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Research Article

Fungicide tolerance of antagonists in the management of mango anthracnose caused by *Colletotrichum gloeosporoides*

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ABSTRACT: In the present study, fungicide tolerance of antagonists (yeast and *Lactobacillus*) with two fungicides *viz.,* Mancozeb and Ridomil gold were conducted using turbidometric method. Findings of study revealed that, ridomil and mancozeb treatments could inhibit the growth of yeasts and *Lactobacillus* to some extent but did not completely inhibit. In this study, it was found that potential yeast and *Lactobacillus* antagonists were tolerant to both mancozeb and ridomil fungicides up to 2000 ppm concentrations. This result implies that the antagonistic yeast and *Lactobacillus* isolates were not adversely affected by both mancozeb and ridomil fungicides. So, these isolates can form an important component of Integrated disease management of mango anthracnose.

KEY WORDS: Antagonists, *Colletotrichum gloeosporoides*, fungicides, mango anthracnose

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INTRODUCTION

Several pests, diseases, and disorders have been recorded on various mango varieties, ultimately resulting in severe loses to all parts of the mango around the world. Approximately 260 pest species including major and minor pests have been recorded from seedlings to mature trees at harvest and postharvest stages (Khaskheli, 2020). Mango suffers from several infectious diseases caused by many phytopathogens. Among them the main diseases are anthracnose (*Colletotrichum gloeosporioides)*, powdery mildew (*Oidium mangiferae)*, malformation (*Fusarium* spp.), bacterial leaf spot (*Erwinia mangiferae),* crown gall (*Agrobacterium tumefaciens)*, sooty mold (*Capnodium mangiferae)*, fruit rot (*C. gloeosporioides* and *Aspergillus niger)*, root rot (*Rhizoctonia solani* and *F. oxysporum)*, dieback, (*Diplodia netalensis* and *Lasiodiplodia theobromae)* and mango sudden decline (Khaskheli, 2020).

Anthracnose, caused by a fungal pathogen *Colletotrichum gloeosporioides*, is a severe disease which can cause huge economic losses at various growth stages of mango production ranging from the blossom period to postharvest. It is considered to be the most important disease of the crops in all mango producing areas worldwide (Arauz, 2000, Chowdhury and Rahim, 2009). It is favored by high relative humidity and abundant rainfall that help in the development of the severe symptoms on leaves, flowers, fruits, and branches of all ages. The disease can cause losses varying from 50 to 100% in unmanaged orchards under a favorable environment (Arauz, 2000).

The use of fungicides for the management of anthracnose disease has been widely practiced worldwide. Fungicides such as benomyl, carbendazim or propiconazole, copper, and mancozeb have been used as the primary means to control mango anthracnose disease (Khaskheli et al., 2020). However, due to the accumulation of chemical residues on agricultural products which poses a direct effect on the consumer's health and the environment, growers and consumers experienced problems using fungicides as sole controlling method. Although growers' complain grew up to reduce the use of pesticides in their crops due to public's growing concern for the negative health effects and environmental pollution associated with pesticide usage, the development of fungicide resistant strains of postharvest pathogens, and the lack of continued approval of some of the most effective fungicides, fungicides and insecticides cannot be discarded from the conventional use due to several pests and still affecting the product, while biological alternatives are not available or not totally effective yet (da Silva *et al.,* 2017). In the absence of fully effective

postharvest fungicides, alternative or integrative measures are becoming increasingly important for controlling losses. Biological control by antagonistic microorganisms, including yeasts, yeast-like fungi and bacteria, appears particularly promising in preventing fungal diseases on various fruits and vegetables (Lima *et al.,* 2008). However, when applied alone or under commercial conditions biocontrol agents (BCAs) are sometimes not sufficient to satisfactorily control Post-Harvest Diseases (PHDs) (Lima *et al.,* 2003). Integrating BCAs with other means of control in order to make their activity more reliable may be the best option for large-scale application of an antagonist (Lima and Cicco, 2006), with a consequent significant reduction of the fungicide amounts used. To this end, several studies have shown that integrating BCAs or biofungicides with small quantities of compatible synthetic fungicides, in comparison with the same treatments applied separately, can exert higher efficacy and persistence against postharvest fungal decays of several important fruits and vegetables *(*Lima *et al.,* 2003, Lima *et al.,* 2008). The knowledge of fungicide effects on antagonist microorganisms is crucial in order to optimize this integrated strategy. Treatments with some fungicides have been shown to alter the population of non-pathogenic epiphytic microorganisms on plant surfaces, which also include potential antagonists (Gildemacher *et al.,* 2004, Legein *et al.,* 2020). Such negative effects are in contrast with the needs of antagonists to fully exert their prophylactic mechanisms based mainly on competition for space and nutrients which, in turn, need high levels of cells on fruit surfaces as well as rapid, timely colonization of wounds produced by handling fruits and vegetables (Castoria *et al.,* 2001). Therefore, the resistance to chemicals currently used on vegetal products as well as to newly developed compounds is important for high efficacy of BCAs. Therefore, to develop an effective disease management program, the compatibility of potential bioagents with fungicides is essential. Integration of compatible bioagents with fungicides can enhance the effectiveness of disease control and provide better management of soil borne diseases (Lima *et al.,* 2008). Several reports are available on the compatibility of biocontrol agents with chemicals (Malathi *et al.,* 2002, Valarmathi *et al.,* 2013, Bhale and Rajkonda, 2015, Basamma and Shripad, 2017, Aynalem and Assefa, 2017a, Lima *et al.,* 2011, Vyas *et al.,* 2020). In several disease management strategies, the addition of fungicide at reduced rate in combination with biocontrol agents has significantly enhanced disease control compared to treatments with biocontrol agents alone (Ons *et al.,* 2020). According to the review findings of Lima *et al.* (2008), Lack of knowledge of the compatibility of antagonist microorganisms with agrochemicals may contribute to the failure of biocontrol to perform as expected, as pre- and post-harvest fungicide applications can affect the survival and population of natural and artificially introduced antagonists on fruits and vegetables. The combination or alternation of synthetic fungicides with antagonist microorganisms may enhance and stabilize the efficacy of BCAs. In addition, this strategy may display even better control of resistant strains of fungal pathogens and may enable commercial growers and packinghouses to reduce the amount of fungicides used, thus lowering the amount of chemical residue on marketed products. Keshgond and Naik (2013) have noted the compatibility of *P. fluorescens* with carbendazium while studying sheath blight in rice. Combined application of *P. fluorescens* + thiophanate methyl resulted in highest plant stand (Malathi *et al.,* 2002). Pereira *et al.* (2010) conducted the pot and field experiments to evaluate the biocontrol potential of *T. harizianum*, *Gliocladium virens* and *P. fluorescens* against *F. oxysporum* f. sp. *lentis* infecting lentils and their compatibility with fungicides. In pots, pre sowing seed treatment (ST) with *P. fluorescens* + carboxin resulted in 62.3 per cent wilt control. Seed treatments with carbendazium + thiram and *G. virens* + *P. fluorescens* + carboxin were effective in the field controlling 48.8 and 44.2 per cent respectively. Keshgond and Naik (2013) reported that compatibility of *P. fluorescens* with fungicides *in vitro.* Crboxin, chlorothalonil and carbendazium were least toxic to *P. fluorescens* strain PFBC-25. In the assessment conducted by Mohiddin and Khan (2013), fungal (*Trichoderma harzianum, Trichoderma virens and Pochonia chlamydosporia*) and bacterial biocontrol agents (*Bacillus subtilis and Pseudomonas fluorescens*) were found to be compatible with carbendazim, mancozeb, metalaxyl, captan, thiram, and nemacur. Moreover, Sameer (2019) reported that carbendazim 50 WP was compatible with *B. subtilis*. On the other hand, Kumar *et al.* (2018) reported that sporulation of *T. harzianum* (C52) was completely inhibited by the tebuconazole (0.05%) and mancozeb (0.1%). Similarly, Pandey *et al.* (2006) reported that both hexaconazole and tebuconazole fungicides showed 100% inhibition of mycelial growth of both *T. viride* and *T. harzianum* under *in vitro* conditions at 500 ppm concentration. Therefore, BCA-fungicide combinations could have potential against populations of fungicide-sensitive and fungicide-resistant populations, which are becoming more and more prevalent (Shao *et al.,* 2021). Many of the compatibility works were, however, limited to fungal *(Trichoderma* spp.) and bacterial biogents (*Pseudomonas* and *Bacillus*). Therefore, this work was initiated to investigate the compatibility of yeast and *Lactobacillus* antagonists (effective against *C. gleosporiodes*) with commonly used general fungicides, mancozeb and ridomil gold.

MATERIALS AND METHODS

Antagonists and Fungicides

Source of antagonists

Regarding antagonists, both yeasts and *Lactobacillus* antagonists were employed in this study. Both yeast and *Lactobacillus* isolates were screened from mango fruits and were labeled as YBC and LBC respectively. The isolated species were identified up to genus level based on colony characters, growth, and structure of mycelium, conidiophores and conidia.

Fungicides

a) **Mancozeb 75% WP:** is a broad spectrum contact fungicide with a protective action which belongs to the dithiocarbamates (Manganese ethylene bisdithiocarbamte) family of chemicals, which also includes maneb.

b) **Ridomil gold:** is a combination of Metalaxyl-M and Mancozeb where Metalaxyl-M is a systemic fungicide which is rapidly taken up by the green plant part and transported upwards in the sap stream and is distributed thus provides control of fungi from within the plant. Mancozeb provides a protective film over plant surfaces hence inhibits germination of the spores.

Fungicide tolerance of antagonists

Tolerance of biocontrol agents was tested to the fungicides Mancozeb and Ridomil gold in order to select tolerant biotypes for compatibility studies with fungicides. The *Lactobacillus* and yeast antagonists were obtained from previous biocontrol screening experiments at Microbiology Laboratory of Bahir Dar University. Two fungicides Mancozeb and Rudmil gold were purchased from local markets and were used for fungicide tolerance test with antagonistic isolates at 0.1%, 0.15% and 0.2% concentrations. Fungicide tolerance test of antagonists (yeast and *Lactobacillus*) with two fungicides *viz.,* Mancozeb and Ridomil gold were conducted using turbdometric method (Valarmathi *et al.,* 2013).

Effect of mancozeb on *Lactobacillus* and yeast isolates

The stock solutions of mancozeb (Limin Chemical Co. Ltd.) were prepared by adding 10 g of mancozeb powder into 1000 mL of distilled water (Aynalem and Assefa, 2017a). Then 1000, 1500, and 2000 ppm of filtered Mancozeb solutions were separately added to sterilized 100 mL of nutrient and yeast peptone dextrose (YPD) broth in 250 mL Erlenmeyer flasks for lactobacillus and yeast antagonists respectively. Then activated 1mL (10^8 cells mL^{-1}) of each isolate was added to each concentration and incubated on shaker at room temperature for 72 h.

Effect of ridomil gold on *Lactobacillus* and yeast isolates

The stock solutions of ridomil gold (Limin Chemical Co. Ltd.) were prepared by adding 10 g of ridomil gold powder into 1000 mL of distilled water (Aynalem and Assefa, 2017a). Filtered 1000, 1500, and 2000 ppm of ridomil gold solutions were separately mixed with sterilized 100 mL of nutrient broth and YPD broth using 250 mL Erlenmeyer flasks for lactobacillus and yeast antagonists respectively. Then efficient 1mL (10^8 cells mL^{-1}) of each isolate was inoculated into broth medium prepared with different concentrations of Ridomil gold and incubated on the shaker at room temperature for 72h. Growth of isolates in different treatments was evaluated through optical density measurement by using UV-7804C spectrophotometer at 600 nm and compared with isolates grown on fungicide-free control. Growth of isolates in different treatments was evaluated through optical density measurement by using spectrophotometer at 600 nm and compared with isolates grown on fungicide-free control. Percent inhibition was performed according to the following formula (Aynalem and Assefa, 2017b).

$$\% I = \frac{\text{OD of control} - \text{OD of treated}}{\text{OD of control}} \times 100$$

Where OD is optical density and % *I* is percentage of inhibition.

Data analysis

Data of response of antagonists (Yeast and *Lactobacillus*) to fungicides of different concentration was analyzed using SPSS version 26. Percent inhibitions from treatments of yeast and *Lactobacillus* isolates were analyzed by Analysis of Variance (ANOVA) and Tukey's test was used to separate the treatment means.

RESULTS AND DISCUSSION

Tolerance of potential antagonistic yeast and *Lactobacillus* isolates to fungicides

In the present study, fungicide tolerance of potential antagonistic *Lactobacillus* and yeasts were evaluated. All antagonistic isolates (*Lactobacillus* and yeasts) were evaluated with chemical fungicides Ridomil and Mancozeb at three different concentrations.

Tolerance of yeasts isolates to ridomil fungicide

Response of yeasts to different concentrations of ridomil: in this experiment, the effect of different concentrations of ridomil on each antagonist isolate and the effect of each ridomil concentration on each antagonist was evaluated. Accordingly, all antagonistic yeasts responded significantly to each ridomil concentrations treated. Results of this experiment revealed that the growth of yeasts was affected as the concentration increases. Hence, maximum growth of all isolates was recorded in 1000 ppm followed by the 1500 ppm. On the other hand, maximum growth was inhibition recorded on 2000 ppm in almost all yeast isolates.

At 1000 ppm, results on the effect of ridomil concentration on each yeast isolates also revealed that there was significant difference between the isolates ($p \leq 0.05$). Hence maximum inhibition was recorded in YBC23 (47.1%) followed by YBC 27(46.1%) and the least inhibition was recorded in YBC19 (30.1%) and YBC33 (30.3) (Table 1). At 1500 ppm, maximum inhibition was recorded in isolate YBC28 (66.66%) followed by YBC27 (64.25%). Minimum percent inhibition, however, was recorded in isolate YBC33 (36.63%). Similarly, at 2000 ppm, maximum inhibition was recorded in yeast isolate YBC28 (68.02%) and minimum inhibition was recorded in isolate YBC16 (37.55%) (Table 1). Genaerally, this study revealed that all yeast isolates could be able to tolerate riodomil fungicide upto 2000 ppm concentration and this implies that this potential antagonistic yeast could be used in combination with ridomil fungicides for mango anthracnose disease management.

Effect of ridomil on the growth of antagonistic *Lactobacillus* isolates

The effect of different concentration of ridomil on the growth of *Lactobacillus* isolates was evaluated invite in this particular experiment.in all cases of isolates maximum inhibition was recorded at 2000 ppm. Moreover, the effect of ridomil on the growth of the isolates increased as the concentration of the fungicide was increased. On the other hand, significant difference was observed between isolates in all concentration treatments. Hence, at 1000 ppm, the least growth inhibition was recorded in LBC2 (1.38%) followed by LBC18 (11.11%) and LBC8 (11.45%). On the other hand, maximum inhibition was recorded in LBC16 (23.26%) (Table 2). At 1500 ppm, minimum growth inhibition was recorded in LBC6 and LBC16 with inhibition percentage of 5.90 % and 9.72% respectively (Table 2). Similarly, at 2000 ppm, minimum inhibition was recorded in LBC16 (12.15%) and maximum inhibition was recorded in LBC6 (39.23%) and LBC2 (38.54%) (Table 2). In lactobacillus isolates, the inhibition percentage was observed to be variable. For instance, the effect of ridomil on the growth of isolates LBC2, LBC19 and LBC21 was observed to be increamental as concentration increases. However, the response of the remaining *Lactobacillus* isolates to the different concentration of ridomil was observed to be variable. This result implies that the antagonistic lactobacillus isolates were not adversely affected by ridomil fungicides. Generally, all LAB isolates were able to tolerate the fungicide and could be used in combination with ridomil as part of mango anthracnose disease integrated management.

Tolerance of potential antagonistic *Lactobacillus* under mancozeb fungicide treatment

Results on the effect of mancozeb on yeast isolates is presented in (Table 3) below. Findings of the present study revealed that, different mancozeb treatments could inhibit the growth of yeasts to some extent but did not completely inhibited (Table 3). Significant difference ($P<0.05$) was observed between mancozeb treatments in the inhibiting yeast isolates. Hence maximum inhibition was recorded at 2000 pmm in all cases and minimum inhibition was recorded at 1000 ppm in all yeast isolates. From all isolates, CYB16 was the most affected of all at 2000 ppm while YBC27 and YBC28 were the least affected at 2000 ppm. Besides, the effect of individual concentrations on the growth of each isolate was compared. Accordingly, at 1000 ppm, the least inhibition was recorded in isolate YBC19 (0.6%) followed by YBC 16 (0.9%). At 1500 ppm, minimum inhibition was recorded in YBC16 (3.93) and maximum inhibition was recorded in YBC28 (36.06). Similarly, at 2000 ppm, the least affected was YBC27 (13.33%) and the most affected isolate was YBC16 (61.21%) (Table 3).

Tolerance of potential antagonistic *Lactobacillus* isolates under mancozeb treatment

Results on the effect of mancozeb on *Lactobacillus* isolates is presented in (Table 4) below. Findings of the present study revealed that, different mancozeb treatments could inhibit the growth of *Lactobacillus* antagonists to some extent but did not completely inhibit (Table 4). Significant difference ($P<0.05$) was observed between mancozeb treatments in the inhibiting *Lactobacillus* isolates. Hence maximum inhibition was recorded at 2000 pmm in all cases and minimum inhibition was recorded at 1000 ppm in all yeast isolates. From all isolates, LBC6 (17.97%) was the most affected of all at 2000 ppm while LBC6 was the least affected at 2000 ppm. In all *Lactobacillus* isolates, there was increment in inhibition as the concentration was increased from 1000ppm to 2000ppm (Table 4). Besides, the effect of

Table 1. Compatibility evaluation of yeast isolates towards different concentrations of Ridomil Gold at 600nm

Isolates	Ridomil gold concentration in ppm						
	1000	% I	1500	% I	2000	%I	Control
YBC16	0.434^{h1}	34.5^{c}	0.426^{h3}	35.74^{c}	0.414^{n2}	37.55^{b}	0.661^{4}
YBC19	0.463^{i3}	30.1^{b}	0.430^{i2}	35.1^{c}	0.328^{g1}	50.52^{j}	0.663^{4}
YBC21	0.432^{gh3}	34.8^{c}	0.260^{c2}	60.78^{k}	0.413^{a1}	37.7^{c}	0.659^{4}
YBC22	0.421^{efg3}	36.5^{e}	0.319^{d2}	51.88^{j}	0.259^{c1}	60.93^{o}	0.660^{4}
YBC23	0.351^{a3}	47.1^{l}	0.341^{e2}	48.56^{i}	0.310^{f1}	53.24^{k}	0.658^{4}
YBC25	0.408^{cd3}	38.4^{g}	0.400^{g2}	39.66^{e}	0.349^{h1}	47.36^{h}	0.663^{4}
YBC27	0.357^{a3}	46.1^{k}	0.237^{b2}	64.25^{l}	0.223^{b1}	66.36^{p}	0.662^{4}
YBC28	0.362^{a2}	45.3^{j}	0.221^{a1}	66.66^{m}	0.212^{j2}	68.02^{q}	0.660^{3}
YBC33	0.462^{i3}	30.3^{b}	0.440^{i2}	33.63^{b}	0.394^{m1}	40.57^{d}	0.660^{4}
YBC34	0.397^{c3}	40.1^{h}	0.358^{f2}	46.00^{h}	0.261^{cd1}	60.63^{g}	0.661^{4}
YBC39	0.412^{de2}	37.8^{f}	0.410^{h2}	38.15^{d}	0.388^{l1}	41.47^{e}	0.659^{3}
YBC42	0.383^{b3}	42.2^{i}	0.364^{f2}	45.09^{g}	0.305^{e1}	53.99^{l}	0.662^{4}
YBC44	0.419^{def3}	36.8^{e}	0.400^{g2}	39.66^{e}	0.354^{i1}	46.60^{g}	0.658^{4}
YBC45	0.425^{fgh}	35.8^{d}	0.418^{h2}	36.95^{e}	0.263^{d1}	60.33^{m}	0.658^{4}
YBC50	0.408^{cd3}	38.4^{g}	0.394^{g2}	40.57^{f}	0.347^{h1}	47.66^{i}	0.664^{4}
YBC56	0.410^{efg2}	38.1^{f}	$0.402^{g1,2}$	39.36^{e}	0.384^{k1}	42.08^{f}	0.663^{3}

Different numbers in the table show significance difference and different letters show that there was significant difference.

Table 2. Tolerance evaluation of *Lactobacillus* isolates towards different concentrations of ridomil gold at 600nm

Isolates	Ridomil gold concentration in ppm						
	1000	% I	1500	% I	2000	% I	Control
LBC2	0.284^{bc3}	1.38^{b}	0.232^{d2}	19.44^{e}	0.177^{a1}	38.54^{f}	0.288^{4}
LBC6	0.225^{cd3}	21.87^{e}	0.271^{g2}	5.90^{b}	0.175^{a1}	39.23^{f}	0.288^{4}
LBC8	0.255^{ab3}	11.45^{c}	0.245^{e2}	14.93^{d}	0.234^{cd1}	18.75^{c}	0.286^{4}
LBC16	0.221^{d3}	23.26^{f}	0.260^{f2}	9.72^{c}	0.253^{d1}	12.15^{b}	0.289^{4}
LBC18	0.256^{ab3}	11.11^{c}	0.179^{a2}	37.84^{h}	$0.22b^{c1}$	23.61^{d}	0.290^{4}
LBC19	0.234^{a3}	18.75^{d}	0.201^{b2}	30.20^{g}	0.185^{b1}	35.76^{e}	0.288^{4}
LBC21	0.225^{a3}	21.87^{e}	0.221^{c2}	23.26^{f}	0.164^{b1}	43.05^{g}	0.288^{4}

Different numbers in the table show significance difference and different letters show that there was significant difference.

individual concentrations on the growth of each isolate was compared. Accordingly, at 1000ppm, the least inhibition was recorded in isolate LBC19 (7.86%) followed by LBC2 (4.86%) (Table 4). At 1500ppm, minimum inhibition was recorded in LBC19 (7.86%) and maximum inhibition was recorded in LBC2 (17.97%). Similarly, at 2000ppm, the least affected was LBC2 (6.74%) and the most affected isolate was LBC6 (17.97% (Table 4). The findings of this research indicated that as the concentration of mancozeb was increased, the inhibition of the isolates was increased in almost all lactobacillus antagonists. However, it was also noted that these isolates were tolerant to mancozeb fungicide even at the highest concentration (2000ppm). So, the use of these isolates as biocontrol in combination with mancozeb for mango anthracnose disease control could be recommended.

DISCUSSION

The present study on fungicide tolerance clearly indicates the selective response of antagonistic microbes to fungicides. The variation in the sensitivity of yeast and *Lactobacillus* isolates to fungicides might be due, heir inherent ability to degrade them (Malathi *et al.,* 2002).

Table 3. Mancozeb tolerance of yeast isolates at different concentrations at 600nm

Isolates	Mancozeb concentration in ppm						
	1000	%I	1500	%I	2000	%I	Control
YBC16	0.327^{j3}	0.9^{b}	0.317^{l2}	3.93^{b}	0.128^{a1}	61.21^{q}	0.330^{4}
YBC19	0.328^{j3}	0.6^{ab}	0.274^{h2}	16.96^{g}	0.271^{l1}	17.87^{d}	0.330^{4}
YBC21	0.314^{h3}	4.84^{c}	0.269^{g2}	18.48^{h}	0.138^{b1}	58.18^{p}	0.329^{4}
YBC22	0.310^{hi2}	6.06^{d}	0.255^{f1}	22.72^{j}	0.246^{k1}	25.45^{g}	0.331
YBC23	0.300^{fg3}	9.09^{g}	0.294^{k2}	10.9^{d}	0.230^{i1}	30.30^{i}	0.329^{4}
YBC25	0.306^{fgh3}	7.27^{e}	0.278^{i2}	15.75^{f}	0.182^{f1}	44.84^{l}	0.330^{4}
YBC27	0.256^{c3}	22.42^{m}	0.296^{k2}	10.30^{c}	0.286^{n1}	13.33^{b}	0.331^{4}
YBC28	0.193^{a3}	41.51^{o}	0.211^{a2}	36.06^{p}	0.279^{m1}	15.45^{c}	0.332^{4}
YBC33	0.294^{f3}	10.9^{i}	0.283^{j2}	14.24^{e}	0.189^{g1}	42.72^{k}	0.329^{4}
YBC34	0.297^{d3}	10^{h}	0.259^{b2}	21.51^{i}	0.262^{h1}	20.60^{e}	0.329^{4}
YBC39	0.306^{fgh3}	7.27^{f}	0.246^{e2}	25.45^{m}	0.239^{j1}	27.57^{h}	0.330^{4}
YBC42	0.263^{cd3}	20.3^{l}	0.233^{c2}	29.39^{o}	0.171^{d1}	48.18^{n}	0.330^{4}
YBC44	0.246^{c3}	25.45^{n}	0.233^{c2}	29.39^{o}	0.145^{c1}	56.06^{o}	0.328^{4}
YBC45	0.276^{e3}	16.36^{j}	0.239^{d2}	27.57^{n}	0.191^{g1}	42.12^{j}	0.329^{4}
YBC50	0.270^{de3}	18.18^{k}	0.253^{f2}	23.33^{k}	0.248^{k1}	24.84^{f}	0.330^{4}
YBC56	0.312^{gh3}	5.45^{c}	0.249^{e2}	24.54^{l}	0.175^{e1}	46.96^{m}	0.330^{4}

Different numbers in the table show significance difference and different letters show that there was significant difference.

Table 4. Mancozeb tolerance of *Lactobacillus* isolates at different concentrations at 600nm

Isolates	Mancozeb concentration in ppm						Control
	1000	% I	1500	% I	2000	% I	
LBC2	0.254^{a3}	4.86^{bc}	0.219^{a2}	17.97^{g}	0.249^{b1}	6.74^{b}	0.264^{4}
LBC6	0.223^{a3}	16.47^{f}	0.235^{d2}	11.98^{dc}	0.219^{b1}	17.97^{f}	0.267^{4}
LBC8	0.240^{a3}	10.11^{e}	0.232^{d2}	13.10^{d}	0.225^{a1}	15.73^{e}	0.267^{4}
LBC16	0.245^{a3}	8.25^{d}	0.229^{b2}	14.23^{e}	0.234^{a1}	12.35^{c}	0.265^{4}
LBC18	0.249^{a2}	6.74^{c}	0.225^{ab1}	15.73^{f}	0.225^{a1}	15.73^{e}	0.267^{3}
LBC19	0.261^{a3}	2.20^{b}	0.246^{c2}	7.86^{b}	0.225^{a1}	15.73^{e}	0.266^{4}
LBC21	0.250^{a3}	6.36^{c}	0.237^{c2}	11.23^{c}	0.228^{a1}	14.60^{d}	0.267^{4}
Control	0.267^{a}	0^{a}	0.267	0^{a}	0.267	0^{a}	

Different numbers in the table show significance difference and different letters show that there was significant difference.

Investigation of the fungicide tolerance of microbial biocontrol and generating data on fungicide tolerance helps to select suitable selective fungicides that are compatible with biocontrol agents. Microbial antagonists with a direct action have reportedly been combined with fungicides to control post-harvest diseases. However most of the reports are confined only to *Trichoderma*, *Pseudomonas* and *Bacillus* antagonists (Malathi *et al.,* 2002, Vyas *et al.,* 2020). Findings of many authors revealed that there are microbial antagonists which are tolerant to fungicides and there others which are completely incompatible. For example, in the study conducted by Vyas *et al.* (2020), Carbendazim at 50, 100, 250 and 500 ppm, and copper oxychloride at 1000, 1500, 2000 and 2500 ppm concentrations, completely inhibited growth of *T. harzianum*. Methyl-o-demeton at all the four concentrations *i.e.* at 250, 500, 1000 and 1500 ppm were found incompatible with 94.44% growth inhibition. Quizalofop-ethyl at 500, 100, 1500 and 2000 ppm produced incompatible reaction with 94.44% growth inhibition of fungal bioagent *T. harzianum*. Moreover, Sarkar *et al.* (2010)

tested the compatibility of propiconazole, hexaconazole and tebuconazole with *T. harzianum* at 5, 10, 25, 50, 100, 200, 300 ppm concentrations and reported that all the three fungicides completely inhibited the growth of bioagent at 200 and 300 ppm concentration. According to these authors, all the fungicides/combination of fungicides, tested at all their concentrations were completely compatible with *Pseudomonas fluorescens* isolate-1. On the contrary to this, some compatibility studies on fungicides with *Trichoderma* revealed that the fungicide at lower concentration improved the antagonistic potential of *Trichoderma* spp. Suseela Bhai and Thomas (2010) stated that *T. harzianum* was not inhibited by copper oxychloride at 0.25% concentration. The enhancements of *Trichoderma* activity against pathogens when combined with fungicide application could be due to weakening of pathogen by fungicide (Thoudam and Dutta, 2014).

In the study conducted by Valarmathi *et al.* (2013), they reported that bacterial biocontrol agents *viz., Pseudomonas fluorescens and Bacillus subtilis* were compatible with copper hydroxide (Kocide 3000) even at a high concentration of 300 ppm. Additionally, Sameer (2019) reported that *B. subtilis* was compatible with propiconazole at 2000 ppm concentration. According to Basamma and Shripad (2017), the compatibility tests revealed that the *B. subtilis* showed more tolerance to Carbendazim. The fungicides *viz.,* Carbendazim, Difenconazole, Hexaconazole and Kresoxim methyl were found to be compatible with *B. subtilis* at concentrations which were recommended for plant disease management. Malathi *et al.* (2002) also reported that *in vitro* growth of P. *fluorescens* (11 strains) was not affected up to 500 ppm of carbendazim and thiophanate methyl when its growth was measured by turbidity value of bacterial growth.

In the present study tolerance of yeasts and *Lactobacillus* against fungicides mancozeb and ridomil was evaluated. The findings of this study revealed that antagonistic yeasts were able to grow under ridomil treatment even at 2000 ppm. This indicates that the antagonistic yeast isolates could be used in combination with ridomil for the control of post-harvest mango anthracnose disease. From this experiment, it was, however, observed that as the concentration of the ridomil was increased the gowth inhibition of the antagonist yeasts was also increased in almost all cases. But at lowest concentration of ridomil, the inhibition percentage of yeast isolates was low. The ability of yeasts to tolerate ridomil even at higher concentration could be due to the ability of yeasts to tolerate extreme environmental conditions. In a study conducted on apples mixure of the biocontrol yeast *Cryptococcus laurentii* and thiabendazole, at 10% of the standard dose, resulted in the highest and longest control of another important post-harvest pathogen, *B. cinerea* (Lima *et al.,* 2006). In the study conducted by Lima and Cicco (2006), the yeast isolate LS28 (*Cryptococcus laurentii*) antagonists were resistant to several fungicides, but they were inhibited by triazoles and dithiocarbamates. In their investigation, the application of the yeast isolate *Cryptococcus laurentii* together with thiabendazole at a low dose provided synergistic effects to control the pathogen and was markedly better than treatments applied separately, whereas the fungicide applied alone at the highest label dose was ineffective in the presence of the isolate of *B. cinerea* resistant to thiabendazole. Lima *et al.,* (2011) also tested the compatibility of the biocontrol yeasts (*Rhodosporidium kratochvilovae* LS11 and *Cryptococcus laurentii* LS28) with the recently developed fungicides boscalid (BOSC), cyprodinil (CYPR) and fenhexamid (FENH) to create an efficient integrated approach to control blue mould on apples. Both the biocontrol agents (BCAs) LS11 and LS28 were compatible in vitro with BOSC and CYPR, whereas they were strongly inhibited by FENH. TBZ was compatible with LS28, while it strongly inhibited LS11. *In vitro* assays with some isolates of *Penicillium expansum* showed that the majority were resistant to TBZ, whereas they were all markedly inhibited by BOSC and CYPR. Lima *et al.,* (2011) also investigated that the combination of a low BCA concentration (5×10^6 cfumL^{-1}) with a low dose (25% of the label dose) of commercial formulates of BOSC or CYPR, resulted in an efficient reduction of blue mould incidence (83–100 % less infection with respect to the control). Conversely, the combination of BCAs with TBZ was less effective (not more than 60% of rot reduction). When applied alone at low dosage, LS11, LS28, BOSC, CYPR and TBZ reduced *Penicillium* rot by 35%, 52%, 67%, 72% and 0%, respectively. They also showed that the integration of biocontrol yeasts with a low rate of the recently commercialized fungicides BOSC or CYPR could be an effective and safer strategy to control *P. expansum* and keep fungicide residues as well as patulin (PAT) contamination in apples low.

The present study also investigated the fungicide tolerance of potential antagonistic lactobacillus isolates with ridomil and mancozeb treatments. The results of this study revealed that even though there were irregularities in some of the treatments, all lactobacillus isolates tolerated both fungicides but there were also an increase in percent inhibition as the concentration of the fungicides were increased from 1000 ppm to 2000 ppm and this experiment was reported for the first time.

CONCLUSION

In this study, it was investigated that potential yeast and lactobacillus antagonists were tolerant to both mancozeb and ridomil fungicides upto 2000 ppm concentrations. It was also observed that percent inhibition of all yeast isolates showed a decreasing trend as the concentration of the fungicides was increased. Accordingly, the least percent inhibition of yeast antagonists was observed at 1000 ppm in both fungicide treatments. However, the trend in percent inhibition in lactobacillus isolates was not regular and this shows that the lactobacillus isolates were least affected by both fungicides as compared to the control. The result of the present study clearly indicated that these potential antagonists could be used with reduced dose of selected fungicides for the control of plant pathogenic fungi. Therefore, rather than applying these chemicals alone, it is very important to use these compatible antagonistic yeasts and lactobacillus isolates in combination with fungicides at lower concentration for effective management of fungal pathogens since they do not have side effect on the environment. Further compatibility study should also be done on pathogen, antagonist and fungicide combinations to see compatibility efficiency of the antagonist and the fungicides.

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