PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 14 (6): 325-329, 2015
ISSN 1680-5194

Isolation and Efficacy of Fungicides and Homeo-Fungicides Against Anthracnose of Chilies Caused by *Colletotrichum capsici*

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Abstract: Chili anthracnose damages chili fruits extensively at pre and postharvest stages causing anthracnose lesions. Even very small lesion of anthracnose on fruits of chili reduces the market value of chili crop. Fungitoxic effects of eight fungicides and homeo-fungicides were tested *in vitro* through poisoned food technique. There was a significant decrease in mycelial growth of the fungus with an increase in fungicide and homeo fungicide concentration in all the tested fungicides over the control. When mycelial growth of *Colletotrichum capsici* in response to fungicides and their concentrations were compared, Benomyl proved to be the best which gave highest control efficiency (100%) on all the concentrations. While Protest proved to be the least effective (48.7%) at highest concentration (1000 µg/ml) tested. Revus had also inhibited the mycelial growth of *Colletotrichum capsici* completely at three concentrations of 300, 500 and 1000 µg/ml, respectively. However, when growth of the fungus in response to homeo-fungicidal concentrations were compared Vampire proved to be the best as it had given the maximum control (83 percent) followed by Rigrous (81%) on 1000 µg/ml concentration. While Vantage proved to be the least effective (76%) at highest concentration (1000 µg/ml). None of the other tested fungicides or any of the homeo-fungicides have completely checked the mycelial growth of *Colletotrichum capsici*. From our results it is clear that with increase in fungicide concentration decreased the mycelial growth of fungus and homeo fungicides can be used as an alternative to the synthetic fungicides to reduce the hazardous impact on the environment.

Key words: *Colletotrichum capsici*, chili anthracnose, fungicides, homeo-fungicides

INTRODUCTION

Chili crop is cultivated in most of the tropics majorly as spice crop. In the year 2011 it was grown on an area of 2032263 ha with a total production of 3457533 tons (FAO, 2013). It is one of the popular spicy herbs in Pakistan. It is supplied as fresh, dry or as food ingredient depending on consumer's requirements domestically and exported to other countries.

Major constraints in chili production are the diseases mainly caused by fungi, bacteria and viruses which limit the successful production of chilies. Chili anthracnose caused by *Colletotrichum capsisci*, wilt caused by bacteria and mosaic caused by chili veinal mottle virus and cucumber mosaic virus are thought to be the most serious and destructive diseases of chili (Than *et al*., 2008). Chili Fruit is most susceptible to diseases like anthracnose and fruit rot.

Chili anthracnose damages chili fruits extensively at pre and postharvest stages causing anthracnose lesions. Even very small lesion of anthracnose on chili fruit reduces the market value of the crop (Manandhar *et al*., 1995). Anthracnose is thought to be the major constraints in production chili crop throughout tropics and subtropics (Than *et al*., 2008). Chili growing in wet tropical and subtropical climates is severely under pre or post-harvest fruit rot which causes extensive crop losses (Than *et al*., 2008). Fruit lesions are the most common aspect of this disease. *C. capsici* generally attack ripped red chili fruit, while *C. gloeosporioides* attacks both ripped and unripe fruits. Acervuli are produced in concentric rings within the lesions produced on fruit. In few instances these lesions turn brown and black from the production of setae and sclerotia (Roberts *et al*., 2001).

Integrated disease management technique are best in reducing the damage to chili crop as no single or specific control measure could eliminate anthracnose of chilies (Agrios, 2005). Effective control of these diseases usually involves the combine use of intrinsic resistance along with cultural, mechanical, biological and chemical control (Wharton and Dieguez-Uribeondo, 2004). Manganese ethylenebisdithiocarbamate (Maneb) is traditionally recommended fungicide for anthracnose management in chili, although it could not control the severe attack of anthracnose consistently and permanently on chili fruit (Smith, 2000). Disease caused by *C. capsici* is successfully controlled by chemicals on all the hosts (Ekbote, 2005). This disease can be controlled efficiently in normal weather conditions with spray of fungicides (Voorrips *et al*., 2004). However, tolerance and resistance against many fungicides often arise quickly, if we rely on single compound heavily (Staub, 1991).

Considering the economic importance of the crop, the disease and its impact on the production of chilies present research project was planned for *in vitro* evaluation of fungicide and homeo-fungicides against *Colletotrichum capsici*.

MATERIALS AND METHODS

This research was conducted in the Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan in 2013-14.

Isolation of pathogen: Infected chili pods/fruits showing characteristic anthracnose symptoms were collected from farmer's fields for the isolation of *Colletotrichum capsici*. Fungi were isolated from seeds and pods of chili. Unsterilized seeds and pieces of anthracnose affected fruit tissues were cut into 8-10 mm long and 8-10 mm wide pieces. These pieces and seeds were then placed on autoclaved potato dextrose agar (PDA) + 0.01% streptomycin media and were incubated at 28±2°C for three days. The mycelial growth and acervuli development around the seeds and pod peels were observed. Conidial masses picked from colonies which were suspected to be *Colletotrichum capsici* were re-streaked on PDA for further purification. *Colletotrichum capsici* was purified by single spore method and was identified on the basis of sporulating acervuli, conidia, setae and disease symptoms on chili pods with the help of relevant literature of the Sutton (1980).

E*valuation* fungicides and homeo-fungicides against mycelial growth of *Colletotrichum capsici*: Sensitivity of mycelial growth of *Colletotrichum capsici* against eight different fungicides viz; Protest (Propeneb 7WP), Score (Difenconazoel), Topas, Benomyle, Thiophenatemethyle, Revis, Anadoul, Pyrine and three homeo-fungicides viz; Rigrous, Ventage and Vampire were evaluated at eight different concentrations that were i.e., 10, 20, 30, 50, 100, 150, 300, 500 and 1000 µg/ml by using modified Borum and Sunclair's technique (1968).

Fungicide and homeo-fungicide concentration served as a single treatment which was replicated for five replications. A weighed quantity of each of the fungicide was amended to autoclave potato dextrose agar medium for obtaining required concentration of fungicides. Potato dextrose agar without fungicide served as control. Twenty five milliliter of amended and non-amended medium was poured in each of the five 90 mm diameter petri dishes. After solidification 5mm agar plugs containing *Colletotrichum capsici* mycelium were cut from 5 days old inoculated potato dextrose agar and were placed in the center of each Petri dish with the help of sterilized needle. The inoculated Petri dishes were incubated at 28±2°C. Data on radial mycelial growth (mm) of *Colletotrichum capsici* were recorded regularly on gap of 24 h till the mycelial colony grows to full growth on the control and data were analyzed statistically to see the difference among various treatments.

RESULTS

Efficacy fungicides against mycelial growth of *Colletotrichum capsici*: Fungitoxic effects of nine fungicides viz Protest, Topaz, Score, Benomyl, Thiophenate methyl, Revus, Anadol, Pyrineand Sentinel at eight concentrations that were i.e., 10, 20, 50, 100, 150, 300, 500 and 1000 µg/ml was tested *in vitro* by applying poisoned food technique. Analysis of variance shows highly significant results of fungicides, their concentrations and the interaction between fungicides and their concentration for reducing the mycelial growth of the *Colletotrichum capsici*. From our results it becomes clear that with increase in fungicide concentration decreased the mycelial growth of fungus. The results obtained on the fungitoxicity of fungicides against *Colletotrichum capsici* in *vitro* are presented in (Table 1). The fungitoxicity of eight fungicides varied greatly among each fungicide used and their concentrations (Table 2). As a general trend, there was a significant decrease in mycelial growth of the fungus with an increase in fungicide concentration in all the tested fungicides over the control. However, when growth of the fungus in response to various fungicide concentrations after eight days of incubation at 28±2°C compared on the basis of comparison of means of all fungicide concentrations Score proved to be the best as it had given the maximum control (100%) on all the concentrations tested. While Protest proved to be the least effective (48.7%) at highest concentration (150 µg/ml) tested. Revus had also inhibited the mycelial growth of *Colletotrichum capsici* completely at two concentrations of 100 and 150 µg/ml, respectively. None of the other tested fungicides have completely checked the mycelial growth of *Colletotrichum capsici*

Thus, the most effective fungicides in inhibiting the growth of the fungus, in descending order were Score, Thiophanate methyl, Revus and Benomyl as they caused 100, 79.1, 74.4 and 72.5% reduction over the control in mycelial growth *Colletotrichum capsici*, respectively. While, Protest, Anadol and Topas were least effective in inhibiting the mycelial growth of fungus as they caused 29.9, 50.9 and 51.9% reduction in mycelial growth over the control, respectively (Table 2).

Efficacy of homeo-fungicides against mycelial growth of *Colletotrichum capsici*: Fungitoxic effects of three homeo-fungicides viz; Rigrous, Ventage and Vampire at eight different concentrations viz; 10, 20, 30, 50, 100, 150, 300, 500 and 1000 µg/ml were tested *in vitro* by applying poisoned food technique. Analysis of variance shows highly significant results homeo-fungicides, their concentration and interaction between fungicides and fungicide concentrations. The results obtained on the fungitoxicity of homeo-fungicides against *Colletotrichum capsici in vitro* are presented in (Table 3). The fungitoxicity of three homeo-fungicides varied greatly among each other and their concentrations (Table 4). In general, there was a significant decrease in mycelial growth of the fungus with an increase in fungicidal

Table 1: Efficacy of fungicides against mycelial growth of *Colletotrichum capsici*

	Fungicide concentrations							
Fungicide	10 ppm	20 ppm	50 ppm	100 ppm	150 ppm	300 ppm	500 ppm	1000 ppm
Protest	6.05^{bcd}	5.13^{efg}	4.71^{fgh}	4.63^{fhg}	4.34^{ijl}	3.34^{jkl}	3.28^{kl}	2.77^{klm}
Benomyl	0.00^{w}	0.00^{w}	0.00^{w}	0.00^{w}	0.00^{w}	0.00^{w}	0.00^{w}	0.00^{w}
Topas	5.36^{e}	3.75^{ij}	3.49^{jk}	3.16^{kl}	2.21^{mno}	2.01^{mnopq}	1.87^{opqrs}	1.53^{opqrst}
Score	5.23^{ef}	1.31^{pqrstu}	1.16^{qrstu}	1.13^{qrstu}	1.01^{stu}	0.87^{tuv}	0.63^{uvw}	0.14^{vw}
Revis	1.95^{nopqr}	1.65^{opqrst}	1.40^{pqrstu}	1.32^{pqrstu}	1.09^{rstu}	0.89^{tuv}	0.85^{tuvw}	0.60^{uvw}
Thiophenate methyl	3.61^{j}	2.34^{mno}	2.00^{mnopq}	1.81^{opqrs}	1.21^{qrstu}	0.00^{w}	0.00^{w}	0.00^{w}
Anadoul	5.69^{ede}	4.51^{gh}	3.79^{ij}	3.58^{j}	2.31^{no}	1.39^{pqrstu}	1.19^{qrstu}	0.89^{tuv}
Pyrine	5.41^{de}	3.81^{ij}	3.07^{kl}	2.72^{lmn}	2.06^{mnop}	1.53^{opqrst}	1.37^{pqrstu}	1.13^{qrstu}
Sentinel	5.51^{de}	4.35^{hi}	3.66^{ij}	3.41^{kl}	2.25^{mno}	1.15^{qrstu}	1.01^{stu}	0.84^{tuvw}
Control	7.04^{a}	6.23^{bc}	6.45^{ab}	6.77^{ab}	6.23^{bc}	6.77^{ab}	6.76^{ab}	6.23^{bc}

CV = 15.03%

Table 2: Efficacy of fungicides against mycelial growth of *Colletotrichum capsici*

	Fungicide concentrations							
Fungicide	10 ppm	20 ppm	50 ppm	100 ppm	150 ppm	300 ppm	500 ppm	1000 ppm
Protest	12^{vw}	17^{vw}	30^{uv}	27^{uv}	30^{uv}	49^{opqrst}	52^{nopqrs}	55^{mnopqr}
Benomyl	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Topas	23^{vw}	40^{stu}	53^{nopqrs}	58^{lmnopqr}	64^{ijklmn}	70^{ghijkl}	71^{fghijkl}	75^{defghij}
Score	24^{vw}	79^{defgh}	83^{cdefg}	83^{cdefg}	84^{bcdefg}	87^{abcdef}	95^{abc}	98^{ab}
Revis	72^{fghijkl}	73^{fghijk}	80^{cdefgh}	80^{cdefgh}	82^{cdefgh}	87^{abcdef}	89^{abcde}	90^{abcd}
Thiophenate methyl	47^{qrst}	62^{klmnop}	73^{fghijk}	75^{defghij}	80^{cdefgh}	100^{a}	100^{a}	100^{a}
Anadoul	17^{vw}	27^{uv}	46^{rst}	49^{opqrst}	63^{ijklmnop}	79^{defgh}	83^{cdefg}	85^{abcdefg}
Pyrine	21^{vw}	38^{tu}	59^{klmnopqr}	61^{klmnopq}	67^{hijklm}	77^{defghi}	80^{cdefgh}	82^{cdefgh}
Sentinel	20^{vw}	30^{uv}	48^{pqrst}	50^{nopqrst}	63^{klmnop}	83^{cdefg}	85^{abcdefg}	86^{abcdefg}

CV = 10.22%

Table 3: Efficacy of homeo-fungicides against mycelial growth of *Colletotrichum capsici*

	Fungicide concentrations								
	10 ppm	20 ppm	30 ppm	50 ppm	100 ppm	150 ppm	300 ppm	500 ppm	1000 ppm
Rigurous	4.44^{abc}	3.47^{bcd}	4.57^{abc}	2.07^{def}	1.85^{def}	1.31^{f}	1.23^{f}	1.13^{f}	1.11^{f}
Ventage	5.37^{a}	4.44^{abc}	4.19^{abc}	4.23^{abc}	3.47^{abc}	1.25^{f}	1.71^{ef}	1.67^{ef}	1.39^{f}
Vampire	2.78^{cdef}	3.30^{cde}	1.03^{f}	5.23^{a}	1.09^{f}	1.57^{f}	1.09^{f}	1.00^{f}	0.98^{f}
Control	5.84^{a}	6.17^{a}	5.84^{a}	6.17^{a}	5.84^{a}	6.17^{a}	5.84^{a}	6.17^{a}	5.84^{a}

CV = 27.14%

Table 4: Efficacy of homeo-fungicides against mycelial growth of *Colletotrichum capsici*

	Fungicide concentrations								
	10 ppm	20 ppm	30 ppm	50 ppm	100 ppm	150 ppm	300 ppm	500 ppm	1000 ppm
Rigurous	23^{def}	39^{bcd}	20^{ef}	65^{a}	71^{a}	78^{a}	79^{a}	81^{a}	81^{a}
Ventage	6^{fg}	23^{def}	29^{cde}	29^{cde}	39^{bcd}	79^{a}	70^{a}	71^{a}	76^{a}
Vampire	51^{b}	44b^{c}	82^{a}	11	82^{a}	73^{a}	82^{a}	83^{a}	83^{a}

CV = 15.70%

concentration. However, when growth of the fungus in response to various fungicidal concentrations at an incubation period of eight days at 28±2°C compared Vampire proved to be the best as it had given the maximum control (83.4%) followed by Rigrous (82.2%) on 1000 µg/ml concentration. While Vantage proved to be the least effective (76.5%) at highest concentration (1000 µg/ml). None of the pother tested fungicides have completely checked the mycelial growth of *Colletotrichum capsici*. There was a continuous trend that with increase in the concentration of homeo-fungicides decrease was seen in the mycelial growth of the *Colletotrichum capsici* (Table 3).

DISCUSSION

Chili anthracnose caused by *Colletotrichum capsici* is a major constraint in chili production throughout the world. The integrated management strategies should include minimum use of chemicals for checking the pathogen population buildup, encouraging the growth of beneficial biological agents to reduce pathogen inoculum, modification of cultural practices and maximum cultivation of resistant varieties (Yoon *et al*., 2004).

Preliminary evaluation of comparative efficacy of fungicides on the mycelial growth of *Colletotrichum capsici* revealed that the effectiveness of fungicides in inhibiting the mycelial growth of the pathogen varied greatly. As a general trend, there was a significant decrease in mycelial growth of the fungus with an increase in fungicide concentration in all the tested fungicides over the control. On the basis of comparison of means of all fungicide concentrations score proved to be the best as it had given the maximum control (100%) on all the concentrations tested. Thiophanate methyl had also inhibited the mycelial growth of *Colletotrichum capsici* completely at two concentrations of 300, 500

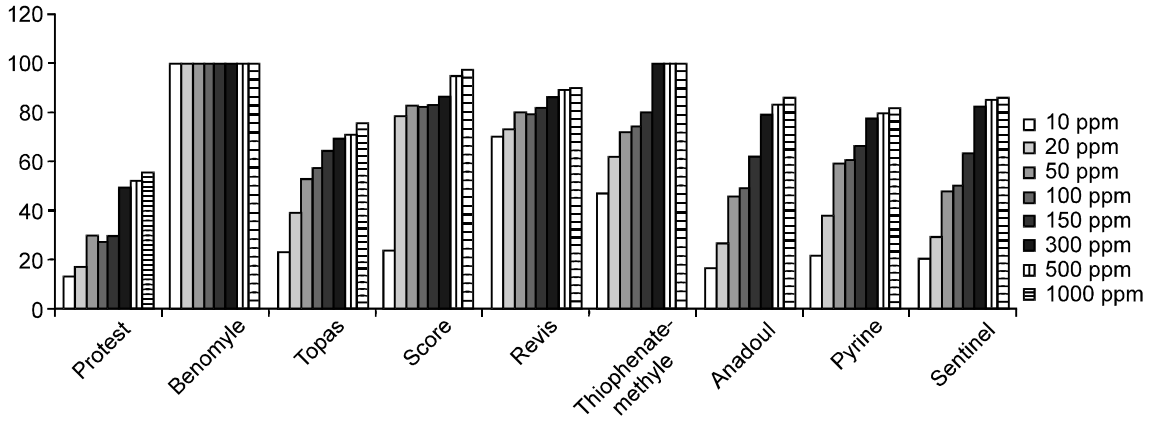

Fig. 1: Efficacy of fungicides against mycelial growth of *Colletotrichum capsici*

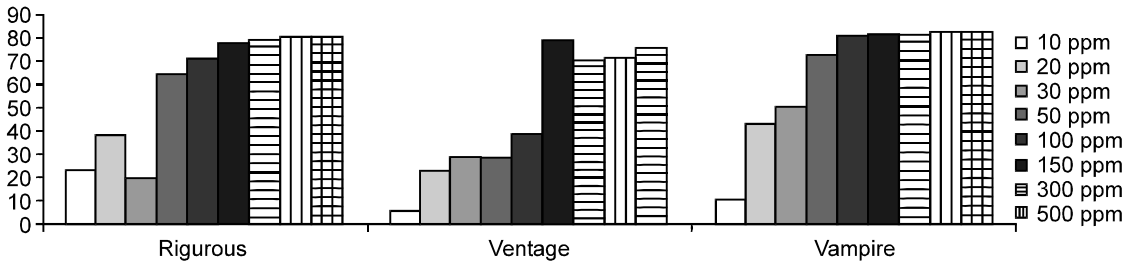

Fig. 2: Efficacy of homeo-fungicides against mycelial growth of *Colletotrichum capsici*

and 1000 µg/ml, respectively. While Protest proved to be the least effective (55%) at highest concentration (1000 µg/ml) tested. None of the other tested fungicides have completely checked the mycelial growth of *Colletotrichum capsici*. Integrated disease management technique are best in reducing the damage to chili crop as no single or specific control measure could eliminate anthracnose of chilies (Agrios, 2005). Effective control of these diseases usually involves the combine use of intrinsic resistance along with cultural, mechanical, biological and chemical control and (Wharton and Dieguez-Uribeondo, 2004). A lot of scientists have proved the efficacy of synthetic fungicides for the control of *C. capsici*. Manganese ethylenebisdithiocarbamate (Maneb) is traditionally recommended fungicide for anthracnose management on chili fruit (Smith, 2000). Ekbote (2005), has successfully controlled this disease by using chemical fungicides on all the hosts. Voorrips *et al*. (2004) says that this disease can be controlled efficiently fungicide sprays. Three fungicides; Benomyl 50 WP, Folicar and Radomil 75 WP completely (100%) inhibited the growth of *C. gloeosporioides*. Shukla and colleagues in 2013 found that Propiconazole, Carbendazim, Benomyl, Mancozeb and Metalaxyl-mancozeb effectively inhibited mycelial growth of *C. capsici* at 2.8, 4.6, 6.0, 9.3 and 11.2 µg/ml.

These results are confirming our results but tolerance and resistance against many fungicides often arise quickly, if we rely heavily on synthetic compound for the control of diseases (Staub, 1991). To minimize the risk of fungicide tolerance and resistance new and alternate ways are to be investigated regularly. In this search homeo-fungicides were tested which were quite effective but less as compared to the synthetic fungicides. These homeo-fungicides can be used singly or as combination with the synthetic fungicides to reduce the concentration of fungicides or the number of fungicides sprays to control the anthracnose of Chili. Homeo-fungicides alone can also be used as an alternative with reduced spray interval to reduce the use of synthetic fungicides and their hazardous implications on environment. Furthermore, *in vivo* investigations are to carried out to assess the effectiveness of homeo-fungicides and their combinability with the synthetic fungicides.

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