International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 05 (C); May 2019; Page No.18703-18706
DOI: http://dx.doi.org/10.24327/ijcar.2019.18706.3583

Research Article

EVALUATION OF BICARBONATES FOR THE CONTROL OF COLLETOTRICHUM GLOEOSPORIOIDES OF MANGO PLANT

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A R T I C L E I N F O

Article History:
Received 10th February, 2019
Received in revised form 2nd March, 2019
Accepted 26th April, 2019
Published online 28th May, 2019

Key words:
Colletotrichum gloeosporioides, bicarbonate, mango

A B S T R A C T

The mango plant suffers from several diseases at all stages of its life. The most important is anthracnose disease caused by Colletotrichum gloeosporioides Penz. The use of fungicides has been increasingly restricted due to public concerns of the accumulation of toxic compounds potentially hazardous to humans and the environment. This in fact has forced producers to evaluate safer alternatives for controlling diseases in the context of sustainable agriculture. Thus, an inorganic solution to this problem is studied. In recent years, there is a broad range of products based on bicarbonates. As reported by the United States Environmental Protection Agency; sodium bicarbonate (SBC),potassium bicarbonate (PBC) and ammonium bicarbonate (ABC) are considered harmless from ecotoxicological and toxicological point of view. The three bicarbonate salts were examined for their effect against C. gloeosporioides. Mycelium growth and percent growth inhibition of C. gloeosporioides is seen to vary among the treatments used at different concentrations of 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2% (w: v) for individual salt testing. Low growth was seen in ABC treatments. Where treatments ABC 0.4%, 0.8%, 1.6% and 3.2% was seen to have a growth performance of C. gloeosporioides at 0.90 cm each. This is due to the inoculation of C. gloeosporioides from the inoculation tool (cork borer) having a diameter of 0.90 cm. In reality, there was no growth recorded for these treatments. Whereas for SBCtreatments, all the concentrations used showed no significant difference, thus not having any success in controlling the pathogen, C. gloeosporioides. The best bicarbonate salt tested on its own, ABC 0.4% is set as the benchmark for testing on mixture of bicarbonate salt at a lower concentration. Mixture of PBC 0.8% + ABC 0.2% together gave significant results from growth and PGI data, with a growth of 0.9 cm and PGI of 90.98%. Thus, we conclude the best treatment to control C. gloeosporioides with the lowest concentration of bicarbonate salt according to PGI is a mixture of PBC 0.8% + ABC 0.2%.

INTRODUCTION

The mango plant is grown all over Malaysia with a planted area of 6,048.29 hectarage with a value estimated at RM 149,475.20 in 2017(Department of Agriculture, Malaysia, 2019). Even though the mango plant is established here in Malaysia, it is prone to attacks of diseases such as anthracnose and stem-end rot (Akhtar *et al.,* 2002). Anthracnose is recognized as the most important fungal disease of mango worldwide caused by *Colletotrichum gloeosporioides* Penz (Sundravadana*et al.,* 2007). The post-harvest phase is the most damaging and economically significant phase of the disease. This phase is directly linked to the field phase where initial infection starts on young leaves and spreads to the flowers, causing blossom blight and destroying the inflorescences and even preventing fruit' set.The use of fungicides has been increasingly restricted due to public concerns of the accumulation of toxic compounds potentially hazardous to humans and the environment. This in fact has forced producers to evaluate safer alternatives for controlling diseases in the context of sustainable agriculture. These alternatives include antagonistic microorganisms, natural compounds, organic and inorganic salts, and physical methods to ensure optimal fruit quality (Ippolito and Sanzani, 2011; Youssef et al., 2012a; Youssef and Roberto, 2014).

In recent years, there is a broad range of products based on bicarbonates. These bicarbonate salts increase osmotic stress, which reduces the fungal cell turgor pressure and results in collapse and shrinkage of hyphae and spores. As reported by the United States Environmental Protection Agency in 2018; potassium bicarbonate, sodium bicarbonate and ammonium bicarbonate are considered harmless from ecotoxicological and toxicological. Bicarbonate salts are considered as good alternative control options, because they have fungical properties with a very low mammalian and environmental toxicity profile (Jamar et al, 2007).

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Thus, the objective of this study is to evaluate the efficacy of three bicarbonate salts to control anthracnose, *C. gloeosporioides* of mango plant as an individual component and mixture at laboratory stage.

MATERIALS AND METHODS

Salts

Three bicarbonates were examined for their effect against *C. gloeosporioides* (Table 1).

Table 1 Bicarbonate salts, purity, chemical formula and manufacturer.

Salt	Purity	Chemical formula	Manufacturer
Sodium bicarbonate (SBC)	99.8-100.3%	$NaHCO_3$	Bendosen
Potassium bicarbonate (PBC)	99%	$KHCO_3$	Bendosen
Ammonium bicarbonate (ABC)	98-101%	NH_4HCO_3	HmbG

Pathogen

The pathogen causing anthracnose on mango plants, *C. gloeosporioides,* was isolated from infected mango leaves and flowers in the Malaysian Agriculture Research and Development Institute (MARDI) Sintok, Kedah. Five samples of diseased tissues were washed and dipped for 10 minutes in 10% clorox. Consequently, the tissue was re-washed with sterile distilled water for three times and subsequently dried. Then it was placed on potato dextrose agar (PDA). The isolates were then identified by using morphological characteristics and molecular PCR (Figure 1).

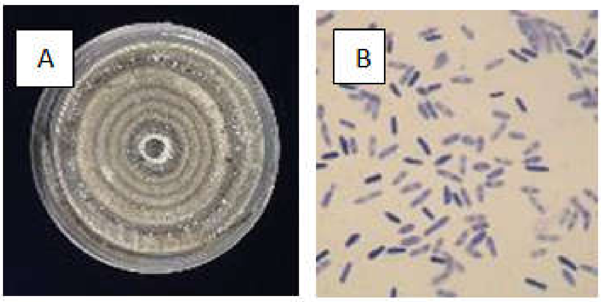


Figure 1. (A) *C. gloeosporioides*; Culture plate on PDA and (B) Spore under microscope observation (40 x)

Effect of Individual salts on Mycelia Growth

Three bicarbonates salts were examined for their effect against *C. gloeosporioides;* Sodium bicarbonate (SBC), Potassium bicarbonate (PBC) and Ammonium bicarbonate (ABC). To assess the effect these bicarbonate salts on the growth of *C. gloeosporioides,* the method of Youssef et al. (2012b) was used. In particular, agar plugs (9 mm in diameter) containing mycelia from growing edge of 14 day old culture of *C. gloeosporioides* were placed facing down in the center of Petri dishes containing PDA amended with bicarbonate salts at different concentrations of 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2% (w: v). Bicarbonate salt solutions were filtered through a 0.45 µm syringe filter before adding them to autoclaved PDA after it had cooled to 55-60°C. Control plates comprised of only PDA with no added amendments. 10 plates were prepared for each treatment and incubated at room temperature (±25˚C).

Effect of Mixed Salts on Mycelia Growth

The best bicarbonate salt tested on its own is then subjected to the next experiment, which is testing on mixture of bicarbonate salt at a lower concentration. The experimental plates are amended with bicarbonate salts using the Youssef et al. (2012b) method according to concentrations of:

Control

T1:	PBC 0.8%	+	ABC 0.1%
T2:	PBC 0.8%	+	ABC 0.2%
T3:	PBC 1.6%	+	ABC 0.1%
T4:	PBC 1.6%	+	ABC 0.2%
T5:	PBC 3.2%	+	ABC 0.1%
T6:	PBC 3.2%	+	ABC 0.2%
T7:	ABC 0.4%		

Data Collection

Growth of *C. gloeosporioides*was taken every 2 days by measuring the diameter of the mycelium growth.
While, data of the percentage growth inhibition (PGI) was calculated using the formula of Zivkovic *et al.,* 2010.

$$PGI (\%) = \frac{R1 - R2}{R1} \times 100$$

Where,

R1 = Growth of pathogen alone without antagonist (control)
R2 = Growth of pathogen along with the antagonist

The effect of the salts on spore germination was tested. Spore suspensions were prepared by removing the spores from sporulating edges of 2 week old culture of the plates tested. A sterile bacteriological loop was used to suspend the plate in sterile distilled water. Suspensions were filtered through cheesecloth to remove fungal mycelia. Spore concentration was determined by using a haemocytometer.

Experimental design. The laboratory experiment for the effect of individual and mixed salts on mycelia growth consisted of 19 treatments and 8 treatments respectively, including control with 10 replications each. The design was completely randomize (CRD) which is used for statistical analysis. Data of the percentage growth inhibition and inhibition zone were analysed by ANOVA) using SAS 9.3 TS Level 1M1. Differences within the means were compared by using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Effect of Individual salts on Growth of C. gloeosporioides

The efficacy of different bicarbonate salts with different concentrations where seen to vary among the treatments for the data taken; growth, PGI and spore count of *C. gloeosporioides*. Results are seen in Figure 2.

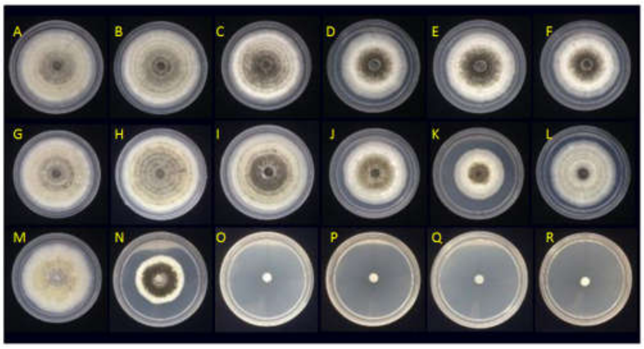


Figure 2 Interaction of*C. gloeosporioides*among the 3 bicarbonate salts at different concentration; (A) SBC 0.1%; (B) SBC 0.2%; (C) SBC 0.4%; (D) SBC 0.8%; (E) SBC 1.6%; (F) SBC 3.2%; (G) PBC 0.1%; (H) PBC 0.2%; (I) PBC 0.4%; (J) PBC 0.8%; (K) PBC 1.6%; (L) PBC 3.2%; (M) ABC 0.1%; (N) ABC 0.2%; (O) ABC 0.4%; (P) ABC 0.8%; (Q) ABC 1.6% and (R) ABC 3.2%.

Sodium bicarbonate. Low mycelium growth of the pathogen indicates success of the treatments used. For SBC treatments, all the concentrations used showed no significant difference compared to control treatments.Thus, not having any success in controlling the pathogen, *C. gloeosporioides.* This is contrary to reports by Smilanick et al (1999), solution treatments prepared using sodium bicarbonates were more effective than those prepared from potassium or ammonium salts in citrus green mold. PGI results for sodium bicarbonate in this study is also found to have no significant difference. There are negative results in treatments 0.1% and 0.4% concentration. This reflects that the concentration used seem to have made the pathogen to grow faster than that compared to control treatments. Results with high spore count were seen in treatments containing Sodium bicarbonate (SBC). Spore count having the highest was found to be from treatment SBC 1.6% with 183.7 spore count. This was then continued by treatment SBC 0.8% and SBC 0.4% respectively having 124.9 and 98.1 spore count.Differing of reports by De Costa, D.M. in 2012 stated in a study on *Colletotrichum musae* on banana; spore production was completely inhibited by using sodium bicarbonates. Nevertheless, bicarbonate salts have produced different results in different plants, making recommendations specific to the plant at hand.

Table 2 Growth (cm) of *C. gloeosporioides* among the 3 inorganic compounds at different concentration.

Sodium bicarbonate	Concentration (%)						
	Control	0.1	0.2	0.4	0.8	1.6	3.2
Mycelium Growth (cm)	7.66 a	7.66 a	7.53 a	7.70 a	7.60 a	7.60 a	7.65 a
Percent in Growth Inhibition (%)	N/A	-0.02 a	1.60 a	-0.58 a	0.73 a	0.73 a	0.07 a
Spore Count	5.9 a	5.2 a	6.5 a	98.1 a	124.9 a	183.7 a	67.7 a

Treatments in a row with the same letters do not differ significantly (P≥0.05) according to the Duncan's Multiple Range Test.

Potassium bicarbonate. As for PBC treatments, significant difference was seen in PBC 1.6%, with a mycelium growth diameter of 6.89 cm. This finding is concurrent with PGI results of 10% for PBC 1.6%. It is typical to have a better protection with a higher concentration. But in this case, PBC 3.2% did not give a better defence against the disease. This is concurrent with a finding by Wenneker, M. and Kanne J. (2010), where low dosage of potassium bicarbonate can be effective in controlling powdery mildew of gooseberries at weekly applications. For spore count, no significant difference was seen in the treatments when compared to control treatment.

Table 3 Growth (cm) of *C. gloeosporioides*among the 3 inorganic compounds at different concentration.

Potassium bicarbonate	Concentration (%)						
	Control	0.1	0.2	0.4	0.8	1.6	3.2
Mycelium Growth (cm)	7.66 a	7.47 a	7.63 a	7.67 a	7.26 ab	6.89 b	7.47 a
Percent in Growth Inhibition (%)	N/A	2.41 b	0.35 b	-0.15 b	5.13 ab	10.00 a	2.47 b
Spore Count	5.9 a	13.1 a	2.9 a	4.7 a	5.7 a	15.3 a	16.3 a

Treatments in a row with the same letters do not differ significantly (P≥0.05) according to the Duncan's Multiple Range Test.

Ammonium bicarbonate. In this study, low growth was seen in treatments ABC (Table 4). Where treatments ABC 0.4%, 0.8%, 1.6% and 3.2% was seen to have a growth performance of *C. gloeosporioides* at 0.90 cm each. This is due to the inoculation of *C. gloeosporioides* from the inoculation tool (cork borer) having a diameter of 0.90 cm. In reality, there was no growth recorded for these treatments.With the addition of higher concentration of bicarbonate salts, percent in growth inhibition (PGI) was seen to increase in ABC treatments. According to the PGI in Table 4, it was concluded that treatment ABC 0.4%, 0.8%, 1.6% and 3.2% was able to significantly control *C. gloeosporioides* at 88.24%. This is due to the fact that ammonium bicarbonate has additional toxicity towards microbes because it is a source of free ammonia gas besides the inhibitory effect of the bicarbonate ions (Mlikota and Smilanick, 2001). No spore count was obtained from treatments ABC 0.4%, 0.8%, 1.6% and 3.2%. This is concurrent with results from the growth of *C. gloeosporioides,* where no growth was found in these treatments stated.

Table 4 Growth (cm) of *C. gloeosporioides* among the 3 inorganic compounds at different concentration

Ammonium bicarbonate	Concentration (%)						
	Control	0.1	0.2	0.4	0.8	1.6	3.2
Mycelium Growth (cm)	7.66 a	7.70 a	2.33 b	0.90 c	0.90 c	0.90 c	0.90 c
Percent in Growth Inhibition (%)	N/A	-0.58 c	69.58 b	88.24 a	88.24 a	88.24 a	88.24 a
Spore Count	5.9 a	0.8 b	0.3 b	0 b	0 b	0 b	0 b

Treatments in a row with the same letters do not differ significantly (P≥0.05) according to the Duncan's Multiple Range Test.

Effect of Mix Salts on Growth of C. Gloeosporioides

Previously, we evaluated the salt solutions through dual culture screenings as individual component against the pathogen. In this experiment, the selected salt concentration having significant differences in controlling the pathogen as mixture of PBC and ABC with a lower concentration in dual culture screening. ABC 0.4% is set as the benchmark for this experiment as is was seen to be the best treatment as individual treatment.

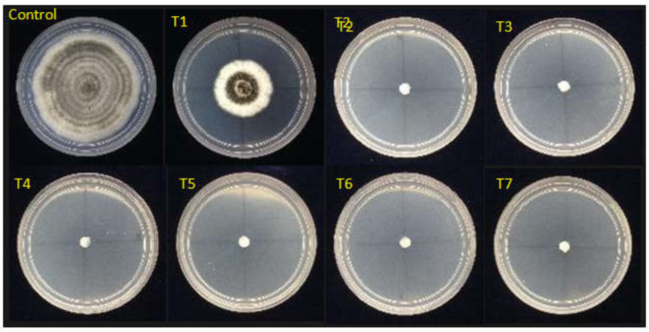
The mean diameter growth of *C. gloeosporioides* on Control treatments are 7.74 cm (Table 5). T1 had a significant difference compared to control treatments having a growth performance of 2.9 cm. Nonetheless, better results was seen by T2 to T7; where growth of pathogen was fixed at 0.9 cm, the width of the cork borer used for this experiment. It can be said no growth was evident. This is concurrent to PGI results where T1 had a significant result compared to the other treatments.

Thus T2 to T7 had a better control of the pathogen at hand. Therefore, it is chosenthe best control with the lowest concentration is proven to be a mixture of PBC 0.8% + ABC 0.2% in T2 with a PGI of 90.98%. The use of PBC and ABC has an added advantage where they provide nitrogen and potassium when used, giving needed nutrient to the plant (Beckerman, 2014).

Table 5 Growth and Percentage in Growth Inhibition of *C. gloeosporioides* using a mixture of inorganic compounds at different concentration.

	Bicarbonate mixture	Growth of *C. gloeosporioides* (cm)	Percentage of Growth Inhibition (%)
Control	-	7.74a	N/A
T1	PBC 0.8% + ABC 0.1%	2.900 b	62.62 b
T2	PBC 0.8% + ABC 0.2%	0.900 c	90.98 a
T3	PBC 1.6% + ABC 0.1%	0.900 c	90.98 a
T4	PBC 1.6% + ABC 0.2%	0.900 c	90.98 a
T5	PBC 3.2% + ABC 0.1%	0.900 c	90.98 a
T6	PBC 3.2% + ABC 0.2%	0.900 c	90.98 a
T7	ABC 0.4%	0.900 c	90.98 a

Treatments with the same letters do not differ significantly (P≥0.05) according to the Duncan's Multiple Range Test.



CONCLUSION

C. gloeosporioides can be significantly reduced by using bicarbonate salts that are safe for consumers and the environment. We conclude the best treatment to control *C. gloeosporioides* with the lowest concentration of bicarbonate salt at laboratory conditions is PBC 0.8% + ABC 0.2%.

Acknowledgement

This work is funded by the Malaysian Agriculture Research and Development Institute (MARDI).

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How to cite this article:

Nor Dalila, N.D *et al* (2019) 'Evaluation of Bicarbonates for the Control of Colletotrichum Gloeosporioides of Mango Plant', *International Journal of Current Advanced Research,* 08(05), pp. 18703-18706.

DOI: http://dx.doi.org/10.24327/ijcar.2019.18706.3583

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