2016. https://en.wikipedia.org/wiki/*Streptomycin*