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IN VITRO ASSESSMENT OF THE EFFICACY OF ORGANIC ACIDS AGAINST *BOTRYTIS GLADIOLORUM* CAUSAING GLADIOLUS LEAF BLIGHT

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ABSTRACT

An experiment was conducted at the Mycology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, to evaluate the efficacy of selected organic acids against *Botrytis gladiolorum*, the causal agent of gladiolus leaf blight. Nine organic acids—tartaric acid, oxalic acid, citric acid, ascorbic acid, acetic acid, benzoic acid, gallic acid, glutamic acid, and orthophosphoric acid—were tested for their effects on the colony growth of *B. gladiolorum*. These acids were applied at 1000 ppm, 2000 ppm, and 3000 ppm concentrations. At 5 days after inoculation (DAI), oxalic acid at 1000 ppm resulted in the minimum radial mycelial growth (26.10 mm), which was statistically similar to acetic acid (26.30 mm), with growth inhibition rates of 44.47% and 40.04%, respectively. At 10 DAI, the highest growth inhibition was observed with acetic acid (46.23%). By 15 DAI, acetic acid again showed the highest growth inhibition (43.22%), followed by benzoic acid (38.11%). When applied at 2000 ppm, acetic acid achieved the highest growth inhibition at 5 DAI (75.65%), followed by oxalic acid (49.41%). At 10 DAI, acetic acid maintained the highest inhibition (71.11%), with benzoic acid following at 54.69%. By 15 DAI, acetic acid continued to exhibit the highest inhibition (57.02%), with benzoic acid at 48.04%. At 3000 ppm, acetic acid inhibited mycelial growth at 5 DAI, followed by benzoic acid, which recorded a mycelial growth of 8.60 mm. At 10 DAI, acetic acid maintained 100% growth inhibition, followed by benzoic acid with 64.69%. At 15 DAI, acetic acid again provided the highest inhibition (86.75%), followed by benzoic acid at 58.54%. Among the organic acids evaluated in vitro, acetic acid, benzoic acid, and oxalic acid at 3000 ppm were found to be the most effective in inhibiting the growth of *Botrytis gladiolorum*.

Keywords: *Botrytis gladiolorum,* Management, Organic acid, Mycelial growth.

INTRODUCTION

Gladiolus (*Gladiolus grandiflorus* L.) is very much popular among all commercial flowers in Bangladesh. The climatic and agroecological conditions of Bangladesh are favorable for Gladiolus cultivation. The major production areas of this flower are Jashore, Dhaka, Manikganj, Narayanganj, Chattogram, Cox's Bazar, Bogura, Rangpur, Gaibandha, and Faridpur. The cultivation process of Gladiolus is comparatively easy and its economic value is more than other field crops. Income from gladiolus flower production is six times higher than that of rice in Bangladesh (Momin, 2006). Gladiolus hybrids are among the preferred cut flowers due to their different sizes, shapes and excellent vase life (Bose *et al.* 1989). Gladiolus is native to South Africa and has been cultivated globally though gladiolus cultivation commenced in Bangladesh around 1992 from India (Mollah *et al*. 2002). It has become popular for use on different occasions and events in Bangladesh. Its demand has been increasing day by day with the eye-catching beautification keeping aristocracy and modernization in Bangladesh.

Disease is one of the most important limiting factors for the commercial cultivation of gladiolus in Bangladesh. Gladiolus plant is attacked by several diseases throughout the world and production is strongly hampered due to disease intensity. Gladiolus plants are affected by fungal pathogens along with bacteria, viruses, and nematodes such as Botrytis leaf blight (*Botrytis gladiolorum*), Corm rot, or Fusarium rot (*Fusarium oxysproum* f. sp. *gladioli*), Curvularia leaf spot (*Curvularia trifolli* f. sp. *gladioli*), Nematodes (*Meloidognye, Pratylenchus, Trichodorus, Belonolaimus, Ditylenchus, Hemicyliophora, Rotylenchus*), Scab (*Pseudomonas marginata*), Stemphylium leaf spot (*Stemphylium botryosum*), Stromatinia dry Rot (*Stromatinia gladioli*), Viruses (Bean yellow mosaic, Cucumber mosaic, Tomato ring spot, Tobacco ring spot), etc (Elmer and Kamo 2018).

Nowadays, Botrytis blight which is caused by *Botrytis gladiolorum* becomes severe in the farmers' field of different cultivated regions in Bangladesh. The disease is manifested by spots on leaf, flower bud, inflorescence, stem and corm. Drayton (1928) reported *Botrytis* disease of gladiolus from Canada in 1928. The disease has also been reported in Holland (Drayton 1929), England (Moore, 1939), New York (Dodge and Laskaris,1941), Australia (Wade 1945), India (Sohi 1992, Singh *et al.* 2005), Pakistan (Mirza and Shakir, 1991) and Iran (Mirzaei *et al*., 2008). Sohi (1992) worked on diseases of ornamental plants and reported *B. gladiolorum* from corms and leaves of gladiolus in India. Blight caused by *B. gladiolorum* is noted as the major threat for gladiolus production in India (Singh *et al.* 2005). Mirza and Shakir (1991) reported *B. gladiolorum* from corm and leaves of gladiolus in Pakistan.

In recent years, disease problems appeared in Bangladesh as one of the major limiting factors for the cultivation of gladiolus. In the 2013-2014 crop seasons, Botrytis leaf blight of gladiolus appeared as a new disease in farmers' fields in the Jashore region (Siddique *et al.* 2013). The disease was manifested by characteristic symptoms of *Botrytis* blight as spots on leaf, flower bud, flower, stem, and corm. The disease incidence and severity were found very high and caused leaf and inflorescence blight. Almost all plants in a field were found to be infected by the disease. Moreover, the market price of flower sticks was reduced.

No attention has been given to botrytis blight of gladiolus and its control earlier in Bangladesh. Farmers depend on chemical pesticides for control of gladiolus leaf blight. It was observed that most farmers used locally available fungicides, but often the diseases were not well managed. Fungicides, and other types of pesticides, have recently been linked to cancer, and respiratory and hormone imbalance diseases (Piel, 2019; Hoppin, 2017; Juntarawijit, 2018).

Apart from the use of fungicides, the disease is controlled in various ways. Organic acids have been reported to be primarily responsible for their antimicrobial activity (Banwart 1981). In this case, we have used organic acids to control *Botrytis gladiolorum* causing botrytis leaf blight *in vitro* condition.

MATERIALS AND METHODS

Experimental site and duration

The experiment was conducted in the Mycology Laboratory, Department of Plant Pathology, Shere-e-Bangla Agricultural University, Dhaka during September, 2018 to October, 2018. Infected leaf samples were collected from gladiolus farmers' field and brought to the laboratory for study. Nine selected organic acids were tested *in vitro* to evaluate their efficacy on colony growth of the fungal isolate. Evaluation was done through the poison food techniques (Hawamdeh and Ahmad 2001).

Used organic acids and preparation of different concentrations

Nine organic acids were evaluated against the colony growth of *Botrytis gladiolorum.* The acids were tartaric acid, oxalic acid, citric acid, ascorbic acid, acetic acid, benzoic acid, galic acid, glutamic acid and ortho phosphoric acid. The concentrations of the organic acids were 1000 ppm, 2000 ppm and 3000 ppm. The organic acids were mixed with PDA media at 50^0C in different quantities to make the desired doses.

Bioassay of organic acids

Organic acids added in autoclaved (Shovan *et al.*, 2008) PDA Media which were distributed in ten conical flasks. The conical flasks without organic acid served as control media. 20 ml of sterilized PDA media were poured in each 9 cm petridishes. After solidification, the plates were inoculated with a 5 mm disk of 16-days-old cultures. Five replications were used for each concentration of organic acids based on CRD design. Radial colony growth was measured after 5 days, 10 days and 15 days of incubation (Plate 7 and plate 8). Diameter of colonies were measured in two directions from the underneath side, perpendicular to each other, taking growth as the mean of the two measures. Percent inhibition of radial growth was computed based on colony diameter on control plate using the following formula (Sundar *et al.* ,1995) and data were analyzed using MSTAT-C program (Khan *et al.*, 2007).

$$\% \text{ Inhibition} = \frac{X-Y}{X} \times 100$$

Where,

X= Growth of control plate

Y= Growth of organic acids treated plate

RESULT AND DISCUSSION

Efficacy of organic acids at 1000 ppm dose in controlling *Botrytis gladiolorum* in the laboratory

Nine selected organic acids were tested with at the rate of 1000 ppm against *Botrytis gladiolorum* and the radial mycelia growth of fungus was measured at 5, 10 and 15 DAI. All organic acids showed significantly different results as compared to control. At 5 DAI, the radial mycelia growth was found minimum (26.10 mm) by oxalic acid which was statistically similar with acetic acid (26.30 mm) followed by benzoic acid (28.80 mm) and the inhibition of growth was 44.47%, 40.04% and 38.72%, respectively. At 10 DAI, the highest inhibition of growth was found by acetic acid (46.23%), which was statistically similar with oxalic acid (42.92%) and benzoic acid (41.87%). At 15 DAI, the growth inhibition was highest (43.22%) by acetic acid followed by benzoic acid (38.11%) and oxalic acid (33.50%), respectively (Table 1 and Plate 1).

Table 1. Efficacy of organic acids at 1000 ppm in mycelial growth inhibition of *Botrytis gladiolorum in vitro* condition at 5, 10 and 15 days after inoculation

Treatments	Radial mycelial growth (mm)					
	5 DAI	% Growth inhibition over control	10 DAI	% Growth inhibition over control	15 DAI	% Growth inhibition over control
T_1=Tartaric acid	38.70 b	17.66	55.00 b	17.17	69.40 b	11.25
T_2=Oxalic acid	26.10 f	44.47	37.90 d	42.92	52.00 e	33.50
T_3=Citric acid	40.30 b	14.26	51.40 c	22.59	62.20 d	20.46
T_4=Ortho phosphoric acid	33.20 d	29.36	51.70 c	22.14	65.80 c	15.86
T_5=Ascorbic acid	35.50 c	24.47	54.70 b	17.62	65.30 c	16.50
T_6=Acetic acid	26.30 f	40.04	35.70 d	46.23	44.40 g	43.22
T_7=Benzoic acid	28.80 e	38.72	38.60 d	41.87	48.40 f	38.11
T_8=Gallic acid	39.50 b	15.96	51.40 c	22.59	69.50 b	11.13
T_9=Glutamic acid	35.00 cd	25.53	51.40 c	22.59	72.30 b	7.54
T_{10}=Control (Untreated)	47.00 a	-	66.90 a	-	78.20a	-
LSD (P=0.01)	2.15	-	2.81	-	2.978	-

Table 2. Efficacy of organic acids at 2000 ppm on mycelial growth inhibition of *Botrytis gladiolorum in vitro* condition at 5, 10 and 15 days after inoculation.

Treatments	Radial mycelial growth (mm)					
	5 DAI	% Growth inhibition over control	10 DAI	% Growth inhibition over control	15 DAI	% Growth inhibition over control
T_1=Tartaric acid	31.80 b	24.82	48.70 b	28.29	64.40 b	18.58
T_2=Oxalic acid	21.40 e	49.41	31.90 e	53.22	45.90 f	41.97
T_3=Citric acid	32.40 b	23.40	44.80 c	34.31	56.80 d	28.19
T_4=Ortho phosphoric acid	28.10 d	33.57	38.00 d	44.28	51.90 e	34.39
T_5=Ascorbic acid	30.50 bc	27.90	47.60 b	30.20	61.00 c	22.88
T_6=Acetic acid	10.30 f	75.65	19.70 f	71.11	34.00 h	57.02
T_7=Benzoic acid	20.50 e	51.53	30.90 e	54.69	41.10 g	48.04
T_8=Gallic acid	32.90 b	22.22	44.60 c	34.60	61.50 bc	22.25
T_9=Glutamic acid	29.20 cd	30.97	43.00 c	36.95	59.70 cd	24.53
T_{10}=Control (Untreated)	42.30 a	-	68.20 a	-	79.10 a	-
LSD (P=0.01)	2.25	-	2.64	-	3.20	-

Table 3. Efficacy of organic acid at 3000 ppm on mycelial growth inhibition of *Botrytis gladiolorum in vitro* condition at 5, 10 and 15 days after inoculation.

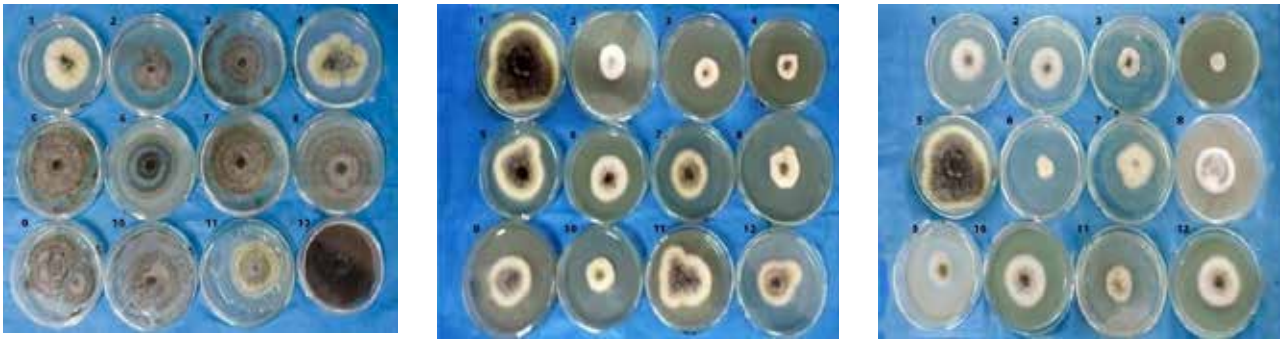
Treatments	Radial mycelial growth (mm)					
	5 DAI	% Growth inhibition over control	10 DAI	% Growth inhibition over control	15 DAI	% Growth inhibition over control
T_1=Tartaric acid	26.50 b	40.05	43.60 b	28.05	58.30 b	22.78
T_2=Oxalic acid	16.40 d	62.90	27.70 f	54.29	40.50 e	46.36
T_3=Citric acid	22.40 c	49.32	35.50 e	41.42	47.00 d	37.75
T_4=Ortho phosphoric acid	24.50 bc	44.57	35.70 e	41.09	47.10 d	37.62
T_5=Ascorbic acid	26.80 b	39.37	40.30 cd	33.50	52.80 c	30.07
T_6=Acetic acid	0.00 f	100	0.00 h	100	10.00 g	86.75
T_7=Benzoic acid	8.60 e	80.54	21.40 g	64.69	31.30 f	58.54
T_8=Gallic acid	27.40 b	38.00	41.90 bc	30.86	55.80 b	26.09
T_9=Glutamic acid	26.00 b	41.18	38.50 d	36.47	50.30 c	32.72
T_{10}=Control (Untreated)	44.20 a	-	60.60 a	-	75.50 a	-
LSD (P=0.01)	2.72	-	2.34	-	2.59	-

Efficacy of organic acids at 2000 ppm on mycelial growth inhibition of *Botrytis gladiolorum.*

Nine selected organic acids were tested with at the rate of 2000 ppm against *Botrytis gladiolorum* and the radial mycelia growth of fungus was measured at 5, 10 and 15 DAI. All organic acids performed significantly different results as compared to control. At 5 DAI, the radial mycelia growth was found minimum in acetic acid (10.30 mm) followed by oxalic acid (21.40 mm). benzoic acid showed (20.50 mm) growth inhibition was 75.65%, 49.41% and 51,53%. At 10 DAI, the highest growth inhibition was found in acetic acid (71.11%) followed by benzoic acid (54.69%). At 15 DAI, highest inhibition of growth found in acetic acid (57.02%) followed by benzoic acid (48.04%) and oxalic acid (41.97%) (Table 2 and Plate 1).

Efficacy of organic acids at 3000 ppm on mycelial growth inhibition of *Botrytis gladiolorum.*

Nine selected organic acids were tested with at the rate of 3000 ppm against *Botrytis gladiolorum* and the radial mycelia growth of fungus was measured at 5, 10 and 15 DAI. All organic acids showed significantly different results as compared to control. At 5 DAI, no radial mycelial growth was found in acetic acid (00) which was followed by benzoic acid (8.60 mm) and oxalic acid (16.40 mm) and the inhibition of growth of these organic acids were 100%, 80.54%, 62.90%, respectively. At 10 DAI, the highest growth inhibition found in acetic acid (100%), which was statistically followed by benzoic acid (64.69%) and oxalic acid (54.29%). At 15 DAI, the growth inhibition (86.75%) was highest in acetic acid followed by benzoic acid (58.54%) and oxalic acid (46.36%), respectively (Table 3 and Plate 1).



A B C

Plate 1. Mycelial growth Inhibition of *B. gladiolorum* by some selected organic acids at 15 days after inoculation (1= Ascorbic acid, 2= Citric acid, 3= Benzoic acid, 4= Acetic acid, 5= control, 6= Acetic acid, 7= Citric acid, 8= Tartaric acid, 9= Orthophosphoric acid, 10= Glutamic acid, 11= Oxalic acid, 12= Galic acid). A= 1000 ppm, B= 2000 ppm and C= 3000 ppm

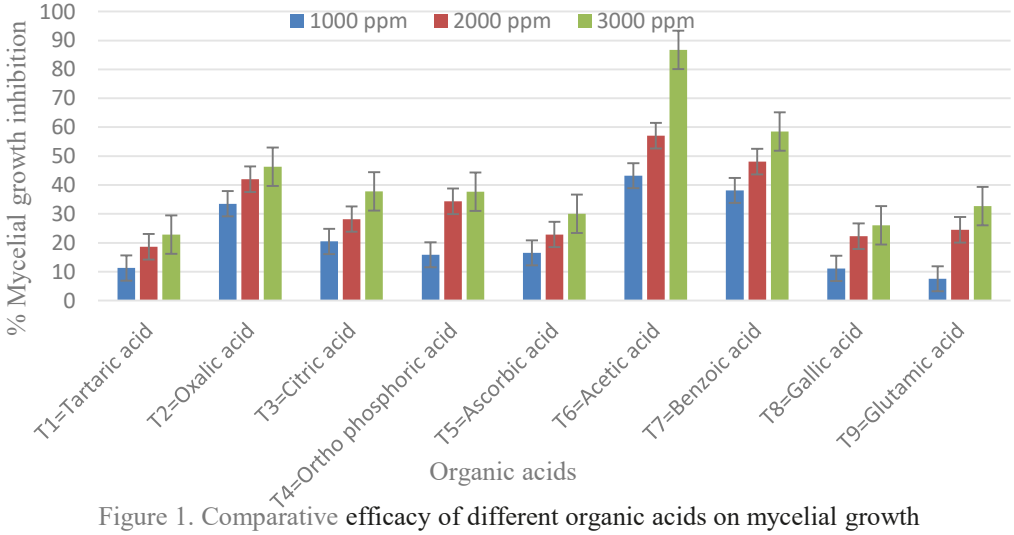


Figure 1. Comparative efficacy of different organic acids on mycelial growth inhibition of *Botrytis gladiolorum* under *in vitro* condition.

Comparative efficacy of different organic acids on mycelial growth inhibition of *Botrytis gladiolorum* at 15 days after inoculation

The highest growth inhibition was found by 3000 ppm in the case of all organic acids at 15 DAI. At 15 DAI, the growth inhibition was highest (86.75%) by acetic acid followed by benzoic acid (58.54%) and oxalic acid (46.36%), respectively (Figure 1). This dose can be used for further study in field conditions.

Abd-El-Kareem (2001) reported that acetic acid vapours caused complete inhibition of linear growth of *Botrytis cinerea* and reduced grey mould incidence of table grapes by more than 84.6% compared with control berries. In another study, Lagopodi *et al.* (2009) recorded the effects of acetic acid fumigation, ethanol fumigation, and steam heat treatment on the growth of *Botrytis cinerea in vitro*. Fumigation with 4 or 6 ⁄ µl acetic acid for 6 min, and 8 µl acetic acid for 3 or 6 min resulted in complete inhibition of fungal growth of *Botrytis cinerea*. Shokri (2011) evaluated the inhibitory effects of citric and tartaric acids and their combination on the growth of *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, *Candida albicans*, and *Malassezia furfur*.

CONCLUSION

The results showed that acetic acid had more fungistatic and fungicidal activities than tartaric acid against *Botrytis gladiolorum*.

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