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Effectiveness of Natural Antifungal Activity of Medicinal Plant Leaf Extracts Against Isolates Of Pathogen Associated On *Cucumis Sativus*

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Abstract: The use of chemical pesticides for controlling various plant diseases is still a common practice especially in developing countries. Although with the application of chemical fungicides, plant diseases can be controlled but the hazardous impacts of such products in human health and environment are well known. Moreover, with their excess applications pest resistance may exist. Natural plant products have been found effective in *Cucumis sativus* plant fruit disease managements and could be safely incorporated as suitable alternatives for synthetic fungicides. The plant leaf extracts revealed marked reduction in mycelial growth and sporulation of the *Phytophthora capsici* fungi isolate. Growth inhibition of *Phytophthora capsici* increased linearly with an increase in concentration of the Plant leaf extract.

Among all the tested botanicals, the pathogen's growth was significantly inhibited by 75% *Withania somnifera* (growth inhibition of 76.9%). Further research and field trials should be conducted to select appropriate concentrations of botanical extracts to con-trol the disease pathogen economically and sustainably.

Keywords: **Antifungal activity Cucumis sativus fruit rot fungi Phytophthora capsici, Withania somnifera, Plant leaf extract, growth inhibition, sporulation.**

INTRODUCTION

Cucumber (*Cucumis sativus*) is belonging to the family of *Cucurbitaceae* and it is native to Asia, where it has been consumed for over 3,000 years (Pandey & Kujur, 2022). Cucumber is probably native to India (Yang & Sagar, 2022). Although it possibly arises from India, other scholars also reported Egyptians knew the crop in the old times and later on cultured by the Greeks and Romans (Goel et al., 2022). However, African horned cucumber is also a traditional food plant indigenous to Southern and Central Africa, and it is cultivated in Namibia, Botswana, South Africa and Swaziland (Omokhua-Uyi & Van

Staden, 2020). Kiwan is also a popular cucumber variety that contains a significant number of vitamins (vitamins C and B6), and minerals (magnesium, calcium, potassium and iron) (Murray & Horswill, 2022). It also contains antioxidants, which can help the body to fight the free radicals (dos Santos et al., 2022). Thus, eating Kiwan is essential to attain the essential nutrients used for healthy life. Cucumbers also have external health benefits which may help to relieve sunburn pain, and swelling (Uzal et al., 2022). *Phytophthora* fruit rot caused by *Phytophthora capsici* is one of the most devastating soil-borne diseases, and it causes severe economic loss (Anandaraj and Sarma, 1995). The isolated pathogen was identified as Phytophthora *capsici* based on its mycelial and sporangial characters through standard mycological keys (Waterhouse, 1963; Hemmes, 1993) and by CMI descriptions.

The aim of this study was to evaluate the antifungal activity of some medicinal plant leaf extract used in ayurveda and traditional medicinal system for treatment of manifestations caused by pathogens.

MATERIAL AND METHODS

Eco-friendly approaches for the management of fungal diseases of *Cucumis sativus* were done by using different plant extracts.

Plant extracts

Collection of plant materials

Fresh leaves of five plant leaf extract *Azadirachta indica*, *Lantana camera*, *Aegle mormelos*, *Adhatoda vassica*, *Withania somnifera* were collected and washed in distilled water.

Fungal pathogens

The pathogens such as *Phytophthora capsici* caused by *Cucumis sativus* fruit rot.

Soxhlet Extraction

In a conventional Soxhlet extraction, 100gm of fresh sterilized plant leaves were placed in a thimble holder. The Soxhlet extraction was placed onto a flask containing the extractor solvent i.e. distilled water (100ml). The Soxhlet was equipped with a condenser. The distilled water was heated to reflux. The water vapours were traveled up in distillation arm and floods into the thimble chamber of solid. The condenser ensures that water vapours cools and driped back down into the thimble holder. The thimble containing solid material was filled slowly with warm water. Some desired compound was dissolved. When the thimble was fulfilled then the thimble chamber is automatically emptied through siphon side arm and extraction running back down to the distillation flask. The operation is repeated until complete extraction is achieved (Wang and Weller, 2006).

Determination of antifungal activity

The toxicity of stock extracted extracts was determined against *Cucumis sativus* plant fruit rot disease isolates of *Phytophthora capsici* by food poisoning technique (Mishra and Tiwari, 1992) at four (25, 50, 75 and 100%) different concentrations.

The isolates of test fungi were multiplied on CZA medium. Petriplates containing CZA supplemented with different plant leaf extracts at four concentrations with their three replications were inoculated with fresh 7 days old culture of test fungi (8 mm) cork borer discs and kept upside down. The above plates were incubated in BOD incubator (at 28 ± 2^0 C). Plates without plant extracts were served as control. Radial growth of the fungus on leaf extracts was measured at regular intervals & calculated by Vincentt method.

RESULTS AND DISCUSSION

Five Plant leaf used in this study were tested against one pathogenic fungi to determine their antifungal activity. Different concentrations of plant leaf (25, 50, 75 and 100%) were tested against pathogenic fungi. Minimum inhibitory concentration (MIC) was measured to determine the antifungal activity. The inhibition effects of the medicinal plant on pathogenic fungi are presented in table 1. Among leaf extracts tested, *Withania somnifera* leaf extract showed reduction of radial growth of *Phytophthora capsici* (75%) (Growth inhibition of fungi 76.9%) (Plate- I). and significantly inhibition of four plant leaf extract. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were reported. In many cases the people claim the good benefit of certain natural or herbal products (Tradit et al., 2011). Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world (Tradit et al., 2011). The antimicrobial activities of plant extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Mahlo et al.,2016). Medicinal plants played a crucial role in the search for components that are capable of neutralizing the multiple mechanisms of fungal resistance. The present paper reported the collection of medicinal plants mentioned in Holly Quran and used traditionally in KSA to treat some human pathogens. (Tsopmbeng et al., 2012) found V6 juice agar (64mm) the best medium for *Phytophthora* as compared to V8-m juice agar (57 mm), onion agar (55 mm), V8 juice agar (54 mm) and potato dextrose agar (54 mm). (Wongkaew and Sinsiri, 2014) tested three plant extracts against different fungi and found effective against *Phytophthora infestan*. (Abayhne and Chauhan 2016) evaluated various plant extracts against Phytophthora infestans and found Eucalyptus highly effective against *Phytophthora* . (Jagtapa, et. al., 2012) tested six plant extracts and found effective against three species of *Phytophthora* . (Sarpong, 2016) evaluated plants extracts against *Phytopthora* and Neem was found effective against *Phytophthora* . Our results are also fortify by the findings of (Bahraminejad et al.,2013), they tested 32 species of plants extracts and found all effective against *Phytophthora* and *Phythium* . Our outcomes are also in line with the findings of (Bahraminejad, et al. 2012), their results showed that plant extracts were effective against *Phytophthora drechsleri*. The findings of (Bekepe, 2012) also substantiate our discoveries; they conclude that 2% Garlic extracts was effective against *Phytophthora*.

PHOTO PLATE-I

Diseased fruit | ***Phytophthora capsici*** | **Control** | **75% Conc. (76.9%)**

Fig. 1. *Phytophthora capsici* against *Withania somnifera*

Table:1. Antifungal activity of plant leaf extracts against pathogenic fungi of *Cucumis sativus* fruit.

Plant species	Family	Conc. (%)	Radial growth of *P. capsici* (mm)	Inhibition (%)
Azadirachta indica	*Meliaceae*	25	40	60.02
		50	38	62.06
		75	31	69.01
		100	30	70.12
Lantana camera	*Verbenaceae*	25	59	40.02
		50	55	45.10
		75	49	51.02
		100	48	52.11
Aegle marmelos	*Rutaceae*	25	60	40.01
		50	55	35.03
		75	50	50.02
		100	45	55.00
Adhatoda vassica	*Acanthaceae*	25	70	30.02
		50	69	31.23
		75	60	30.05
		100	55	45.00
Withania somnifera	*Solanaceae*	25	50	50.01
		50	37	60.20
		75	29	76.09
		100	29	76.09
Mean	-		**85.677**	**90.20**
SEm± CD(p=0.05)			**12.10**	**37.05**

CONCLUSION

There is growing body of evidence indicating the benefits of medicinal plants for their use against pathogenic microorganisms. Plant based remedies used in human and animal medicine are an essential part of the primary health care system in many countries. Extensive screening programmes of plants mainly used in traditional medicine have resulted in the discovery of thousand of phytochemicals with inhibitory *Phytophthora capsici* of microorganism in vitro. There is need to exploit these bioactive compound in diseases caused by pathogenic fungi. The extract of the plant part used showed prominent antifungal activity against the fungi which are severe pathogen. Thus the use of these plants in the treatment of pathogenic diseases associated with the infection of this pathogen is validated, scientifically supported by the results which are obtained in these studies. However, the further studies are to gain more clarity as to the specificity and biochemical mechanism responsible for the antifungal properties of these nine plants in the different concentrations and on the other strains of these fungi. Natural plant derived fungicides may be a source of new alternative compounds, in particular with antifungal activity. New milestone in the development of pharmaceutical products can be achieved by discovering bioactive natural products from these medicinal plants that address unfulfilled therapeutic needs against these fungi. Further investigations are warranted for safety, cost effectiveness have to be conducted. This study paves the way for the development of bioactive natural products with the added benefit of an environmentally safe and economically viable product. So these plants can be used to discover bioactive natural products that may serve as feed for the development of new pharmaceuticals compounds. Development of phytomedicine is relatively inexpensive and also suitable to our economic conditions.

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