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IN VITRO EVALUATION OF BOTANICAL EXTRACTS AND FUNGICIDES AGAINST PHYTOPHTHORABLIGHT OF CHILI PEPPER (*CAPSICUM ANNUM* L.)

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A B S T R A C T

Recently, an increasing interest has been observed in utilization of botanicals to manage phytopathogenic fungi. An investigation was undertaken to evaluate six different aqueous botanical extracts *in vitro* for their antifungal activity against *Phytophthora capsici* associated with chili blight. Aqueous botanical extracts of *Parthenium hysterophorus* revealed promising antifungal activity against the pathogen andexhibited up to 100% inhibition compared to control. While, aqueous extracts of *Azadirachta indica*, *Calotropis gigantea, Cassia fistula, Nerium oleander* and *Oscimum basilicum* were not effective against the pathogen. Instead, they revealed a stimulatory effect on colony growth of the pathogen with negative values of percent inhibition. *Cassia fistula* had the most stimulatory effect on colony growth of the pathogen. Three different fungicides Ridomil (Metalaxyl + Mancozeb), Curzate (Cymoxanil + Mancozeb) and Proctor (Difenconazole) were also tested *in vitro* at three different concentrations 50, 100 and 150 ppm against the colony growth of the pathogen. Ridomil and Proctor produced 100% inhibition of the pathogen at all tested concentrations followed by Curzate.

Keywords: *Phytophthora psici,* Vegetable, Aqeuous extracts, *Parthenium hysterophorus,* Antifungal, Inhibition.

INTRODUCTION

Chili is a famous and most important vegetable cash crop in the world, (Sang *et al.,* 2013). It is commonly consumed in Pakistan and have significant economic value with 1.5% share in GDP of Pakistan (Hussain and Abid, 2011). Chili production is reduced up to 33% by the attack of multiple pathogens. *Phytopthoraca psici* (Leonian) associated with *Phytophthora* Blight of chili is a destructive soil-borne oomycete pathogen can spread zoospores rapidly through watersplashes infecting all plant parts such as crown, root, foliage and fruits (French-Monar*et al.,* 2006) in almost all chili pepper growing countries of the world including major areas of Pakistan i.e., KPK (Hassan, 1994) and Punjab (Saleem*et al.,* 1997). This pathogen has a broad host range that includes members of *Leguminosae, Solanaceae* and *Cucurbitaceae*(Hwang and Kim, 1945; Hausbeck and Lamour, 2004). It is continuously a serious threat to chili production around the globe and can cause losses up to100% leading to complete destruction of the crop (Babadoost, 2004).As far as, management is concerned *Phytophthora* blight of chili pepper is always difficult to manage because it can survive for long time in the soil as oospores, sporangia and mycelia produced by the pathogen in infected host plants (Lamour and Hausbeck, 2003). Even resistance breeding sometimes becomes insufficient to overcome losses caused by this notorious pathogen (Shah *et al.,* 2013).

This disease has been attempted to be managed by using various strategies such as avoidance to cultivate new crop in previously infected fields; cultivation in well drained soils and crop rotation practices. However, all these strategies have failed to provide an effective control over this disease (Bosland and Lindsey, 1991). Due to devastating nature of this pathogen it would be more appropriate to manage this disease through integration of multiple management strategies.Fungicides have been extensively used in developed and developing countries of the world as a quick source to get rid of multiple

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economically important pathogens (Alkhail, 2005). Extracts of different botanicals have been reported to exhibit antifungal properties against plant pathogenic fungi associated with threatening plant diseases. Plants produce antimicrobial compounds that are toxic to phytopathogens and can be used to control plant diseases (Ngadze, 2014). Plants have the natural ability to produce aromatic secondary metabolites such as quinones, saponins, flavones, phenols, tannins, flavonols and coumarins (Cowan, 1999). Botanical pesticides are preferred over chemical pesticides, as they have least damage to human health and environment (Varma and Dubey, 1999). Hence, botanical extracts are regarded as a bio-safe management strategy and a remarkable alternate to chemical management strategy. Botanical and chemical pesticides can be used in combinations to overcome the damage caused by this devastating pathogen.The present study was undertaken to in evaluate the antifungal potential of different aqueous botanical extracts and fungicides against *P. capsici*. *In vitro* evaluation of fungicides was carried out at different concentrations while, aqueous botanicals were evaluated at 10% concentration against *P. capsici*.

MATERIALS AND METHODS

Isolation and Purification of the Pathogen: Infected chili leaves exhibiting typical disease symptoms were randomly sampled from Ayub Agricultural Research Institute, Faisalabad. Pathogen isolation was carried out by following standard isolation procedures (Pathak, 1987). Infected leaves were washed with distilled water, cleaned with sodium hypochlorite and surface sterilized with 70% ethanol to free the samples from surface borne saprophytic micro-organisms. These leaves were then cut into very small pieces (5mm) and plated on PARP (Pimaricin, Ampicllin, Rifampicin and Pentachloronitrobenzene) medium followed by incubation at 22±1°C under 12-hour light and 12 hour dark conditions. Colony growth was observed after 24-hour of incubation period and transferred to PARP medium slants for purification. The fungus was identified microscopically based on typical identification structures documented in literature and keys (Sutton, 1980). The presence of lemoniform sporangia detached hyphae and sporangiophore that was not swollen and typical colony growth characters such as floral pattern and submerged growth in the medium.

Pathogenicity Test: Pathogenicity was tested to satisfy Koch's postulates. Experiment was conducted in completely randomized design in greenhouse (Steel *et al.,* 1997). Chili seeds were pre-treated by washing with sterilized water and surface sterilization by sodium hypochlorite. Sterilized seeds were sown in earthen pots of 15-cm-diameter. Inoculum was prepared by multiplying the pathogen on PDA medium and scrapping the colony growth in sterilized distilled water. Spore density was adjusted using a hemocytometer to 1×10^6 sporesmL^{-1} (Omar *et al.,* 2006). Healthy plant leaves were inoculated at the age of 5-weeks-old with 20µL zoospore suspension of the pathogen following the technique given by Pliakhnevich and Ivaniuk, (2008). Inoculated plants along with control plants were kept in greenhouse under controlled conditions. The plants were observed regularly for the development of infection symptoms and severity. Pathogen was re-isolated from infected plants and grown on PDA medium.

In vitro evaluation of botanical extracts against *P. capsici:*Fresh healthy leaves of six different plants i.e., Gajjar booti (*Parthenium hysterophorus)*, Neem (*Azadirachta indica)*, Niazboo (*Oscimumbasilicum)*, Kaner (*Nerium oleander)*, Akk (*Calotropis gigantea*) and Amaltas (*Cassia fistula*) well known for their antifungal activity and medicinal properties were collected form Ayub Agricultural Research Institute, Faisalabad. Healthy leaves were washed with sterilized distilled water followed by cleaning with sodium hypochlorite and drying for 24 hours by keeping them pressed in blotter papers.

Preparation of aqueous botanical extracts: Each plant sample was taken 25g by weight and crushed in 75 mL sterilized, distilled water (Sahi *et al.,* 2012). Crushed material was initially filtered through a coarse sieve followed by filtration through double layer of filter papers. Initial pH of all botanical extracts was recorded and final pH was maintained at 5.5 (Hassan *et al.,* 2011) by adding acidic HCL or basic NaOH buffer solution. This filtrate was stored for further use at 4°C to avoid contamination.

Poisoned food technique: The antifungal potential of aqueous botanical extracts against the pathogen was evaluated using poisoned food technique (Nene and Thapliyal, 2000).PDA medium 100mL was poisoned with 10mL of each botanical extract separately and poured into sterilized petri plates. In case of control

treatment 100mL PDA was amended with 10mL sterilized distilled water. The amended medium after solidification was inoculated with culture blocks 5-mm-diameter of the pathogen and incubated at 22±1°C for 7 days. Each treatment was replicated five times and one treatment was maintained as control. Colony growth was observed after every 24-hour interval and colony diameter was recorded. Experiment was repeated twice. Percent inhibition of the pathogen compared to control was calculated by using the formula of Vincent (1927).

$$I = (C-T)/C \times 100$$

In vitro evaluation of fungicides against *P. capsici*:

In vitro investigation was carried out to evaluate antifungal action of three different fungicides i.e., Ridomil Gold 68WP (Metalaxyl + Mancozeb), Proctor 25EC (Difenconazole) and Curzate M8 72WP (Cymoxanil + Mancozeb) against *Phytophthora capsici*.

Preparation of stock solutions: Stock solution of each fungicide was prepared by dissolving 100mg active ingredient of each fungicide in sterilized distilled water following Borum and Sinclair Technique (1968). Required quantity of each fungicide was calculated according to the formula to take 100mg active ingredient. Ridomil (14.7), Proctor (400mg) and Curzate (138.9) were taken and each was dissolved separately in 100mL of sterilized distilled water.

Poisoned food technique: *In vitro* inhibitory action of three different fungicides against *P. capsici*was evaluated by poisoned food technique (Nene and Thapliyal, 2000). PDA was amended with three different concentrations i.e., 50, 100 and 150ppm of each fungicide and poured into sterilized petri plates. In case of control treatment PDA was amended with equal concentration of sterilized distilled water. Poisoned medium after solidification was inoculated with culture blocks 5-mm-diameter of the pathogen and incubated at 22±1°C. Each treatment was replicated five times and one treatment was maintained as control. Experiment was repeated twice. Colony growth was observed at every 24-hour interval and colony diameter was recorded. Percent inhibition of the pathogen compared to control was calculated by using the formula of Vincent (1927).

$$I = (C-T)/C \times 100$$

Statistical analysis: Experiments were conducted in completely randomized design (CRD).

Experimental data was subjected to statistical analysis using a personal computer as per the Statistical Analysis System (SAS). Transformation of the data was calculated by using least significant difference test (LSD) (SAS, 1988).

RESULTS AND DISCUSSION

In vitro evaluation of botanical extracts against *P. capsici*:Six different botanical extracts were evaluated at 10% concentration against the pathogen. Mean colony diameter was recorded and percent inhibition of the pathogen compared to control was calculated. It was found that aqueous botanical extract of *P. hysterophorus* produced the best inhibition up to 100% of colony growth of the pathogen (Table 1). *N. oleander* exhibited 10.13% inhibition of colony growth of the pathogen (Table 1). While, all other botanical extracts were not effective against the pathogen and revealed stimulatory effect on colony growth of the pathogen with negative values of percent inhibition that is clearly indicated by trend line (Figure 1). Aqueous botanical extract of *C. fistula* was highly ineffective against the pathogen with highest value of negative inhibition up to -110.53% indicating a strong stimulatory effect on growth of the pathogen (Table 1). Present investigations revealed that four plant extracts had a stimulatory effect on the growth of pathogen. It might be because plant pathogens feed on organic material of the plant species being used as botanical extracts (Linderman, 1989).According to reports of Hegde, (1983) and Subramanian, (1993) *A. indica* exhibited stimulatory effect on mycelial growth of *P. capsici.* Present findings are also supported by the investigation of Chamount, (1979) who found that out of 51 fungi *Phytophthora*and *Pythium* were among the most resistant ones against botanical extracts. Present finding is not supported by the finding of Shashidhara *et al.* (2008) where *A. indica* showed significant inhibition of colony growth of *P. capsici*.

In vitro evaluation of fungicides against *P. capsici*:Three different fungicides were tested *in vitro*at three different concentrations (50, 100 and 150ppm). All fungicides were highly inhibitory against colony growth of the pathogen. Ridomil and Proctor were equally effective exhibiting 100% inhibition at all concentrations followed by Curzate showing up to 93.8% inhibition at 150ppm concentration (Table 2; Figure 2). Fungicides have been extensively evaluated as a mean to provide quick control of many diseases worldwide. Present investigation is in line with the findings of Nair and Sasikumaran (1991) and Subramanian (1993) in which they have reported promising antifungal activity of

fungicides against *P. capsici*. *In vitro* evaluation of Ridomil against *P. parasitica*var*nicotiana*erevealed significant reduction in colony growth and sporulation of the fungus at 0.1, 0.2, 0.3 and 0.4%concentrations (Reddy and Nagarajan, 1980). Shashidhara *et al.* (2008) also reported that Ridomil showed 100% inhibition of *P. capsici* associated with foot rot of black pepper at all tested concentrations. Jahagirdar, (1998) and Veena and Sarma (2000) also reported similar results. According to present investigation Ridomil, Proctor and Curzate exhibited promising inhibition of the pathogen, so all of these fungicides can be recommended as a quick mean to manage *Phytophthora* blight of chili pepper.

Table 1.*In vitro* effect of botanical extracts against *P. capsici.*

Treatment	Concentration (%)	Mean Colony Diameter (mm)	Inhibition (%)
Azadirachta indica	10	39.52 b[a]	-20.00 d[a]
Parthenium hysterophorus	10	0.00 e	100 a
Nerium oleander	10	29.62 d	10.13 b
Calotropis gigantea	10	39.30 b	-19.21 d
Oscimum basilicum	10	34.00 c	-3.12 c
Cassia fistula	10	69.30 a	-110.53
Control	10	33.00 c	0 c

[a]Mean values in columns followed by same letters are not significantly different at $P<0.05$, analyzed using LSD test; mean values are average of 5 replicates.

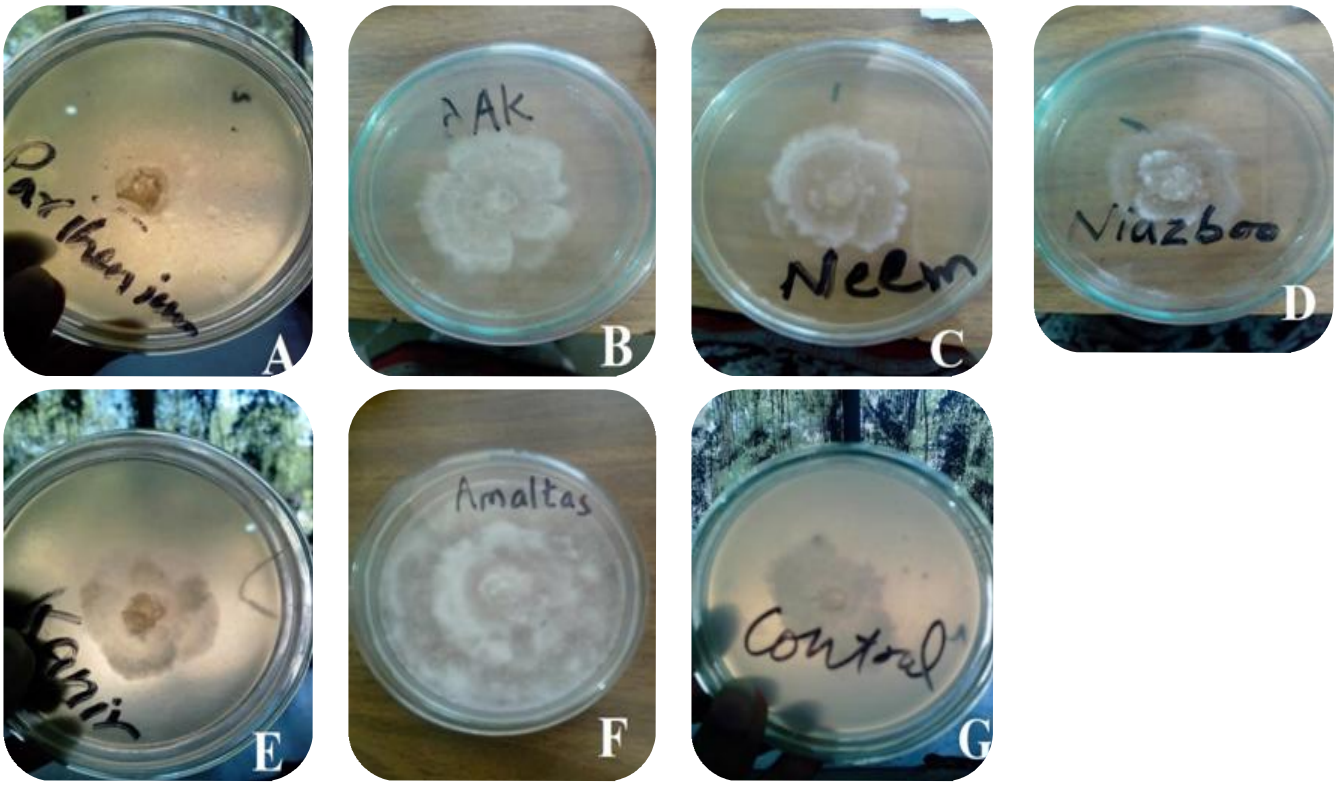

Figure 3. Effect of botanical extracts on mean colony diameter (mm) of *P. capsici*on Day 7 after incubation A: *P. hysterophorus,* B: *C. gigantea,* C: *A. indica*, D: *O. basilicum,* E: *N. oleander*, F: *C. fistula*, G: Control.

Table 2.*In vitro* effect of fungicides against *P. capsici.*

Treatment	Concentration (ppm)	Mean Colony Diameter(mm)	Percent Inhibition (%)
Ridomil	50	0.00 e[a]	100 a[a]
	100	0.00 e	100 a
	150	0.00 e	100 a
Proctor	50	0.00 e	100 a
	100	0.00 e	100 a
	150	0.00 e	100 a
Curzate	50	13.87 b	80.1 d
	100	7.17 c	89.7 c
	150	4.32 d	93.8 b
Control		69.78 a	0.00 e

[a]Mean values in columns followed by same letters are not significantly different at $P<0.05$, analyzed using LSD test; mean values are average of 5 replicates.

CONCLUSION

Promising antifungal potential of *P. hysterophorus* against *P.capsici* should be further investigated to find active ingredient of this plant and it may be able to be formulated as a reliable biopesticide product against this disease. It can be recommended as a control measure against *Phytophthora* blight of chili pepper. Resistance of the pathogen against all other plant extracts indicates that these extracts cannot be further recommended for control of *P. capsici*. Ridomil, Proctor and Curzate can be recommended for practical control of this disease *in vivo.*

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