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Antiphytopathogenic Potential of *Allium sativum* L.: An In Vitro Study

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Abstract: ***The available information on the phytochemical composition and antifungal activity of garlic clove against phytopathogens is very limited. The main aim of this research paper is to determine the in vitro antifungal activity of Allium cloves extract against some isolated fungi viz., F. oxysporum, F. solani, F. moniliforme, H. sativum, C. lindemuthianum, C. lunata, R. solani, A. solani from different vegetables. Both the aqueous and alcoholic extracts of Allium were screened for the phytochemical constituents and were tested against fungal pathogens. Result of phytochemical screening, revealed the presence of different metabolites in different extracts. A. sativum found to be highly effective against all eight fungal pathogens and highest inhibition of mycelial growth was recorded in F. oxysporum, F. solani, F. moniliforme, H. sativum, C. lindemuthianum, C. lunata with 92.63% While methanol extract of A. sativum when tested highest inhibition on myceliall growth showed by F. solani with 89.58%***

Keywords: Allium sativum L, Phytochemical Screening, Fungal Pathogens, Aqueous extract, Methanolic Extract.

I. INTRODUCTION

Agriculture is the backbone of Indian Economy. But in India, chemicals like fertilizers and pesticides are extensively used to boost crop yields and protect against pests and diseases. However, the excessive and unregulated use of these chemicals has led to significant environmental and health problems. These chemicals in addition kill various beneficial organisms and their toxity can persist in the soil (Shaikh and Sahera.,2021). Excessive use of synthetic pesticides has been implicated in their negative effects on the environment such as soil and water pollutions, long periods of degradation, residual accumulation in the food chain, and less control efficacy against pathogenic microbes with long-term usage (Nega, 2014; Bhavaniramya et al., 2019).

The increasing resistance by these microorganisms against these chemicals has been a great concern. Among the various alternatives, natural plant products are used having no side effect and are been used by scientists. Extracts obtained from these valuable plants have gained attention as scientific interest for having antifungal activity (Santas et al., 2010). *Allium sativum* commonly known as garlic, is a member of Liliaceae family (Shruti S, 2018). When crushed, *Allium sativum* yields allicin and phytonicide which serve as antimicrobial compounds (Najeeb et al. 2016).

It also contains other compounds including allinin, ajoene, diallylsulfide, B-vitamins, proteins and many phytochemicals (Momoh et al.2016). Phytochemicals are bio-active chemicals of plant origin. They are regarded as secondary metabolites because the plant that manufactures them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components (Tiwari et al. 2010). Bioactive compounds in the plants which confer protection on them against bacteria, fungi and viruses have been linked to the antimicrobial activities of the extracts of such plants. (El-Mahmood and Amey, 2007). *Allium sativum* consists of various kinds of sulfur-containing compounds with different significances and these compounds give garlic its characteristic odor and favor as well as some favorable effects. Sulfur-containing compounds present in garlic include alliin, allicin, ajoene, 2- Vinyl-4H-1,3-dithiin, Diallyl sulfide, Diallyl disulfide, Diallyl trisulfide and Allyl methyl sulfide (Mardomi, et al. 2017).

II. MATERIAL AND METHOD

A. Preparation of Aqueous Plant Extract

Thoroughly washed fresh plant material (50 g) was macerated with 50 ml sterile distilled water in a blender for 10 min. The macerate was first filtered through double-layered muslin cloth, and then centrifuged at 4000 g for 30 min; the supernatant was filtered through Whatman No. 1 filter paper and sterilized at 120^{o}C for 30 min. The extract was preserved aseptically in a brown bottle at 5^{o}C until further use.

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B. Preparation of solvent plant extract:

Extracts were prepared in methanol at room temperature by simple extraction method (Deshpande *et al.*, 2004). Collected plant parts were shade dried and ground to a fine powder using grinder mixer. Dried powder of plant parts (10g) was mixed with 100 ml solvent in 250 ml conical flask. The flasks were plugged tightly with cotton and wrapped with papers. All conical flasks were kept on shaker for 24 h then it was allowed to stand for five hours to settle the plant materials. Thereafter, it was filtered and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated at 45 °C in vacuum evaporator to make the final volume 1/5th of the original volume. It was stored at 4 °C in airtight bottles for further studies.

These aqueous and methanolic extracts were further used for screening of antifungal activity against eight fungal pathogens causing vegetable fruit diseases.

C. Antifungal Activity of Plant Extracts

The plant extracts were evaluated *in vitro* through Poison food technique (Nene and Thapliyal, 2000). The supernatant was taken as standard plant extract solution (100%). Further, the extract was diluted by adding sterilized water to get different per cent concentrations. The plant extracts were subjected to boiling temperature of 50°C in water bath to avoid contamination and then incorporated into PDA media by transferring 2ml of each type of plant extract in to a Petridish containing 20 ml melted warm PDA medium and gently shaken for thorough mixing of the extract. The PDA plates containing the plant extracts were inoculated aseptically with different pathogens by transferring 6mm diameter agar disc of 07 days old culture of the pathogen to the centre of PDA medium in Petridish. Three replications were maintained for each treatment. The basal medium (PDA) without any phytoextract served as control. All the inoculated Petridishes were incubated at 25±1°C.The radial growth of the test fungus in the treated plates was measured in all treatments when the pathogen growth touched the periphery in the control Petridishes. The per cent inhibition of fungal growth was estimated by using the formula given by Vincent (1927).

$$I = \frac{C - T}{C} \times 100$$

Where, I = per cent inhibition.

C = Colony diameter in control.

T = Colony diameter in treatment.

III. RESULT

Present study reveals that tannins, alkaloids and flavonoids were absent in aqueous extract of *A. sativum* bulbs. *A. sativum* found to be highly effective against all eight fungal pathogens and highest inhibition of mycelial growth was recorded in *F. oxysporum, F. solani, F. moniliforme, H. sativum, C. lindemuthianum, C. lunata* with 92.63% but *A. solani* showed resistant with 87.57% inhibition. Whereas *R. solani* showed 92.60% inhibition of mycelial growth over control. While methanol extract of *A. sativum* when tested highest inhibition on myceliall growth showed by *F. solani* with 89.58% and lowest inhibition by *H. sativum* with 61.38%. Methanol extract of *A. sativum* were significantly effective and revealed 73.94% inhibition of mycelial growth of all eight tested pathogens.

Table 1: Phytochemical Analysis of *Allium* extracts.

Secondary Metabolites	Extracts	
	H2O	MeOH
Tannin	A	A
Alkaloid	A	P
Saponins	P	P
Cardiac Glycosides	P	P
Steroids	P	P
Flavonoids	A	A

A-Absent, P-Present

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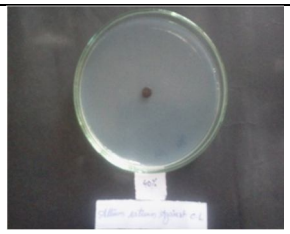
Table 2- Inhibitory effect of plant extracts of different concentrations on mycelial growth of fungal pathogens of vegetables.

Pathogens/Medicinal Plant	Mycelial growth and percent inhibition	*Allium sativum*	
		H2O Extract	MeOH Extract
A. solani	Mycelial Growth (mm)	6.6	21.3
	% Inhibition over control	87.57±0.54	76.22±1.43
C. lindemuthianum	Mycelial Growth (mm)	6.6	22.6
	% Inhibition over control	92.63±0.54	70.91±0.94
C. lunata	Mycelial Growth (mm)	6.6	31.3
	% Inhibition over control	92.63±0.54	64.94±0.71
F. moniliforme	Mycelial Growth (mm)	6.6	13.3
	% Inhibition over control	92.63±0.54	85.10±1.18
F. oxysporum	Mycelial Growth (mm)	6.6	23.3
	% Inhibition over control	92.63±0.54	73.99±0.71
F. solani	Mycelial Growth (mm)	6.6	9.3
	% Inhibition over control	92.63±0.54	89.58±0.54
R. solani	Mycelial Growth (mm)	6.6	27.3
	% Inhibition over control	92.60±0.94	69.42±0.97
H. sativum	Mycelial Growth (mm)	6.6	34.6
	% Inhibition over control	92.63±0.54	61.38±1.18

Value expressed in mean ± S. E. M of triplicate



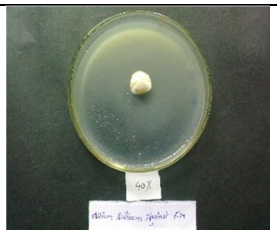
Allium sativum against A. solani



Allium sativum against C. lindemuthianum



Allium sativum against C. lunata



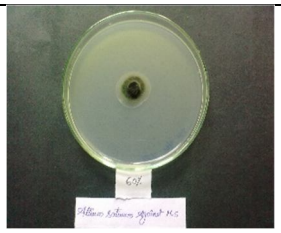
Allium sativum against F. moniliforme



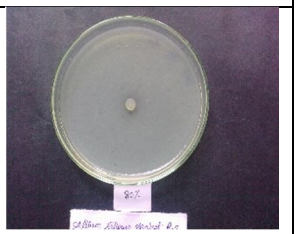
Allium sativum against F. oxysporum



Allium sativum against F solani



Allium sativum against H. sativum



Allium sativum against R. solani

Fig- Aqueous Extract of *Allium sativum* against different plant pathogens

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Allium sativum against A. solani


Allium sativum against C. lindemuthianum


Allium sativum against C. lunata


Allium sativum against F. moniliforme


Allium sativum against F. oxysporum


Allium sativum against F solani


Allium sativum against H. sativum


Allium sativum against R. solani

Fig- Methanolic Extract of *Allium sativum* against different plant pathogens

IV. DISCUSSION

Present study revealed the presence of Saponin, Steroids and cardiac glycosides in different extracts [methanol extract and distilled water extract of garlic. A recent study on phytochemical analysis of *Allium sativum* (garlic) revealed the presence of saponins, terpenoids, flavonoids, amino acids and cardiac glycosides (Arify et al.,2018). Another study on phytochemical profile of aqueous extract of *Allium sativum* L., bulbs showed the presence of saponins, steroids, tannins, carbohydrates and cardiac glycosides (Mikail H.G.2010).

The extract possesses antimicrobial properties that can suppress the growth of fungi due to the presence of active sulfur-containing bioactive compounds such as allicin (Bayan *et al.,* 2014). These compounds act by interfering with fungal cell metabolism and inhibit spore germination (Redondo-Blanco et al., 2020),

The finding of this study correlate with the finding of (Abaoab et al.2011) which found that clove extract possessed a broad spectrum of antimicrobial activity exhibited for both bacteria and fungi due to presence saponin, tannin, flavonoid and terpenoid. The result of this study on Phytochemistry of Garlic supported the study conducted by (Deresse.,2010).

This inhibition was concentration dependent. A similar survey by Alhussaen *et al.,* (2011) indicated that garlic extract had a concentration dependent activity against *Pythium ultimum* isolated from tomato seedlings. Undiluted garlic extract showed a high control activity with no growth as compared to the biotic control without the extract whereas diluted garlic extracts 10% and 5% reduced the fungal growth to 15.5% and 41% respectively (Alhussaen *et al.,* 2011).

The mechanism of action of phytochemicals against microorganism vary and depend on these phytochemicals (Aly and Bafiel.,2008). In conclusion, we have shown that garlic extracts can have a significant effect on preventing the growth of *almost all targeted pathogens*. However, growth of *H. sativum* was not significantly suppressed by the methanolic extract of garlic. The solvent used for dilution concentrations (water or Methanol) had an influence on the antifungal activity of the garlic extract. Aqueous dilutions of the extract had greater antifungal activity than Methanol diluted extracts, possibly because the longer the extracts were exposed to ethanol, the more the antifungal activity was reduced which was in support of the findings of Chanel et al.2014.

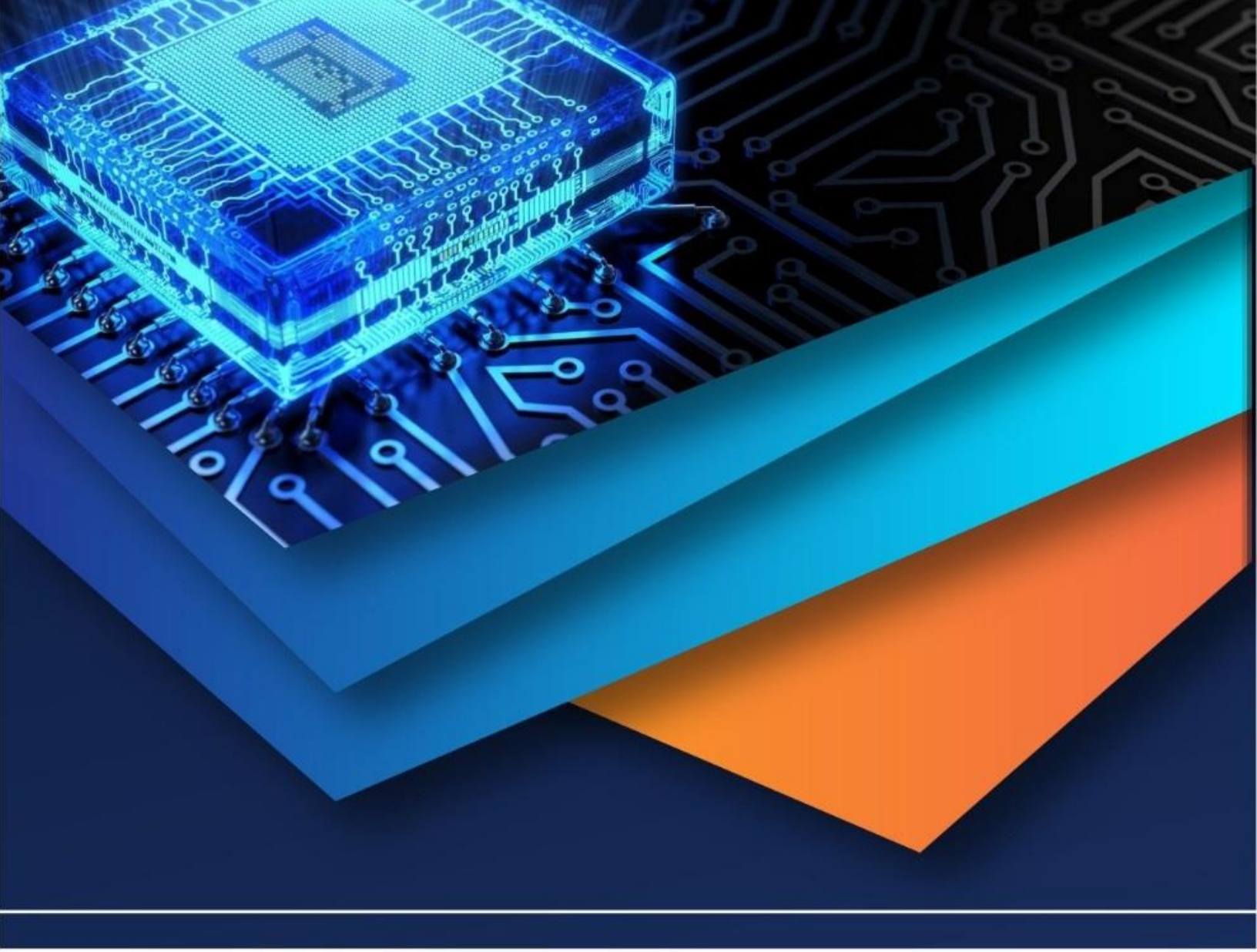
V. CONCLUSION

In conclusion, the in vitro results of this study confirmed the potentiality of *A. sativum* as one of the best sources for controlling fungal growth. Hence, further work is necessary to evaluate its potentiality in vivo on targeted pathogens. This can provide an alternative means for the control of vegetable diseases by farmers. Investigations are also needed to characterize, formulate and market the active principles of these extracts which may provide avenues for the discovery of novel antifungal compounds. These biofungicidal botanicals are environmentally safe; therefore, they could successfully replace the toxic and hazardous synthetic compounds and be exploited as ideal treatment for future plant disease management programs.

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