

EVALUATION OF BOTANICALS AGAINST *FUSARIUM PALLIDOROSEUM* CAUSING WILT DISEASE IN CHILLI

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ABSTRACT

With the increase in awareness about the ill effects of the indiscriminate use of synthetic chemicals in agriculture, the demand for safe and residue free food is increasing. In the present study, we evaluated aqueous extracts of willow (*Salix alba* L.) and cherry plum (*Prunus cerasifera* f. *rosaceae*) at different concentrations (5, 10, 15 and 20%) under *in vitro* conditions using poisoned food technique for their efficacy against *Fusarium pallidoroeseum* causing wilt disease of chilli. The efficacy of the extracts was compared with synthetic fungicide, carbendazim 50 WP, which at 0.05% concentration resulted in 100% inhibition. Both the tested botanical extracts significantly inhibited mycelial growth at all the test concentrations with 100 per cent inhibition at 20% concentration. The present study showed that both the botanicals have the potential to be used against *F. pallidoroeseum*.

Keywords: Antifungal activity, Cherry plum, Eco-friendly, Willow

There are numerous concerns affecting agriculture, significantly hindering crop productivity. Among these concerns are biotic and abiotic stresses, encompassing climate change, declining soil fertility, and the proliferation of pests and diseases. (Pereira *et al.*, 2021). The projected global population is anticipated to reach 9-10 billion by 2050, indicating a potential increase in food demand ranging from 25 to 70% compared to current levels (Scott *et al.*, 2018). As a result, the increasing population requires an increase in agricultural produce that may involve use of excessive agrochemicals to overcome the yield losses due to pests and diseases. As such, these synthetic agrochemicals could provide the solution to overcome the problems in growing food demands, however, indiscriminate use has a number of consequences, including adverse effect on environment, human and animal health, disruption of natural biological control and the development of pesticide resistance (Marco *et al.*, 1987; Perry *et al.*, 1998; National Research Council, 2000; de Oliveira *et al.*, 2014). It has been discovered that 64% of world farmlands (approximately 24.5 million km²) are at threat of pesticide pollution from multiple active compounds, with 31% at high risk (de Oliveira

et al., 2014). Approximately, 34% of threatened regions are in high biodiversity areas, 5% are in drought hit regions, and 19% are in less and lesser-middle income countries (Tang *et al.*, 2021).

Chilli peppers (*Capsicum* spp.), long employed as a culinary spice and domesticated for centuries, are renowned for their abundance of health-enhancing bioactive phytochemicals and significant medicinal qualities within the human diet (Wahyuni *et al.*, 2013; Chopan *et al.*, 2017; Saleh *et al.*, 2018). Chilli peppers are under attack by a multitude of pathogens, with *Fusarium oxysporum* being the most prevalent. This pathogen, known for causing vascular wilt, is responsible for significant crop losses worldwide, ranging from 10% to 50% in different parts of the world and up to 80% in India (Bai *et al.*, 2018). To manage these diseases, a wide range of pesticides are used which have their associated issues (Kulkarni *et al.*, 2017). Thus, there is need to explore the alternatives to synthetic chemicals. One such solution is the conversion of botanical waste into the potential pesticides which are economical, environmental friendly and sustainable. Moreover, it will also take care of agricultural wastes that, otherwise, are difficult to manage leading to environmental pollution. In this case, the agricultural wastes include stalks and stems, leaves, and seed pods (Bhuvaneshwari *et al.*,

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2019). The plant derived pesticides are gaining much attention because they are biodegradable, easily available, environmental friendly, safe for users as well as natural pollinators providing a sustainable solution for pest management. Botanical pesticides have been used in horticulture in ancient China, Egypt, Greece, and India for at least two millennia (Isman *et al.*, 2006). The presence of secondary metabolites in plants has been found to have diverse biological activities including the antifungal activity (Ribera and Zuñiga 2012). Bioactive compounds in plants are classified into three major groups based on their biosynthetic origin: terpenoids, nitrogen-containing compounds (alkaloids, glucosinolates, and cyanohydrins), and phenylpropanoids, also known as phenolic compounds (Croteau *et al.*, 2000). Many secondary metabolites, including terpenoids and alkaloids, have been identified as potential insecticides (Rattan, 2010). Some examples of the botanical pesticides include; Neem (*Azadirachta indica*), Marigold (*Tagetes erecta*), Soursop (*Annona moricata*), Chrysanthemum (*Chrysanthemum cinerariaefolium*), Tobacco (*Nicotiana glauca*), Lemongrass (*Cymbopogon nardus*) (Pangihutan *et al.*, 2016) and extracts of *Clitoria ternatea* (butterfly pea), olive mill wastes (Damalas and Koutroubas, 2018). The present study was, therefore, conducted to test the antifungal activity of some locally available botanicals viz., willow and cherry plum against *Fusarium pallidoseum* an important plant pathogen, causing wilt disease of cherry.

MATERIALS AND METHODS

Isolation and purification of test Pathogens:

Diseased plants of chilli showing typical symptoms of wilt disease were collected from the field and diseased root parts were used for the isolation of associated causal pathogens using standard tissue bit technique on Potato Dextrose Agar (PDA) medium. The isolated culture was further purified by single spore technique.

Collection of plant material:

Disease free leaves and soft twigs of willow tree and leaves of cherry plum were collected from the experimental field of SKUAST-Kashmir at Shalimar campus. The leaves were washed with tap water thoroughly to eliminate the dirt and dust. After washing,

the leaves were sundried and grinded into a fine powder using the high power mechanical grinder.

Preparation of plant extract:

Efficacy of aqueous extracts of two botanicals (Table 1) was evaluated at 5, 10, 15 and 20 per cent concentrations against the test fungus by poisoned food technique. Plant parts to be used were first washed with sterilized distilled water and then shade dried. One hundred gram of each plant material was crushed in 100 ml of sterilized distilled water for crude extract preparation. The extract was homogenized, filtered and centrifuged at 5000 rpm for 15 minutes. The supernatant was filtered through Whatman's filter paper no. 1. The clear extract so obtained, referred to as 100 per cent stock solution, was used for experiment.

In vitro evaluation of botanicals:

The 50 ml of double the desired concentration of respective bio-pesticide, prepared by adding appropriate amount of sterilized distilled water and standard stock solution was thoroughly mixed with equal volume of double the strength molten sterilized PDA medium contained in 100 ml Erlenmeyer flasks, under aseptic conditions to make a desired concentration. After thorough mixing, 20 ml of poisoned food, thus prepared, was poured in 90 mm diameter Petri plates. The mycelial disc of 3 mm diameter, taken from 7 days old pure culture of the test fungus, with the help of sterilized cork borer, was aseptically placed in the center of solidified poisoned PDA. One such mycelial disc was used. For the comparison of inhibitory efficacy of test botanicals, carbendazim 50WP, a commonly used synthetic fungicide for the management of chilli wilt was also used @ 0.05 per cent as standard check. The Petri plates were incubated at 25±1°C and observation on mean radial growth was recorded in terms of diameter of mycelial growth in Petri plate when the fungus in the control plate (non poisoned medium) attained maximum growth (90 mm). The per cent inhibition due to various aqueous extracts of test botanicals and standard check fungicide, carbendazim was calculated by using the following formula:

$$\text{Per cent Inhibition} = \frac{(\text{Radial growth in check} - \text{Radial growth in treatment})}{\text{Radial growth in check}} \times 100$$

Table 1. Botanicals evaluated in the current study

S. No.	Botanical name	Common name	Local name	Plant part used
1.	Salix alba L.	Willow	Veer	Soft twigs and leaves
2.	Prunus cerasifera, f. rosaceae	Cherry plum	Cherry plum	Soft twigs and leaves

Biochemical Estimation

Estimation of flavonoids:

A 5g sample was dissolved in an 80% solvent mixture (ethanol, methanol, and ethyl acetate) and incubated for 72 hours. The resulting extract was adjusted to 50 ml. In each test tube, 1 ml of the extract was mixed with 0.3 ml of 5% NaNO₂, followed by 0.3 ml of 10% AlCl₃ after 2 minutes. After another 2 minutes, 3.4 ml of 4N NaOH was added, and the mixture was kept at room temperature for 10 minutes. Absorbance of the pink solution was measured at 510 nm against a blank, using Quercetin as the standard

Total flavonoid content was calculated as:

$$\text{Total flavonoid content (mg Catechin equivalents/100 g)} = \frac{\text{OD}_{510} * \text{std value (mg/OD)} * \text{Total volume of extract} * 100}{\text{Assay volume} * \text{weight of sample (g)}}$$

Estimation of phenols:

A 5g sample was incubated in 80% solvent mixture (ethanol, methanol and ethyl acetate), homogenized, and adjusted to a final volume of 50 ml. In each test tube, 0.5 ml of extract was mixed with 0.2 ml of Folin Ciocalteu's phenol reagent, followed by 3.3 ml of distilled water. After adding 1 ml of 20% sodium carbonate solution, the solution stood at room temperature for 30 minutes. The absorbance of the blue solution was measured at 700 nm against a blank.

Total phenol content was calculated as:

$$\text{Total phenol content (mg Gallic acid equivalents/100g)} = \frac{\text{OD}_{700} * \text{std value (ug/OD)} * \text{Total volume of extract} * 100}{\text{Assay volume} * \text{weight of sample (g)} * 1000}$$

RESULTS AND DISCUSSION

In vitro evaluation of botanical extracts:

The isolated pathogen was identified as *Fusarium pallidoroseum*. In vitro assay through poisoned food technique was carried out to observe the antifungal activity of different concentrations viz., 5, 10, 15, and 20 per cent of willow and cherry plum extracts against the isolated pathogen, *F. pallidoroseum*. The plant extracts inhibited the mycelial growth of *F. pallidoroseum* (Table 2). Both the botanicals significantly inhibited the mycelial growth as against the control (Fig. 1). The inhibition increased with the increase in concentration (Fig. 2). The per cent mycelial growth inhibition achieved by willow extract at 5 per cent concentration was 23.37 which increased to 42.91 at 10 per cent, 73.56 at 15 per cent and 100 at 20 per cent. Similarly, the per cent mycelial growth inhibition achieved by cherry plum extract at 5% concentration was 39.27 per cent which increased to 59.96 at 10%, 83.33 at 15% and 100 per cent at 20% concentration, respectively. Fillinger and Elad (2016) and Franco *et al.* (2018) also reported 100 per cent inhibition of the test pathogen by onion extract. Several other plant extracts were also been tested as potential bio-fungicides against various plant pathogenic fungi such as *Parthenium* extract against *Alternaria* sp. (Srivastava and Singh, 2011), *Moringa oleifera* leaf extract against *Fusarium* wilt in wheat (Yadav *et al.*, 2023).

The phenol and flavonoid content in the extracts of cherry plum and willow might have role in the inhibition of test pathogen. The higher efficacy of cherry plum extract as against willow extract at 5, 10 and 15% concentrations can be attributed to the concentration of phenols and flavonoids (Table 3) in these extracts as higher concentration was recorded in cherry plum extract

Table 2. Per cent mycelial growth inhibition of *Fusarium pallidroseum* by aqueous crude extracts of Willow, Cherry Plum

Treatment	Concentration (%)	Radial mycelial growth (mm)	Per cent mycelial growth inhibition
Willow Extract	5	66.7	23.37
	10	49.7	42.91
	15	23.0	73.56
	20	0.0	100.00
Cherry Plum Extract	5	52.8	39.27
	10	34.8	59.96
	15	14.5	83.33
	20	0.0	100.00
Carbendazim 50 WP (Standard Check)	0.05	0.0	100.00
Control	-	87.0	-
CD (p=0.05)	-	1.48	

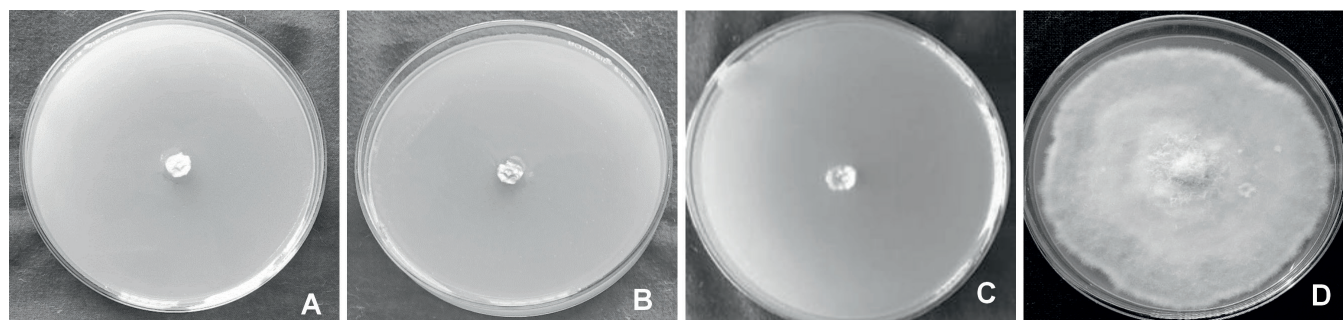


Fig. 1. Pictures of Petri plates showing per cent mycelial growth inhibition of *F. pallidoroseum* by aqueous plant extracts A) Willow extract (20%) B) Cherry plum extract (20%) C) Carbendazim 50 WP (0.05%) D) Control

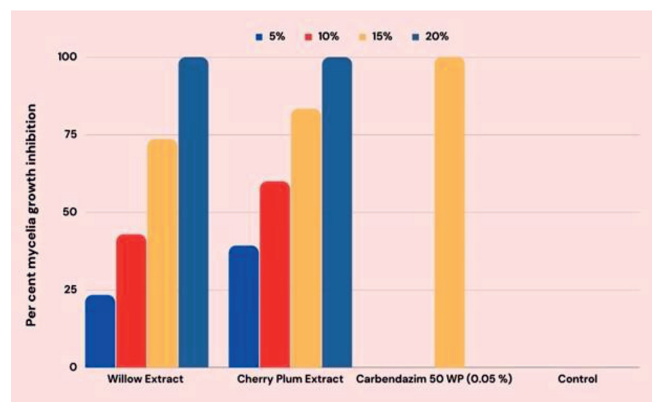


Fig. 2. Graph showing increasing per cent mycelial growth inhibition of *Fusarium pallidoroseum* by crude extracts of cherry plum and Willow with increase in concentraion from 5 to 20% in comparison to Control and Standard Control (Carbendazim 50 WP)

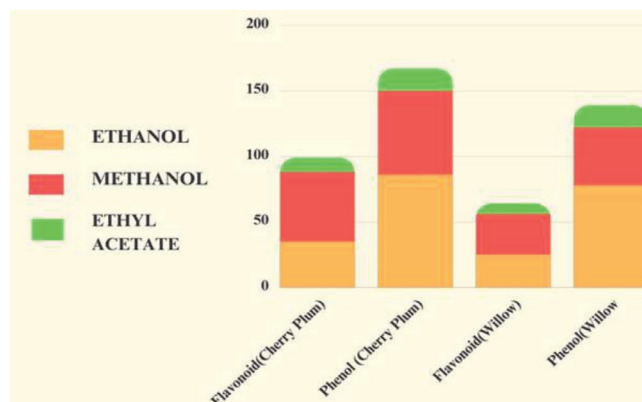


Fig. 3. Graph showing concentration of Flavonoids and phenols in willow and cherry plum extracts in solvents including ethanol, methanol and ethyl acetate

than the willow extract (Fig. 3). Reports also suggested that phenolic compounds, flavonoids, tannins etc. in the extracts of *M. oleifera* were responsible for its antifungal activity (Pinto *et al.*, 2015; Fillinger and Elad 2016). The difference in inhibition percentage of test plant extracts can be attributed to the difference in the amount of higher molecular weight polar molecules, especially those belonging to the phenols and flavonols. It has been established that there is a link between the extracts' antifungal efficacy and their bioactive components (Ghani *et al.*, 2008). Thus, the results are in favour of willow and cherry plum extract as alternative for the management of plant diseases. However, their

in vivo assays need to be carried out to validate their *in vitro* antifungal activity and their impact on plant growth and development.

The bio-fungicides are the suitable alternatives to synthetic fungicides to manage the plant pathogenic fungi as their source waste materials are easily available. They are ecofriendly, economical as compared to their commercial synthetic counterparts and their usage can help in the management of crop waste that is generated every year and the burning of which, otherwise, causes air pollution (Mittal *et al.*, 2009; Zhang *et al.*, 2011; Bhuvaneshwari *et al.*, 2019).

Table 3. Concentration (mg/g) of flavonoids and phenols in ethanolic, methanolic and ethyl acetate extracts of willow and cherry plum

Sample	Flavonoids (mg/g)			Phenols (mg/g)		
	Ethanol	Methanol	Ethyl acetate	Ethanol	Methanol	Ethyl acetate
Willow Extract	25.07±0.43	31.502±1.01	8.312±0.09	78.813±1.86	44.00±1.31	16.27 ±0.08
Cherry	35.31±0.01	00.010.01	53.498±1.73	86.473±0.08	64.02±0.34	17.073±0.31

All values are means of three replicates

CONCLUSION

The cherry plum extract exhibited higher efficacy than willow extract. These botanicals have the potential to be used as alternatives to synthetic fungicides. However, more research is needed to identify the active inhibitory molecules from these bio-pesticides so that high efficacy commercial bio-pesticide formulations can be developed.

Authors' Contribution:

Execution of lab experiments and manuscript writing (AB); data analysis of flavonoids and phenols (GK); statistical analysis (SSM); statistical analysis, and finalization of the draft (MN); guidance in bioassay of cultures and review of manuscript (ZAB); supervision of research work (MAB); conceptualization of research work, supervision of the experimentation work and finalization the manuscript (SMZ).

Declaration:

The authors declare that they have no conflict of interest.

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