International Journal of Agricultural and Environmental Research
FREE AND OPEN ACCESS
Available online at www.ijaaer.com
ISSN 2414-8245 (Online)
ISSN 2518-6116 (Print)

IN VITRO CHEMICAL CONTROL OF *ALTERNARIA* SP, THE CAUSE OF LEAF SPOT OF BITTER GOURD (*MOMORDICA CHARANTIA* L)

HAKIM KHAN[1], JAWAD UR REHMAN[1] AND MARIA[1]

[1]*Department of Plant Pathology, The University of Agriculture, Peshawar*
Corresponding author's email: hakimkhan74@yahoo.com

Abstract

An *in vitro* study was conducted to determine the efficacy of different concentrations (0, 500 and 1000 ppm) of Dithane M-45 against six different isolates of *Alternaria* sp, the cause of leaf spot of bitter gourd, in the Department of Plant Pathology, The University of Agriculture, Peshawar during 2010. The experiment was conducted by using Completely Randomized Design (CRD). The responses of six different isolates (Yaseen Abad, Nasirpur, Taimalpura, Jabba Daudzai, Zakhi Miana and Ghari Momin) of *Alternaria* sp against different concentrations of Dithan M-45 were significantly different from one another. After 10 days of incubation at 25^0C, Dithane M-45 @ 1000 ppm greatly (66.3%) affected the mycelia growth of Taimalpura isolate of *Alternaria* sp. Moreover, Yaseen Abad isolate showed partial resistance at that concentration of Dithane M-45, which inhibited the mycelia growth by 41.4% as compared to check.

Key words: *In vitro*, chemical contol, *Alternaria* sp, leaf spot, Bitter gourd, Dithane M-45.

INTRODUCTION

Bitter gourd (*Momordica charantia* L), locally called Karela, is regarded as one of the World's major vegetable crops and has great economic importance (Bakhru, 1997). Bitter gourd has been used in various Asian traditional medicine systems for a long time. Like most bitter tasting foods, bitter gourd stimulates digestion. This is helpful in people with sluggish digestion, dyspepsia and constipation (Grover and Yadav, 2004). It has been claimed that bitter gourd's bitterness comes from quinine. Bitter gourd is traditionally regarded by Asians, as well as Panamanians and Colombians, as useful for preventing and treating malaria. Laboratory studies have confirmed that various species of bitter gourd have anti-malarial activity (Waako *et al*, 2005). Laboratory tests suggest that compounds in bitter gourd might be effective for treating HIV infection (Nerurkar *et al*, 2006). As most compounds isolated from bitter gourd that impact HIV have either been proteins of glycoprotein and lectins. In 2007, the Philippine Department of Health issued a circular that bitter gourd can lower blood sugar levels (Abscal and Yarmell, 2005). It is also a promising candidate as a remedy that can help millions in the developing world who suffer from metabolic disorders such as diabetes (Miura *et al*, 2001).

It is planted on more than 60000 ha area annually but major insect transmitted diseases such as Cucurbit aphid borne yellow virus (CABYV), Papaya ring spot virus (PRSV) and Zucchini yellow mosaic virus (ZYMV) are spreading quickly, causing significant yield reduction. *Fusarium* wilt, powdery mildew, bacterial wilt and leaf spots (*Alternaria* sp) also cause considerable damage. Diseases reported on bitter

gourd are leaf spots (*Alternaria* sp, *Cercospora* sp and *Myrothecium roridunt*), powdery mildew, white rot of fruit and *Rizoctonia solani* fruit rot (Khan & Kamal, 1963, Maholay, 1989, Ali *et al*, 1989, Nair, 1982 and Mathur, 1990). *Alternaria* species are known as major plant pathogens. They are normal agents of decay and decomposition. The spores are airborne and found in the soil and water, as well as indoors and on objects. The club shaped spores are singly or form long chains. They can grow thick colonies which are usually black or gray. At least 20% of agricultural spoilage is caused by *Alternaria* spp (Krik *et al*, 2008).

This disease causes tiny brown spots on leaves which enlarge causing target spots with concentric rings. Older lesions will develop dark color in the concentric pattern. The dark color is caused by spore production which can cause new infection sites, if no protective measure were followed (Pati *et al*, 2008). The objective of this research was to check the efficacy of different concentrations of Dithane M-45 against *Alternaria* sp, the cause of leaf spot of bitter gourd.

MATERIALS AND METHODS

Affected leaf samples of bitter gourd were collected from Peshawar and Nowshera districts of Khyber Pakhtunkhwa during 2010 growing season of the crop. These were brought to the laboratory of Plant Pathology Department, The University of Agriculture, Peshawar. All these samples were preserved and packed in paper bags.

Isolation and Identification of the Pathogen: Pathogen from all the six locations of district Peshawar and Nowshera were isolated on general purpose media PDA (Potato Dextrose Agar) under aseptic conditions. Media was prepared using the standard procedure (for 1 liter of PDA, 250g potato, 20g agar and 20g dextrose) and was sterilized in autoclave for 20 minutes at 121^0C. Streptomycin was added before pouring to inhibit the bacterial growth. Infected bitter gourd leaves having leaf spot were cut into small pieces, surface sterilized with $HgCl_2$ (0.1%) for 15-30 seconds and thoroughly rinsed in sterilized distilled water. The specimens were then placed on Petri plates having PDA and kept at 25^0C in incubator for growth. After obtaining pure culture, temporary slides were made from that and observed under the microscope. Help was taken from the key of Barnet and Hunter (1970) for identification of the pathogen.

***In vitro* Study:** Poison food technique (PFT) was used to test the *in vitro* efficacy of different concentrations of Dithane M-45 (0, 500 and 1000 ppm). Different quantities of Dithane M-45 were mixed with PDA medium before pouring. Completely Randomized design (CRD) was used for the experiment, with three replications. Uniform inoculum size of the pathogen was placed in the centre of Petri plate having poisoned PDA. One treatment was kept as check, to assess the efficacy of different doses of Dithane M-45 on the six different isolates of Peshawar and Nowshera. The Petri plates were kept at 25^0C in incubator, for growth. Data was recorded by measuring colony diameter (cm) after five and ten days of incubation. All the recorded data were then analyzed by using analysis of variance and least significant difference test (Dana, 2001).

RESULTS AND DISCUSSION

Identification of the Pathogen: After thorough observation under the microscope and taking help the key of Barnet and Hunter (1970) the pathogen was identified up to genus level as *Alternaria* sp.

Growth of different isolates of *Alternaria* sp on poisoned PDA: Significant differences ($p<0.05$) were observed among the colony growth of *Alternaria* sp isolated from different locations of Peshawar and Nowshera after five days of incubation at 25^0C (Table 1). Dithane M-45 @ 500 ppm reduced the growth of *Alternaria* sp by 4.1-43.1%, with the highest (43.1%) reduction in isolate Nasirpur while the lowest (4.1%) of Jabba Daudzai as compared to check. Dithane M-45 @ 1000 ppm reduced the growth of *Alternaria* sp by 32.7-51.0%, with the highest reduction in isolate Nasirpur and lowest (32.7%) in Jabba Daudzai.

Data presented in Table 2 indicated significant ($p<0.05$) differences among the response of isolates of *Alternaria* sp to Dithane M-45 applied @ 500 ppm after 10 days of incubation. The colony diameter of all the six isolates were maximum (8.6-8.0cm) and non

significant among each other of the untreated check. Dithan M-45 @ 500 ppm reduced the colony diameter by 27.6-39.1% with highest (39.1%) reduction in isolate Ghari Momin and lowest (27.6%) in Nasirpur. Dithane M-45 @ 1000 ppm reduced the colony diameter of six different isolates by 41.4-66.3%, with the highest (66.3%) reduction in Taimalpura and lowest (41.4%) in Yaseen Abad (Table 2). The maximum (6.3cm) colony diameter was of the isolate Nasirpur while minimum (5.3cm) was of isolate of Ghari Momin, when PDA was poisoned with Dithane M-45 @ 500 ppm. Moreover, when PDA was poisoned with Dithane M-45 @ 1000 ppm, the maximum (5.1cm) colony diameter was of isolate Yaseen Abad and minimum (2.9cm) of isolate Taimalpura (Table 2).

Vegetables belonging to family cucurbitaceae are important due to their nutritional as well as economical values. However, the farmers face heavy yield losses both in their quality and quantity of these crops due to various diseases. Early blight diseases caused by fungal pathogen *Alternaria* sp inflict serious damage to these crops. Different *Alternaria* species were found to be associated with various angiospermic families but *A. alternata* usually inflicts members of cucurbitaceae. Beside these, pathogenic infections are reported due to *A. tenuissima* and *A. cucumerina* on cucurbitaceous plants (Neeraj and Varma, 2010). In treatment where fungicides were used, the mycelia growth of *Alternaria* sp was depressed substantially as compared to the treatment where no fungicides were used. This indicated the importance of fungicide used in controlling the disease.

Table 1. Effect of different doses of Dithane M-45 on the growth of different isolates of *Alternaria* sp after 5 days of incubation.

Districts (D)	Locations (L)	Treatments	Colony diameter (cm)	Decrease than check (%)
Peshawar (D_1)	Yaseen Abad (L_1)	No fungicide	5.1 a*	---
		500 ppm	3.5 cd	31.4[1]
		1000 ppm	2.9 de	43.1
	Nasirpue (L_2)	No fungicide	5.1 a	---
		500 ppm	2.9 de	43.1
		1000 ppm	2.5 e	51.0
	Taimalpura (L_3)	No fungicide	4.4 ab	---
		500 ppm	2.8 de	36.4
		1000 ppm	2.4 e	44.5
Nowshera (D_2)	Jabba Daudzai (L_1)	No fungicide	4.9 ab	---
		500 ppm	4.7 ab	4.1
		1000 ppm	3.3 cde	32.7
	Zakhi Miana (L_2)	No fungicide	4.8 ab	---
		500 ppm	4.4 ab	8.3
		1000 ppm	2.6 de	45.8
	Ghari Momin (L_3)	No fungicide	5.1 a	---
		500 ppm	4.1bc	19.6
		1000 ppm	3.1 de	39.2
LSD Value	---	---	0.9	---
CV (%)	---	---	14.0	---

**Values followed by different letter(s) are significantly different from one another at 5% level of significance.*
[1]Decrease (%) than their respective control.

Table 2. Effect of different doses of Dithane M-45 on the growth of different isolates of *Alternaria* sp after 10 days of incubation.

Districts (D)	Locations (L)	Treatments	Colony diameter (cm)	Decrease than check (%)
Peshawar (D_1)	Yaseen Abad (L_1)	No fungicide	8.7 a*	---
		500 ppm	5.5 bc	36.8[1]
		1000 ppm	5.1 c	41.4
	Nasirpue (L_2)	No fungicide	8.6 a	---
		500 ppm	6.3 b	27.6
		1000 ppm	3.9 d	55.2
	Taimalpura (L_3)	No fungicide	8.6 a	---
		500 ppm	5.9 bc	31.4
		1000 ppm	2.9 e	66.3
Nowshera (D_2)	Jabba Daudzai (L_1)	No fungicide	8.8 a	---
		500 ppm	5.5 bc	38.6
		1000 ppm	3.5 de	59.1
	Zakhi Miana (L_2)	No fungicide	8.6 a	---
		500 ppm	5.6 bc	34.9
		1000 ppm	3.1 de	64.0
	Ghari Momin (L_3)	No fungicide	8.7 a	---
		500 ppm	5.3 c	39.1
		1000 ppm	3.4 de	61.0
LSD Value	---	---	1.6	---
CV (%)	---	---	10.7	---

**Values followed by different letter(s) are significantly different from one another at 5% level of significance.*
[1]Decrease (%) than their respective control.

CONCLUSION AND RECOMMENDATION

All the six isolates were susceptible to Dithane M-45 both applied @ 500 & 1000 ppm in in vitro study. The mycelial growth of Taimalpura isolate of Alternaria sp was greatly affected by Dithane M-45 applied @ 1000 ppm. This reduced the mycelia growth by 66.3% as compared to check. Moreover, Yaseen Abad isolate of *Alternaria* sp showed resistance at this concentration of Dithane M-45, whose mycelia growth was reduced was reduced by 41.4% as compared to check. However, on the basis of these results and as far as the problem is concerned, I would like to recommend detail research work on the problem to solve the long-lasting problem of the farmers community.

LITERATURE CITED

Abascal, K.E. and E. Yarnell. 2005. Using bitter gourd to treat diabetes. Alternative and Cimplementary Therapies. 11(4):179-184.

Ali, S., A. Wahid, M. Murtaza and A. Nadeem. 1988. *Myrothecium* leaf spot of bitter gourd in Pakistan. Pakistan Journal of Agricultural Research. 9:598-600.

Avenor, H.F. and T.J. Michailides. 2007. Resistance of Boscalid Fungicides in *Alternaria alternata* isolates from Pistachio in California. Plant Disease. 91(10):1345-1350.

Bakhru, H.K. 1997. Foods that Heal: The Natural Way to Good Health. Orient aperbacks.

Barnet, H.L. and B.B. Hunter. 1970. Illustrated Genera of Imperfect Fungi. 3rd Edition. Burgess Publishing Company, Minneopollis, Minnesotar, USA. Pp.203.

Dana, S.D. 2001. Statistical and data analysis for the behavioral sciences. 1st Edition, Von Hofmann Press Incharge, New York, USA. 11:411-454.

Grover, J.K. and S.P. Yadav. 2004. Pharmacological action and potential uses of *Momordica charantia*: a review. Journal of Ethnopharmacology. 93(1):123-132.

Kaiser, B. and N. Labuschagne. 2006. *In vitro* inhibition of several hytopathogenic Fungi from avocado by soluble potassium silicate, South African Avocado Growers' Association Yearbook. Pp.29.

Kamal, M. and S.M. Moghal. 1968. Studies on plant diseases of South West Pakistan. Agricutural College, Tandojam, Pakistan. Pp.207.

Kepczynska, E. and J. Kepczynski, 2005. Inhibitory effect of methyl jamesonate on development of phytopathogen *Alternaria alternata* (Fr.) Kiessl. and its reversal by ethephon and ACC. 27(4):491-496.

Khan, S.A. and M. Kamal. 1963. Cercosporae of Sind region including 35 new records. Pakistan Journal of Science and Industrial Research. 6:118-119.

Kirk, P.M., P.F. Cannon, D.W. Minter and J.A. Stalpers. 2008. Dictionary of the Fungi. 10th Edition. Wallingford. CABI. Pp.22.

Maholay, M.N. 1989. Seed borne diseases of cucurbits. III. Bottle gourd (*Lagenaria siceraria* Mol.) Standl. Seed and Farm. 15:30-31.

Manthachitra, P. 1971. Investigations on seed borne fungi of some vegetable crops of Thailand. Summaries of research projects (1967-1988). S.B. Mathur. 1990. Danish Government Institute of Seed Pathology for Developing Countries, Denmark. Pp.18.

Mathur, S.B. 1990. Summaries of Research Projects 1967-1988. Danish Government Institute of Seed Pathology for Developing Countries, Denmark. Pp.111.

Miura, T., C. Itoh, N. Iwamoto, M. Kato, M. Kawai, S.R. Park and I. Suzuki. 2001. Hypoglycemic activity of the fruit of the *Momordica charantia* in type 2 diabetic mice. Journal of Nutritional Sciences and Vitaminology (Tokyo). 47(5):340-344.

Nair, L.N. 1982. Studies on mycoflora of seeds: some cucurbitaceous vegetables. Journal of Indian Botanical Society. 61:342-345.

Neeraj and S. Verma. 2010. *Alternaria* diseases of vegetable crops and new approaches for its control. Asian Journal of Experimental Biological Sciences. 1(3):681-692.

Neerurkar, P.V., Y.K. Lee and E.H. Linden. 2006. Lipid lowering effects of *Momordica charantia* (bitter gourd) in HIV-1-protease inhibitor treated human hepatoma cells, HepG2. British Journal of Pharmacology. 148(8):1156-1164.

Pati, P.K., Sharma, M. Salar, R.K. Sharma, A.P. Gupta and B. Singh. 2008. Studies on leaf spot disease of Withania somnifera and its impact on secondary metabolites. Indian Journal of Microbiology. 48:432-437.

Surviliene, E. and E. Dambrauskiene. 2006. Effect of different active ingredients of fungicides on *Alternaria* sp growth in vitro. Agronomy Research. 4(special issue):403-406.

Waako, P.J., B. Gumede, P. Smith and P.L. Folb. 2005. The *in vitro* and in vivo antimalarial activity of *Cardiospermum halicabum* L and *Momordica foetida* Schumch. Et Thonn. Journal of Ethnopharmacology. 99(1):137-143.