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INHIBITORY EFFECT OF MEDIATIONAL BOTANICAL EXTRACTS AGAINST ROOT ROT OF MUSKMELON CAUSED BY *PHYTOPHTHORA DRECHSLERI* (TUCKER)

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ABSTRACT

Phytophthora drechsleri (Tucker) causing root rot of muskmelon is a threatening pathogen in Pakistan. Non-chemical control based on the use of plant extracts are considered the best alternatives of noxious chemical pesticides as they are considered to be eco-friendly with no or minimal residual effects. The results of all tested botanicals suppressed the mycelial growth of pathogen. Extract of garlic (*Allium sativum*) showed maximum (86.5%) mycelial growth inhibition of root rot pathogen, followed by parthenium (*Parthenium hysterophorus*) (82.8%) and datura (*Datura stramonium*) (82%) at 30% concentration, while minimum inhibition was shown by Aak (*Calatropis procera*) (42.8%). These results suggested that plant extracts can be used as an alternative to chemicals for the management of root rot of muskmelon.

Keywords: antifungal, muskmelon, plant extracts, *Phytophthora drechsleri*

INTRODUCTION

Muskmelon (*Cucumis melo* L.) is an important fruit in world's fresh fruit market (Mabalaha *et al.,* 2007). Many diseases cause severe losses to this crop but root rot caused by *Phytophthora drechsleri* (Tucker) is economically important disease of muskmelon in Pakistan (Majid *et al*., 1994). Management of fruit crop diseases through chemicals gave rapid control but their regular usage creates resistance in pathogen, hazardous to human health and environmental problems. World is moving to organic farming, so there is a need of alternatives which are non-toxic, environmental friendly and having long term effectiveness against pathogens. Plant extracts have great potential as an alternative to noxious chemicals. Antibacterial and antifungal potential of plant extracts gained the attention of researchers throughout the world. During last two decades, many scientists worked on biocidal potential of botanicals (Tegegne *et al*., 2008). Use of plant extracts is environmentally safe approach as compared to chemicals (Kumar *et al*., 2008; Masih *et al*., 2014). Plant extracts such as *Xanthium strumarium* (Kim *et al*., 2002), *Cinnamomum zelanicum* (Abdolmaleki *et al*., 2008) and *Carumcarvi, Pinus halepensis, Zataria multiflora* (Abdolmaleki *et al*., 2010) have been reported to suppress the mycelial growth of *P. drechesleri.* Cabbage, garlic and alfalfa extracts have showed antifungal activity against *P. capsici* (Demirci and Dolar, 2006). The objective of present study was to evaluate the plant extracts against root rot of muskmelon for its sustainable management.

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MATERIALS AND METHODS

Collection, Isolation and identification of pathogen

Diseased samples were collected from muskmelon growing area of district Jhang $30^{\circ}35'$N and $71^{\circ}39'$E, Punjab, Pakistan. The samples were kept in zip bags and brought to laboratory for further processing. Isolation was performed from root samples on PARP medium (corn meal agar 17 g L^{-1}, 0.4 ml Pimaricin L^{-1}, 0.25 g Ampicilline L^{-1}, 0.01 g Rifampcin L^{-1}, 5 mL Pentachloronitrobenzene (PCNB) L^{-1}, 1 mL Dimethylsulphooxide (DMSO) L^{-1}) by using tissue segment method (Rangaswamy, 1958). The pathogen was identified based on cultural and morphological characteristics described in literature (Bush *et al*., 2006; Jadesha, 2014). Culture was maintained on PDA (potato starch 4 g L^{-1}, agar 20 g $L^{-1}$and dextrose 20 g L^{-1}) and stored at 4°C for further use.

Preparation of plant extracts

Fresh leaves of eight different plants, healthy fruits of chilli and cloves of garlic were collected locally from the area of University College of Agriculture, University of Sargodha (Table 1). The plant parts were washed with tap water in order to remove surface dust/ pollutants. Leaves of all tested plants were blended in water with 1:1 (w/v) to obtain its crude extract. Chilli fruits and garlic gloves purchased from local market were blended in water with 1:1 (w/v) and crude extracts were passed through double layer of muslin cloth and filtered with Whatman filter paper No.1 in 250 ml Pyrex flasks. The extracts were centrifuged at 10000 rpm for 5 minutes and supernatant was sterilized at 40°C for 10 min to avoid the risk of contamination and stored at 4ºC for further use (Jaganathun and Narasimhan, 1988; Ramaiah and Garampalli, 2015).

Table 1. List of plant species used for evaluating their antifungal activity against mycelial growth of *P. drechsleri*

Common/ Local name	Botanical name	Family	Plant part used
Aak	*Calatropis procera*	Asclepiadaceae	Leaves
Beri	*Ziziphus mauritiana*	Rhamnaceae	Leaves
Chilli	*Capsicum annuum*	Solanaceae	Fruit
Datura	*Datura stramonium*	Solanaceae	Leaves
Garlic	*Allium sativum*	Liliaceae	Cloves
Kaner	*Nerium oleander*	Apocynaceae	Leaves
Niazbo	*Ocimum basilicum*	Lamiaceae	Leaves
Parthenium	*Parthenium hysterophorus*	Asteraceae	Leaves
Sisal	*Agave sisalana*	Asparagaceae	Leaves
Thyme	*Thymus vulgaris*	Lamiaceae	Leaves

***In Vitro* assay**

Antifungal activity of plant extracts at three concentrations (10, 20 and 30%) was evaluated by using food poison technique (Bhutia *et al*., 2016). To obtain 10, 20 and 30% concentrations, 10, 20 and 30 ml extract was added into 90, 80 and 70 ml molten PDA, respectively. After solidification of the media, a bore in center of PDA plate was made with the help of sterilized cork borer. A5 mm mycelial bit of *P. drechsleri* was placed aseptically in the center of each plate. The plates were covered with para film and incubated at 24 ± 1^{o}C. The PDA petri plate containing media without plant extract served as control.

Statistical analysis

The statistical analysis was carried out using R.3.0.3- Statistical package. Two factor factorial analyses were used for the interpretation of the results. Each set of experiment was repeated three times independently. The inhibition over control percentage (%) was determined by using the formula and the treatment means were separated by using LSD (Gomez and Gomez, 1984). Inhibition over control percentage (%)= C-T/C×100 where C= mycelial growth of pathogen in control, and T= mycelial growth of pathogen in dual culture.

RESULTS

Pathogen inhibition by plant extracts

Aqueous extracts of ten different plant species were tested at different concentrations (10, 20 and 30%) against *P. drechsleri* under laboratory conditions. Extracts ($F_{9, 180}$ = 1534.16, P< 0.001), concentrations ($F_{2, 180}$ = 3053.46, P< 0.001) and days ($F_{2, 180}$= 947.19, P< 0.001) effect was highly significant. Interactions of extract and concentrations ($F_{18, 180}$ = 17.05, P< 0.001), interaction of extracts and days ($F_{18, 180}$ = 59.14, P< 0.001), interaction of concentrations and days ($F_{4, 180}$ = 69.70, P< 0.001) and interaction among extracts, concentrations and days ($F_{36, 180}$ = 5.92, P< 0.001) were also significant. According to our findings, extracts of all tested plant spp. inhibited the mycelial growth of *P. drechsleri.* Maximum inhibition showed by *A. sativum* (86.5%), *P. hysterophorus* (82.8%) and *D. stramonium* extracts (82.0%), followed by *A. sisalana* (77.5%), *T. vulgaris* (74.7%), *C. annuum* (69%) and *O. basilicum* (51.5%). While less than 50% mycelial inhibition showed by *N. oleander* (47.7%), *Z. mauritiana* (43.5%) and *C. procera* (42.8%), respectively. Results also showed that concentrations at 10% of *P. hysterophorus* and *A. sativum* extracts have great potential to inhibit the pathogen (Table 2).

DISCUSSION

Antifungal ability of different plant species has been tested on different soil borne fungi and viruses (Kuc and Shain, 1977; Beckman *et al*., 1981; Bahraminejad *et al*., 2012; Shafique, 2012; Bahraminejad *et al*., 2013). The present results provided insights that plant extracts (Table 1) significantly inhibited the mycelial growth of pathogen.

Table 2. Efficacy of different botanical extracts against *Phytophthora drechsleri* at three concentrations (10, 20 and 30%) under the laboratory conditions by using poison food technique

Treatment	% inhibition with time intervals								
	3rd day			5th day			8th day		
	10%	20%	30%	10%	20%	30%	10%	20%	30%
C. procera	26.40±1.03c	39.36±0.89e	52.83±1.37e	19.82±0.49f	32.46±0.76f	49.42±0.57g	27.16±0.50f	32.82±0.50h	42.82±0.32g
Z. mauritiana	20.70±2.37d	34.18±1.37fg	49.20±1.37f	37.64±1.25e	52.01±1.15e	51.72±0.86g	29.24±0.65f	39.05±0.67g	43.58±0.82g
C. annuum	27.96±1.37c	42.99±1.37d	56.98±1.37d	38.50±0.99e	52.29±0.57e	64.93±1.52e	45.27±0.67d	54.33±0.99e	69.05±0.37d
D. stramonium	36.25±0.89b	48.68±0.89c	72.52±0.51ab	55.45±0.75c	61.77±1.25d	80.45±0.28ab	60.55±0.49c	71.50±0.49c	82.07±0.49b
A. sativum	48.17±0.52a	64.23±0.89a	75.12±0.00a	60.91±1.25ab	75.57±0.76a	83.04±0.29a	65.84±0.49b	81.50±0.37a	86.59±0.49a
N. oleander	26.40±1.03c	36.25±2.37f	51.79±0.89ef	39.36±1.25e	50.85±0.86e	56.60±0.75f	34.52±0.49e	43.20±0.49f	47.73±0.67f
O. basilicum	19.66±1.37d	32.10±1.86g	45.57±0.89g	39.07±0.57e	35.05±1.79f	43.09±1.31h	34.89±0.56e	42.63±0.68f	51.50±0.68e
P. hysterophorus	46.61±1.86a	58.53±2.74b	71.49±0.51b	61.77±1.03a	69.24±0.28b	78.73±0.57b	72.44±0.37a	75.84±0.37b	82.82±0.18b
A. sisalana	45.57±2.37a	56.98±1.86b	66.31±1.37c	58.04±1.52bc	65.80±1.03c	75.57±0.28c	64.52±0.37b	73.95±0.65bc	77.54±0.19c
T. vulgaris	37.29±1.86b	49.72±1.37c	63.72±0.52c	50.86±0.49d	61.77±1.03d	70.97±0.76d	58.29±0.18c	66.59±0.56d	74.71±0.37c

Mean sharing similar letter in a row are statistically non-significant (P>0.01). Small letters represent comparison among interaction means

The aqueous extracts of *P. hysterophorus*, *A. sativum, T. vulgaris* and *D. stramonium showed mycelial growth inhibition of P. drechsleri*, which directly correlates to results of different authors (Tariq and Magee, 1990; Kshemkalyani *et al*., 1990; Singh *et al*., 1992; Reimers *et al*., 1993; Naganawa *et al*., 1996; Avato *et al*., 2000; Kyung and Lee, 2001), who described the antifungal effects of *P. hysterophorus, A. sativum, D. stramonium* and *T. vulgaris* leaf extracts of these species and found that these plants have a great potential to control the soil borne fungal pathogens. Ajoene is *A. sativum* derived compound that have ability to restrict the growth of many fungi. In present study, extract of *A. sativum showed maximum inhibition of P. drechsleri mycelial growth,* which may be due to ajoene. Results of Bekepe *et al.* (2006) also matched with our findings, they concluded that 2% garlic extract inhibited the growth of Phytophthora isolates. Aqueous extract of Parthenium have great potential to inhibit the growth of fungal pathogens, it gave maximum mycelium inhibition zone at 1% concentration against *F. solani* (Bajwa *et al*., 2003; Shafique and Shafique, 2012). Antifungal activity of datura and thyme against soil borne pathogens has been reported by Zaker (2014), Sahu *et al*. (2015). Sarpong (2016) reported the antifungal impact of neem extract against Phytophthora species, which also correlates to the results of present study. *Z. mauritiana* contains many bioactive compounds like cardiac glycosides, tannins, polyphenols and saponins, and its leaf extract totally inhibits the spore formation of *F. solani* at 20% concentration (Abu-Taleb *et al*., 2011). The current investigation showed that extract of *Z. mauritiana* could not inhibit the mycelial growth of *P. drechsleri* significantly even at 30% concentration which might be due to less effectiveness of bioactive compounds produced by *Z. mauritiana* against tested pathogen. Datura leaf extract inhibited the mycelial growth and spore germination of *Alternaria alternate* (Bagri *et al*., 2011) and *Phytophthora infestans* (Abayhne and Chauhan, 2016). Overall, the present study provided insights into the utilization of plant extracts to manage the plant pathogenic *P. drechsleri* and potential replacement of hazardous chemical fungicides.

CONCLUSION

The present study demonstrated that crude extracts prepared from different plants provide significant suppression of fungal pathogen and showed the potential to combat the soil borne pathogens. The utilization of plant extracts to control fungal diseases is opening new insights to replace the health hazardous synthetic chemicals. The results suggested the use of these environmental friendly methods to develop a healthy society and resolve the food security issues.

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