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Compatibility of Polyoxin D zinc salt 5% SC against various biocontrol agents used in grapes

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Polyoxin D zinc salt 5%SC is a new, medium risk fungicide.The compatibility of the fungicide @ 600 and 1200 ppm was investigated with *Trichoderma viride, Bacillus subtilis*, *Pseudomonas fluorescence, Ampelomyces quisqualis, Beauveria bassiana, Metarhizium anisopliae and Paecilomyces lilacinus* under *in vitro* conditions. It was observed that Polyoxin –D zinc salt @ 600 and 1200 ppm were compatible with *Trichoderma viride*, *Bacillus subtilis* and *Pseudomonas fluorescens, Ampelomyces quisqualis*, *Metarhizium anisopliae* and *Paecilomyces lilacinus* but in case of *Beauveria bassiana* the fungicide was incompatible. Thus Polyoxin D zinc salt 5% SC @ 600 ppm may be recommended against powdery mildew of grapes and may be used in tandem with the bio control agents used in grapes.

Key words : Grape, Diseases, Biocontrol agents, PolyoxinD, Compatibility

INTRODUCTION

Grape is cultivated as an important commercial fruit crop in India. The production of grapes in India is 2951 thousand MT from an area of 137 thousand has with 21.54 MT/ha productivity in 2018-19 (Anon 2018). About 80% of the production comes from Maharashtra followed by Karnataka and Tamil Nadu.Total exports of grapes from India was 2,46,132.27 MT during 2018-19 valued at Rs. 2,335.25 crores. Biotic constraints like diseases and insect pests are the major bottle neck of grapes cultivation (Downy mildew, Powdery mildew, Mealy bugs, Thrips etc.). Out of these to control powdery mildew there is an extensive use of pesticides. However, voluminous application of these pesticides causes serious environmental problems and also results in pesticide residues. Integrated pest management strategy (IPM) is an important approach for control of diseases and pest in which use of pesticide is reduced by employing more biological control agents and maintaining good agricultural practices. This approach can be successful only if there is compatibility between the biological and non-biological components of the IPM.

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In viticulture, the biocontrol agents used largely includes *Trichoderma* spp, *Bacillus subtilis*, *Pseudomonas fluorescens, Ampelomyces quisqualis* and entomopathogens such as *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyce slilacinus*. They inhibit the pathogens through the process of antagonism, competition and parasitism. Polyoxin D zinc salt 5 % SC (FRAC code 19) belongs to peptidylpyrimidin nucleoside chemical group of fungicide and inhibit formation of chitin, a vital component of fungal cell wall. They are produced by *Streptomyces cacaoi* var. *asoensis* is a competitive inhibitor of chitin-UDP acetylglucoseaminyl transferase, leading to an imbalance between the growth of the cell wall and other cellular elements and are relatively non- toxic to the environment and humans (Osada 2019). The biochemically active ingredient controls pathogenic plant fungi by inhibiting the formation of chitin which is a vital component of fungal cell walls. Due to these advantages this compound has a good scope to be included in IPM program.

Therefore, the present study was conducted to assess the compatibility of Polyoxin D zinc salt 5 % SC with different biological control agents (BCA) used in grape farming. To our knowledge this is the first report of compatibility of Polyoxin D with different BCAs, which would help in development of effect IPM strategy to control grape diseases.

MATERIAL AND METHODS

Biocontrol agents

Four antifungal agents, *Trichoderma viride*, *Ampelomyces quisqualis, Bacillus subtilis* and *Pseudomonas fluorescens* and three entomopathogens, *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces lilacinus* were used for the study. All the BCAs were procured from culture collection of ICAR- National Research Centre for Grapes. Fungal strains were cultured and preserved on Potato Dextrose Agar (Hi Media MH096) and bacterial cultures were maintained on Nutrient Agar (Hi Media MM012).

Fungicide

Two doses of Polyoxin D zinc salt 5 % SC @ 600 ppm (parts per million) and 1200 ppm were used for *in vitro* compatibility study.

In vitro compatibility study Poison food technique

Fungal biocontrol agents were tested for compatibility by Poison food technique (Dhingra and Sinclair 1995). Pre sterilised PDA medium was amended with concentrations of 600ppm and 1200ppmof Polyoxin D zinc salt 5 % SC separately. After the media had solidified it was seeded centrally with fungal disc of 5 mm diameter cut from actively growing fungus (*Trichoderma viride*, *Paecilomyces lilacinus, Metarhizium anisopliae*, *Beauveria bassiana* and *Ampelomyces quisqualis*) plate aseptically with the help of a sterilized cork borer. Unamended media served as control. The plates were incubated at 27 ± 2 °C for5 days. Five replications were maintained and experiment was repeated twice. After five days of incubation radial mycelium growth for each treatment was measured with respect to control. Percent growth inhibition was calculated using the formula:

Percent growth inhibition (I)= C-T/C×100
where, I = Percent growth inhibition,T=colony diameter with treatment, C=colony diameter in the control Vincent (1947)

Disc diffusion method

The compatibility of bacteria with Polyoxin D was studied using disc diffusion assay (Mohiddin *et al*, 2013). Sterile NA plate was spread with overnight grown culture of *Bacillus subtilis* and *Pseudomonas fluorescens*. Polyoxin D solutions of concentration 600 ppm ad 1200 ppm was prepared. Sterilised 5mm filter paper disc was dipped in two test concentration of fungicide and were placed on nutrient agar plate. Disc dipped in sterile nutrient agar served as control. Plates were incubated at 27 ± 2 °C for 48 hr. Five replications were maintained and experiment was repeated twice. Zone of bacterial inhibition was measured in millimetre and was compared to control.

Statistical Analysis

The data were analysed in Completely Randomised Design (CRD) with Analysis of Variance (ANOVA) using SAS (ver. 9.3; SAS Institute Inc., Cary, North Carolina, USA). Means were compared by the Tukey's test ($P < 0.05$).

RESULT AND DISCUSSION

Trichoderma spp. are the most effective biocontrol agents against several diseases of grapes and is reported to show a higher tolerance to fungicides. Polyoxin D zinc salt has a non-toxic mode of action. Polyoxin D zinc salt does not kill the target fungal plant pathogens. Instead, it stops the growth of the fungal plant pathogen. The results manifested that *Trichoderma viride* was compatible with Polyoxin D zinc salt 5 % SC @ 600 and 1200 ppm (Table 1) Similarly Shashikumar *et al*, (2019) and Ranganathswamy *et al, (*2012) reported that systemic fungicides Azoxystrobin was highly compatible with *Trichoderma viride.* It was also not inhibited by Carpropamid 300 SC and and Myclobutanil 10 WP (Saha *et al*, 2011)

Although bacterial cells are devoid of chitin, the above mode of action of Polyoxin D is consistent with the observed absence of activity of Polyoxin D in bacteria. Similar observations were recorded in case of *Bacillus subtilis* and *Pseudomonas fluorescens* at 600 ppm and 1200 ppm and they were found moderately compatible with Polyoxin D (Table 1). It was a clear testimony that the test chemical supported approximately 50 % of the growth of the biocontrol agents. The present study was in cognizance with Rajkumar *et al*. (2018) who reported *P. fluorescens and B. subtilis* were compatible with Azoxystrobin 25 SC at 5, 10, 50, 100 and 250 ppm concentrations and Valarmathi et al. (2013) who reported that copper hydroxide

(Kocide 3000) was compatible with *P. fluorescens and B. subtilis.*

In case of *Ampelomyces quisqualis* although, growth inhibiton was around 66.33% at 600 ppm, and approximately 101 colonies of the fungus were visible which reduced to 87 at 1200 ppm. The untreated control exhibited around 300 colonies (Table 1).

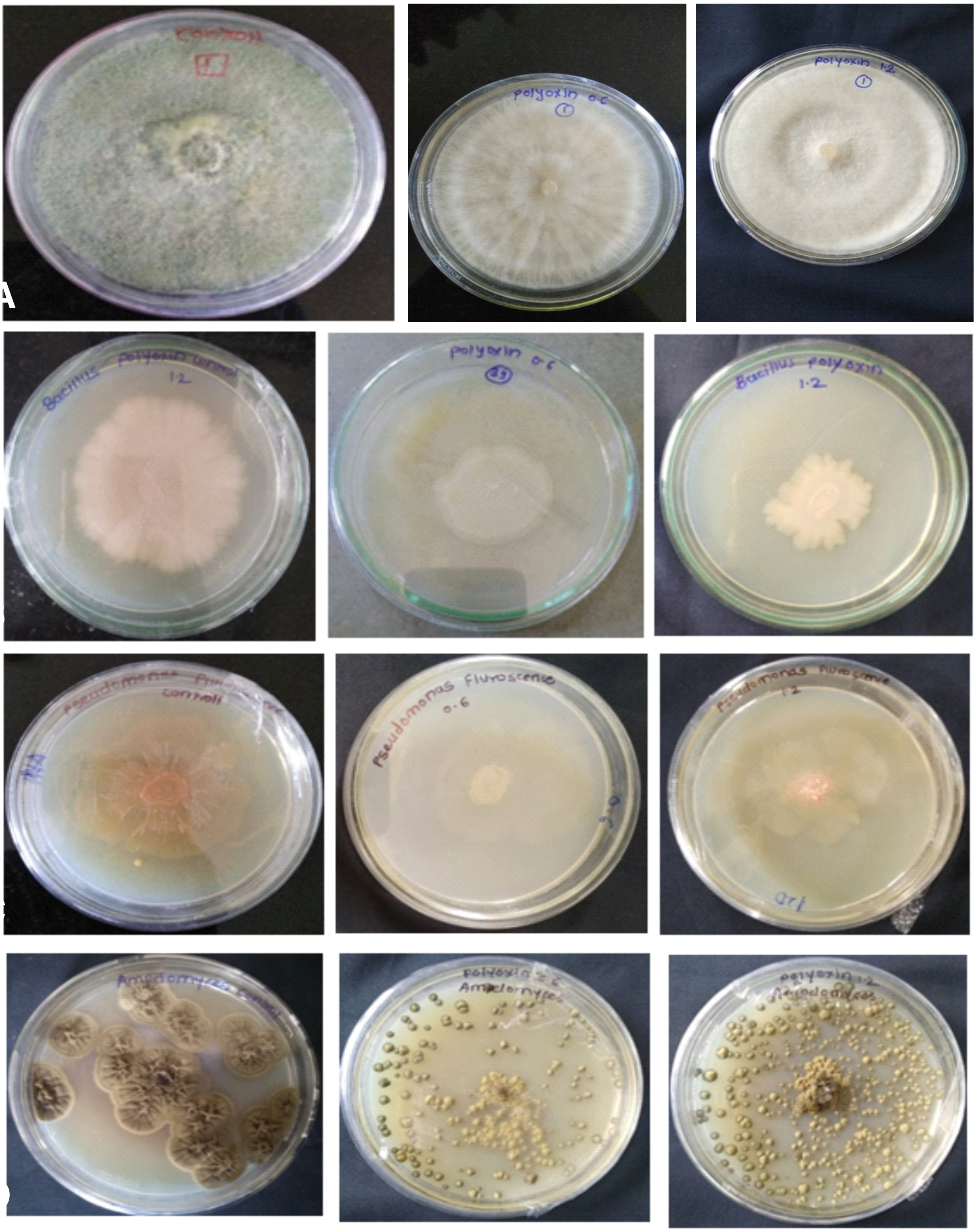
Roberti *et al*, (2010) reported that *A. quisqualis* showed high compatibility with boscalid 50%, fenamidone 4.4%+fosetyl aluminium 66.7%, dimethomorph 50%, cyazofamid 2.03%, fluazinam 39.5%, spiroxamine 30.9%. Among all the fungal biocontrol agents tested *Paecilomyces lilacinus* (2.56 and 5.12 %) and *Metarhizium anisopliae* (6.89 and 24.13 %) and were found to be highly compatible followed by *Trichoderma viride* (18.00 and 38.44 %), at both the concentrations when tested against mycelium growth of fungi. Significant inhibition of growth was observed in *Beauveria bassiana* (71.42 and 78.57 %) indicating that Polyoxin D had negative effect on the growth of this fungi.

Earlier studies had reported compatibility of BCAs with different fungicide such as thiram, copper oxychloride, mancozeb, cymoxanil + Mancozeb, captan and azoxystrobin (Wedajo *et al*, 2015).There is no published data on compatibility of BCAs with Polyoxin D which in chitin synthesis inhibitor. However, some literature on compatibility of chitin synthesis inhibitors like Diflubenzuron, etoxazole, hexythiazox, clofentezine, captan, buprofezin and nikkomycins with BCAs proved their efficiency to be included in planning effective IPM (Merzendorfer, 2013). Negative impact on growth of *Beauveria* and *Metarhizium* by captan and diflubenzuron was reported earlier (Shah *et al,* 2009, Olson and Oetting 1999, Dara, 2017). Hexythiazox inhibited the conidia germination of *Beauveria* as compared to vegetative growth (Oliveira 2014). Present results were in accordance with the above mentioned studies as Polyoxin D in the present study also inhibited the

Table 1: Compatibility of *Trichoderma viride*, *Bacillus subtilis,Pseudomonas fluroscence*, *Ampelomyces quisqualis, Beauveria bassiana,Metarhizium anisopliae* and *Paecilomyces lilacinus* with Polyoxin D zinc salt 5% SC .

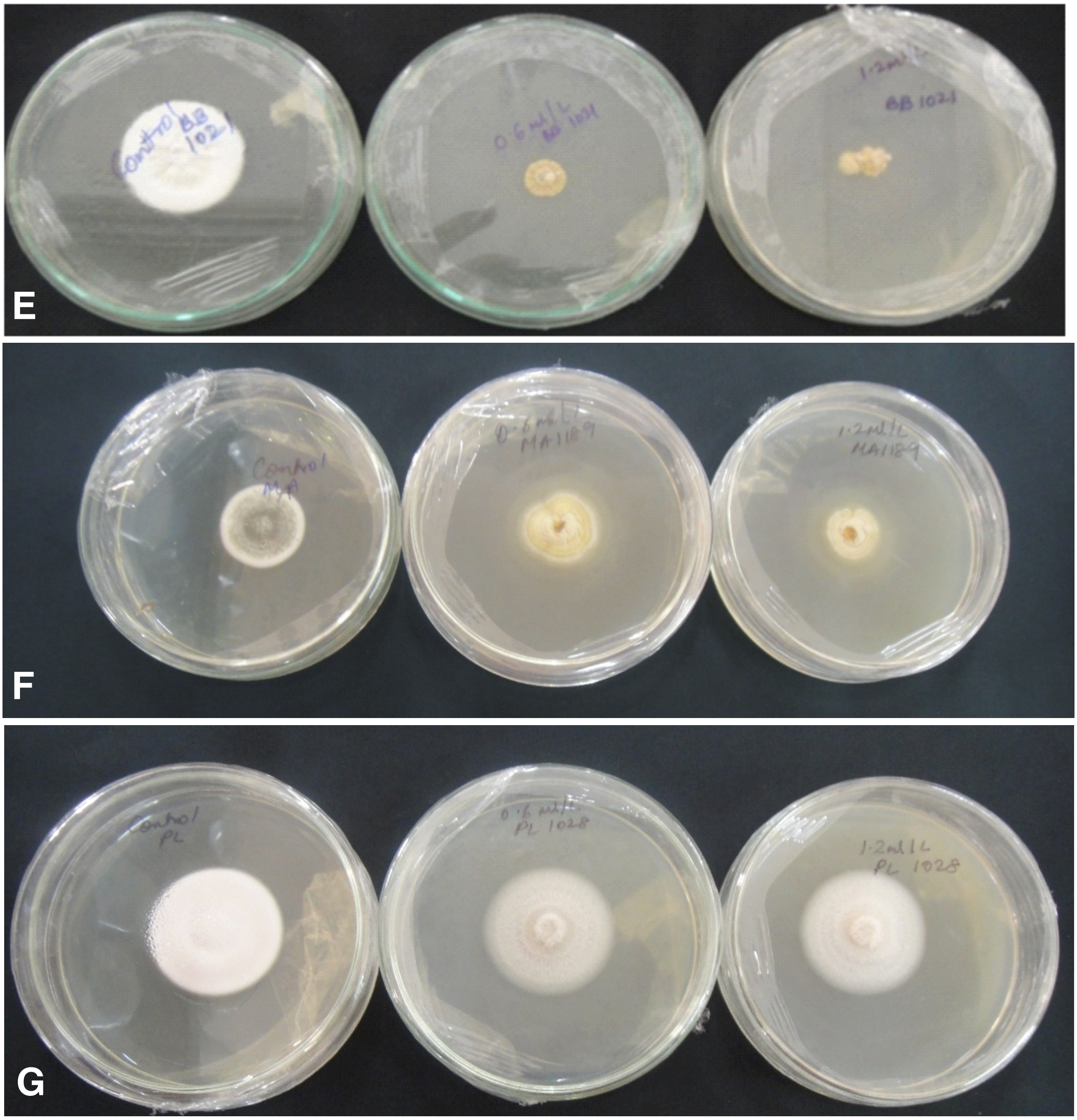
Treatment	Biocontrol agent	Polyoxin D zinc 5 % SC Concentration(ppm)	Mean colony diameter (mm)	Per cent growth inhibition of bio control agents
1	*Trichoderma viride*	600	64.8	18.00(18.55) b
		1200	55.4	38.44(22.75) a
	Control	--	90	0.0
	CD(P = 0.05)	--	--	2.65
2	*Bacillus subtilis*	600	43	52.22(46.03) b
		1200	33	63.33(52.79) a
	Control	--	90	0.0
	CD(P = 0.05)	--	--	2.88
3	*Pseudomonas*	600	52	42.22(46.03) b
	fluorescence	1200	45	50.00(52.79) a
	Control	--	90	0.0
	CD(P = 0.05)	--	--	2.88
4	*Ampelomyces*	600	101	66.33(101.40) b
	quisqualis	1200	87	71.00(86.80) a
	Control	--	300	0.0
	CD(P = 0.05)	--	--	7.27
5	*Beauveria bassiana*	600	8	71.42 (46.03) b
		1200	6	78.57 (52.79) a
	Control	--	28	0.0
	CD(P = 0.05)	--	--	2.88
6	*Metarhizium anisopliae*	600	27	6.89 (46.03) b
		1200	22	24.13 (52.79) a
	Control	--	29	0.0
	CD(P = 0.05)	--	--	2.88
7	*Paecilomyces*	600	39	2.56 (46.03) b
	lilacinus	1200	38	5.12 (52.79) a
	Control	--	40	0.0
	CD(P = 0.05)	--	--	2.88

* Figure in parenthesis indicates the angular transformed values
**means with the same letter are not significantly different



growth of *Beauveria*. However, in contrast to the study by Shah *et al*, (2009), the growth of *Metarhizium* was not inhibited by Polyoxin D. Mohamed et al, (1987) reported the compatibility of *Metarhizium* species with diflubenzuron. Compatibility of buprofezin with both *Beauveria* and *Metarhizium* was reported by Yadav *et al*, (2019). More than 80% inhibition of *Trichoderma viride* by captan was reported by Kumar *et al*, (2017). Similar inhibition of *Trichoderma* was reported by Mishra (2019).

The findings of the present study indicated that although Polyoxin D is a competitive inhibitor of



(a) *Trichoderma viride* control	Polyoxin 600 ppm	Polyoxin 1200 ppm
(b) *Bacillus subtilis* control	Polyoxin 600 ppm	Polyoxin 1200 ppm
(c) *Pseudomonas fluroscence* control	Polyoxin 600ppm	Polyoxin 1200ppm
(d) *Ampleomyces quisqualis* control	Polyoxin 600 ppm	Polyoxin 120 ppm
(e) *Beauveria bassiana* control	polyoxin 600 ppm	Polyoxin 1200 ppm
(f) *Metarhizium anisopliae* control	Polyoxin 600 ppm	Polyoxin 1200ppm
(g) *Paecilomyces lilacinus* control	Polyoxin 600 ppm	Polyoxin 1200 ppm

Fig. 1 : Compatibility of Polyoxin D with (a) *Trichoderma viride* (b) *Bacillus subtilis* (C) *Pseudomonas fluorescence* (d) *Ampelomyces quisqualis* (e) *Beauveria bassiana* (f) *Metarhizium anisopliae* (g) *Paecilomyces lilacinus*

chitin synthase enzyme it was found to be compatible with most of the biocontrol agents used and can be included in IPM program of grapes. However, it affected the growth of *Beauveria* and therefore mixing of Polyoxin with this fungi should be avoided. Further studies on effect of Polyoxin on sporulation and conidia formation is required.

CONCLUSION

The biocontrol agents *Trichoderma viride*, *Bacillus subtilis*, *Pseudomonas fluroscence*, *Ampelomyces quisqualis, Metarhizium anisopliae* and *Paecilomyces lilacinus* were biologically compatible to Polyoxin D zinc salt 5 % SC at both 600 and 1200 ppm. *Beauveria bassiana* was incompatible to Polyoxin D zinc salt % SC.

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