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GENETIC AND BIO-CHEMICAL STRETIGIES FOR THE MANAGEMENT OF POWDERY MILDEW DISEASE OF WATERMELON (*CITRULLUS LANATUS*)

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A B S T R A C T

Watermelon is a highly nutritious crop and its yield is affected by powdery mildew worldwide. Powdery mildew incidence has increased in recent years due to the tunnel farming trend. Conventional strategies like cultural practices e.g. sowing time and over fertigation have not been effective to control the powdery mildew. So, present study was carried out to evaluate different watermelon cultivars against powdery mildew and to provide information about the effectiveness of different fungicides and plant extracts for its control. The experiments were carried out in research area of Department of Plant Pathology, University of Agriculture Faisalabad during 2013-2014. Twelve varieties of watermelon were sown in randomized complete block design with three replications. Inoculation was done by spray method. After the onset of disease, fungicides and plant extract treatments were applied weekly. Data were analyzed statistically and treatment means were compared by using Least Significant Difference (LSD) test. Significant differences for powdery mildew resistance were observed in 12 genotypes and varieties WT2257 and Zcugma F1 showed only 3% disease incidence showing higher disease resistance. Other resistant watermelon verieties were Panther, Pata Nagra and Ayesha 1 with disease incidence of 5, 7 and 9% respectively. On the other hand Sugar Baby and Anar Kali varieties showed 78 and 67% disease. Among the six extracts, neem extract showed least disease incidence of 17.56%. Garlic and sohanjana extracts showed 25.78 and 30.44% disease occurrence. Among the six fungicides, the least disease incidence was recorded in Gemstar with mean of 9.78%. Raydar and Crest fungicide sprayed crop had 15 and 19% disease. Highest disease incidence was recorded in control with mean of 69.11%. It is concluded that genetic resistance, pesticide and plant extract application showed significant variation in disease incidence.

Keywords: Watermelon, Genetic resistance, Cultivars, Fungicides, Botanicals.

INTRODUCTION

Watermelon (*Citrulus lanatus*) belongs to the family cucurbitaceae and is widely cultivated throughout the world especially in subtropical and tropical areas for consumption of its sweet and juicy flesh (Simmons *et al*., 2010). It is a highly nutritious fruit which is consumed in the form of chunks, slices, juice, preserved rind, edible seeds and glace candy. It contains 93% water, the highest amount in any fruit and also has minute quantities of fat, vitamins, protein and minerals. Its seed is a source of iron, zinc and protein (1 gram per 24 seeds). It is a very good source of water in summer and helps to avoid dehydration. Watermelon flesh has vitamin A, carbohydrates and lycopene and lycopene is an anti carcinogenic compound conferring resistance against cancers of pancreas, stomach and prostate (Edwards *et al*., 2003). Watemelon is of the most important cucurbit crop as it is a potential source of proteins, carotenoids and lycopene with an antioxidant potential (Melger *et al*., 2008), nutrients and minerals (Bolin and Brandenberger, 2001).

Various diseases attack the watermelon crop, among these some fungal diseases like stem blight, anthracnose, fusarium wilt and powdery mildew are the most important due to the significant yield losses caused by them. In the last two decates, most of watermelon cultivars were developed from few lines and this narrow germplam or genetic base resulted in susceptibility to various pests and diseases (Levi *et al*., 2000). Four most important fungal diseases of watermelon are stem blight, anthracnose, fusarium

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wilt and powdery mildew. Powdery mildew is an important disease of cucurbits like cucumbers, muskmelons, watermelons, squash, gourds, and pumpkins (McGrath, 2001b). It causes damage to cucurbits and not only limits their yield but also enhances the input cast due to continuous use of fungicide. The fungus *Podosphaera xanthii*is the causal agent of the disease in watermelon and 50-70% infection and 30-50% yield losses are reported due to this disease (Robinson and Provvidenti, 1975). A powdery mildew infection lowers plant photosynthates and cause decline in plant growth and development, cause premature leaf loss, and accordingly a reduced yield. The disease severity and length is positively correlated with yield reduction (Mossler and Nesheim, 2005).

White spots of mycelium are formed on plant stem, leaf and petioles which can be easily recognized as conidial spores and hyphae are produced on leaf surfaces due to disease. Older leaves are more susceptible to this disease and small concentrated brown circular spots which turn to white on later stages. Leaves are adversely damaged and changes to pale yellow and later to brown and finally the leaves are shriveled and expose cucurbits fruits to sunburn which minimize crop yield due to reduced size, number, quality, flavor and storage life of fruit. Under favorable conditions, the disease occurs so rapidly that entire field may appear white within a week to ten days (Keinath and DuBose, 2004).

The best method of growing disease free cucurbit crops is to grow varieties which have genetic resistance towards powdery mildew disease Rehman *et al*., 2014. Consequently, the simplest way to deal with powdery mildew infections is to select a suitable resistant variety. However, because of the presence of different fungal races of the disease, a specific fungal race may counteract the effect of resistant cultivar (Zitter *et al*., 1996). Fungicide is the most efficient mean of controlling powdery mildew when disease occurs due to the need to manage powdery mildew disease in cucurbits (Konstantinidou-Doltsinis *et al*., 2007). Most of the fungicides which are used against powdery mildew disease are preventive; i.e. to be operative they must be applied before the occurrence of infection (Brown 2002). Systemic and translaminar fungicides have special importance against powdery because they provide enough protection on both sides of the leaf surface (McGrath, 2001a).

Bio-rational materials with low phytotoxicity may have a central part in disease management programmes. These compounds do not have a toxic effect on plants; in fact in many cases the mechanism by which these agents suppress the disease is still not known. Among these biorational materials which are used in managing powdery mildew disease are mineral and natural oils, cow's milk, silicon, peroxigens and salts of monovalent cations such as potassium, sodium and ammonium (Belanger and Labbe, 2002).

Keeping in the view of damage caused by powdery mildew to watermelon, identification of resistant cultivars, best fungicides and botanicals is very important. The present study was carried out to provide basic knowledge about controlling of powdery mildew by fungicides and botanicals.

MATERIALS AND METHODS

Screening of watermelon germplasm against powdery mildew: A set of 12 varieties of watermelon were evaluated under field conditions for resistance to powdery mildew at Research Area of Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan during crop season 2013-2014. The seeds were obtained from Ayub Agricultural Research Institute and local grain market, Faisalabad. The varieties were Anar kali, Panther, Charleston- Grey, Asia Black, Sugarbaby, Ayesha 1, Black magic, Patanagra, Augusta, Othello, Zeugma F1 and WT 2257.

Seeds were sown directly in the field on August 6, 2013 under Randomized Complete Block Design (RCBD) with plant to plant (P×P) 40cm and bed to bed (B×B) 120cm distance. In order to raise a good crop recommended dose of fertilizers and water were applied. Symptoms appeared on plants 47 days after sowing of seeds and continuing until at the end of the harvesting. After every three varieties, the check variety was repeated and all the varieties were compared with check. Data regarding disease incidence (D.I) recorded after seven days interval by using modified Horsfall-Barratt scale 0-12 (Horsfall and Barratt, 1945).

Disease rating scale used against powdery mildew of watermelon: Percentage of powdery mildew coverage (PMC) was estimate and disease severity was scored using the 12-grade scale described by Horsfall and Barratt (1945) with minor modifications of Gafni *et al.,* 2015, 0 = 0%, 1 = 0–3%, 2 = 3–6%, 3 = 6–12%, 4 = 12–25%, 5 = 25–50%, 6 = 50–75%, 7 = 75–87%, 8 = 87–94%, 9 = 94–97%, 10 = 97–100%, 11 = 100%

Disease incidence was calculated by using following formula:

$$\% \text{ Disease incidence} = \frac{\text{No. of infected plant}}{\text{Total number of plant}} \times 100$$

Inoculation of powdery mildew: Disease infected material was obtained from Ayub Agriculture Research Institute, Faisalabad. Inoculum was obtained from fresh sporulating colonies on infected leaves 9-12 days after inoculation. Conidia were gently brushed into a small quantity of distilled water and then taking two drops from it and counted with a hemocytometer to maintain a suspension of 3×10^4 conidia ml^{-1} for inoculation. The upper surface of each leaf of each plant was uniformly sprayed with a conidial suspension delivered from a hand sprayer. At 5-6 leaves stage disease started appearing on lower side of the leaves. At this stage some selected plants sprayed with tested fungicides and plant extracts while some non-treated considered as check. Data regarding disease incidence were recorded after the spray of chemicals and plant extracts five, ten and fifteen days after each spray.

Efficacy of different plant extracts against powdery mildew under field conditions: Five plant extracts, Neem (*Azadirachtaindica*), Garlic (*Allium Sativa*), Sohanjana (*Moringaolifera*), Aak (*Calotropisprocera*) and Shisham (*Dalbergiasisu*) were used against powdery mildew disease at standard doses. The leaves of test plants were collected from UAF campus area and Plant extracts were prepared by grinding them with manual plant extraction machine. The extract obtained was considered as standard and was stored at -20°C until use. Three foliar sprays of plant extracts at 20, 30 and 40% concentrations were applied at an interval of 10 days. After each spray disease incidence data was collected two times at five days interval.

Efficacy of different chemicals against powdery mildew under field conditions: After the initiation of disease selective fungicides were applied on weekly basis. Fungicides were prepared at standard labeled rates in 20 L of water and were applied to seedlings with a hand held knap-sack sprayer. Non chemical water sprayed controls were also included in the experiment as check. The fungicides used in the trial were Crest, Raydar, Gemstar, Nativo and Curzate.

Statistical analysis: Data were analysed statistically and means were compared by using Least Significant Difference (LSD) test. ANOVA was used to determine the effect of plant extracts and chemicals on the disease incidence (Steel *et al.,* 1997).

RESULTS

Screening of watermelon varieties against powdery mildew disease: The experiment was conducted to screen 12 watermelon varities for resistance against powdery mildew disease showed significant differences in watermelon varieties for powdery mildew resistance (Table 1.). The least disease incidence was recorded in two varities WT 2257 and Zcugma F1 with mean of 3% showing highest resistance against powdery mildew. Other watermelon varities which showed resistance were Panther, Pata Nagra, Ayesha 1 and Othello with mean disease incidence of 5, 7, 9 and 10% respectively. On the other hand, Sugar Baby variety showed highest incidence of powdery mildew disease with mean of 78% followed by Anar Kali, Asia Black and Charleston-Grey with means of 67, 47 and 45 respectively. These results showed that there are notable differences present in watermelon germplasm for powdery mildew disease resistance which can be utilized in breeding procedures for development of powdery mildew resistant varieties.

Table 1. Genotypic response of watermelon against powdery mildew disease

Name Variety	Percent Incidence	Rating Index	Response
WT 2257 and Zcugma F1	3%	1	Highly Resistance
Panther, Pata Nagra, Ayesha 1 and Othello	5-10%	3	Moderatly Resistance
Augusta and Black magic	15-28%	4	Resistance
Charleston-Grey, Anar Kali and Asia Black	45-67%	5-6	Succeptible
Sugar Baby	78%	7	Highly Succeptible

Effect of different plant extract concentrations on disease incidence: The mean values of disease incidence showed that treatments and plant extract concentrations were highly significant. While treatment and concentration interactions were non-significant (Table 2) implicating that main effects are more important than interaction effects for disease incidence. Among the six treatments the minimum disease incidence was observed in case of neem extract with mean value of 17.56%. Other treatments in which lower disease incidence values was recorded were garlic, sohanjana and aak with mean values of 25.78%, 30.44% and 34.00% respectively. Whereas highest disease incidence was showed in control and shisham extract with mean values of 69.56% and 49.67% respectively. Among the three concentrations the 40% gave significant results (35.56%) as compared to others 20% (40.11%) (Table 3).

Table 2. Means for plant extracts concentration on disease incidence.

Sample	Concentration			Mean
	C1	C2	C3	
Sohanjna	32.33 ± 0.88	31.00 ± 1.15	28.00 ± 0.58	30.44 ± 0.78D
Garic	28.00 ± 1.15	25.67 ± 1.20	23.67 ± 1.76	25.78 ± 0.94E
Neem	19.00 ± 1.15	17.33 ± 1.20	16.33 ± 1.76	17.56 ± 0.80F
Shisham	54.00 ± 1.73	49.67 ± 1.20	45.33 ± 1.45	49.67 ± 1.45B
Aak	36.33 ± 1.45	34.00 ± 1.73	31.67 ± 2.03	34.00 ± 1.11C
Control	71.00 ± 1.73	69.33 ± 2.03	68.33 ± 1.76	69.56 ± 1.00A
Mean	40.11 ± 4.24A	37.83 ± 4.19B	35.56 ± 4.18C	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Effect of different plant extracts spray intervals on disease incidence: The mean values (Table 3) clearly indicate the non-significant interaction of different plant extracts with spray intervals (S). Among the three spray intervals the lowest disease incidence was recorded on 3rd spray and the highest disease incidence was recorded at 1st spray with the mean values of 34.78% and 37.06% respectively.

Table 3. Means for plant extract sprays at various intervals on disease incidence.

Sample	Sprays			Mean
	S1	S2	S3	
Sohanjna	31.00 ± 1.15	30.00 ± 1.15	29.00 ± 1.15	30.00 ± 0.65CD
Garic	29.33 ± 0.88	28.67 ± 0.88	27.67 ± 0.88	28.56 ± 0.50D
Neem	22.67 ± 0.88	21.67 ± 1.45	19.00 ± 1.15	21.11 ± 0.81E
Shisham	38.33 ± 0.88	36.33 ± 0.88	33.67 ± 1.45	36.11 ± 0.87B
Aak	33.67 ± 1.20	31.33 ± 1.45	29.33 ± 1.45	31.44 ± 0.93C
Control	67.33 ± 0.88	69.33 ± 0.88	70.00 ± 1.53	68.89 ± 0.70A
Mean	37.06 ± 3.49A	36.22 ± 3.76A	34.78 ± 3.99B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Effect of chemical concentrations on disease incidence: The mean value (Table 4) showed that type of chemicals and concentrations were highly significant. Among the six treatments the least disease incidence was noted 9.78% in case of Gemstar. Other treatments in which lower disease incidence was recorded were Raydar, Crest and Nativo with mean values of 15.00%, 19.00% and 26.22% respectively. On the other hand Curzate stands at lowest level (30.89%) and control at 69.11%. Among the three chemical concentrations used lowest disease incidence was recorded in 15% chemical concentration and the highest disease incidence was recorded at 5% chemical concentration with the mean values of 26.72% and 29.83% respectively.

Table 4: Means for effect of different chemicals concentration on disease incidence

Chemical	Concentration			Mean
	5%	10%	15%	
Crest	21.00 ± 1.15	19.00 ± 1.15	17.00 ± 1.15	19.00 ± 0.82D
Raydar	17.00 ± 0.58	15.00 ± 0.58	13.00 ± 0.58	15.00 ± 0.65E
Gemstar	11.67 ± 1.45	10.00 ± 1.15	7.67 ± 1.45	9.78 ± 0.89F
Nativo	27.67 ± 0.88	26.33 ± 1.45	24.67 ± 1.45	26.22 ± 0.78C
Curzate	32.67 ± 1.20	31.00 ± 1.15	29.00 ± 1.15	30.89 ± 0.79B
Control	69.00 ± 1.15	69.33 ± 2.03	69.05 ± 1.15	69.11 ± 0.75A
Mean	29.83 ± 4.57A	28.44 ± 4.76B	26.72 ± 4.91C	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Effect of different chemical sprays at various intervals on disease incidence: Disease incidence mean value in relation to different spray intervals showed non-significant interaction (Table 5). Among the three sprays intervals used, the lowest disease incidence was recorded on 3rd spray of chemicals and the highest disease incidence was recorded at 1st spray with the mean values of 27.39% and 29.56% respectively.

Table 5. Means for chemical sprays at various intervals on disease incidence.

Chemical	Spray			Mean
	1st	2nd	3rd	
Crest	20.67 ± 0.88	19.33 ± 2.03	17.33 ± 2.03	19.11 ± 0.99D
Raydar	16.67 ± 0.88	14.67 ± 1.20	13.33 ± 1.45	14.89 ± 0.77E
Gemstar	13.00 ± 1.15	14.00 ± 1.73	11.67 ± 0.88	12.89 ± 0.73E
Nativo	27.00 ± 1.15	28.67 ± 2.60	26.67 ± 2.60	27.44 ± 1.16C
Curzate	31.00 ± 1.15	30.00 ± 1.53	28.00 ± 1.53	29.67 ± 0.83B
Control	69.00 ± 1.15	69.67 ± 0.88	67.33 ± 0.88	68.67 ± 0.60A
Mean	29.56 ± 4.53A	29.39 ± 4.66A	27.39 ± 4.62B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

DISCUSSION

In the present study a significant differences for powdery mildew resistance were observed in 12 genotypes where varities WT2257 and Zcugma F1 showed only 3% disease incidence showing higher disease resistance. Other Moderatly resistant watermelon varities were Panther, Pata Nagra, Ayesha 1 and Othello with disease incidence of 5, 7, 9 and 10% respectively. Such higher degree of resistance have been reported by (Tripathi *et al*., 2003; Sales *et al*. 2011). On the other hand Sugar Baby, Anar Kali, Asia Black and Charleston-Grey varieties showed 78, 67, 47 and 45%disease incidence. Such susceptibility of watermelon varieties was reported by various researchers (Wang *et al*., 2004; Tomason and Gibson, 2006; Sales *et al*., 2011).

This showed a varying response of watermelon germplasm against powdery mildew resistance i.e. significant genetic variabaility was found in watermelon for disease resitance. Presence of such genetic variation against powdery mildew has been reported by previous studies (Mccreight, 2006; Zhang *et al.,* 2011). Such variation is a valuable source which can be utilized for the development of new cultivars resistant to powdery mildew (Henning *et al*., 2005; Margaret *et al*., 2011). Such cultivar development may be followed by different procedures like haploid breeding (Kuzuya *et al.,* 2003), hybrids (Henning *et al*., 2005) or cultivar development by hybridization (Margaret *et al*., 2011). So, these findings will be helpful in future to develop resistant cultivars against powdery mildew disease in watermelon. Plant extracts have been used by various researchers (Rettinassababady*et al*. 2000; Kiran and Ahmad, 2005; Morsy *et al*. 2009; Moharam and Ali, 2012) as disease control measure. In another experiment, effect of various plant extracts and their concentration was studied in order to check their efficacy for disease control. Five plants extracts namely aak, neem, garlic, sohanjna and shisham in 20, 30 and 40% concentrations were used as a disease control measure. These were compared with control i.e. no disease control measure was taken. Among the six treatments, neem extract showed least disease incidence of 17.56%. Efficacy of neem extract as one of the most eficient extract for disease control was also reported by other scientists (Rettinassababady*et al*. 2000; Kiran and Ahmad, 2005; Gurjar *et al.*, 2012). Other extracts i.e. garlic, sohanjana and aak with showed 25.78%, 30.44% and 34.00% disease occurrence respectively. Previous studies (Kiran and Ahmad, 2005; Ahmad and Din, 2006) also showed moderate ability of garlic as plant extract to control powdery mildew. Highest disease incidence was recorded in control and shisham extract with means of 69.56% and 49.67% respectively. Highest disease occurrence was seen in 20% concentration and lowest disease incidence was at 40% concentration of plant extracts with disease incidence of 40.11% and 35.56%. This showed that all the plant extracts significantly reduced the disease occurrence as compared to control. Poor performance of shisham extract in controlling powdery mildew was reported by Daayf *et al.* (1995).

Our findings on evaluation of five different fungicides (Curzate, Nativo, Crest, Raydar and Gemstar) in line with other researchers where they concluded that the use of synthetic chemical always help in reducing the disease incidence significaly (Rani *et al*. 2005; Prasad and

Dwivedi, 2007; Keinath and DuBose, 2012). It is quite interesting that Gemstar resulted in 9.78% and Curzate in 30.89% disease incidence showing variation in pesticide efficiency in disease control. Such findings were reported by previous studies (Romero *et al*. 2007; Haynes *et al.* 2008; Kader *et al.* 2012). It can be concluded that cultivars selection, proper dose of chemicals and plant extract application can be an integrated approach to manage the powdery mildew problems of cucurbits.

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